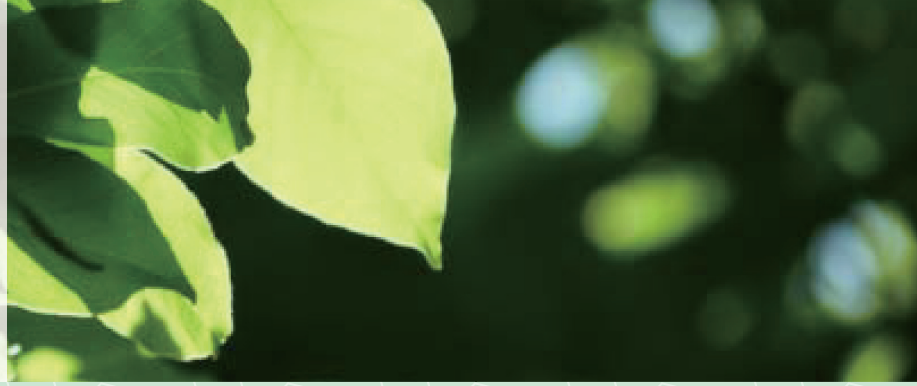
ICPM5



# 5th International Conference on Plasma Medicine (ICPM5)

## Book of Abstracts

Nara, Japan

May 18-23, 2014

# Time Table

| | Sun (May 18) | | Mon (May 19) | | | Tue (May 20) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Room A (Noh Theatre) | Room B (Conference Room) | | Room A (Noh Theatre) | Room B (Conference Room) | |
| | | | **Chair: Masaharu Shiratani** | | | **Chair: Mark Kushner** | | |
| 9:00 | | 09:00-09:15 | Opening 9:00-9:15 (RoomA) | | | *<Plenary>* Eun Ha Choi 9:00-9:45 (Room A) | | 09:00-09:45 |
| 9:30 | | 09:15-10:00 | *<Plenary>* Masaru Hori 9:15-10:00 (Room A) | | | | | |
| | | 10:00-10:20 | Break (20min) | | | *<Tutorial>* David Graves 9:45-10:15 (Room A) | | 9:45-10:15 |
| 10:00 | | | **Chair: Kai Mazur** | **Chair: Victor Vasilets** | | Group Photo and Break (20min) | | 10:15-10:35 |
| 10:15 | | 10:20-10:50 | *<Invited>* Hans-Robert Metelmann 10:20-10:50 | *<Invited>* Masaharu Shiratani 10:20-10:50 | | **Chair: Svetlana A. Ermolaeva** | **Chair: Farzaneh Arefi-Khonsari** | |
| | | 10:50-11:20 | *<Invited>* Tomoko Oshima 10:50-11:20 | *<Invited>* Steven Shannon 10:50-11:20 | | *<Invited>* Akira Myoui 10:35-11:05 | *<Invited>* Krasimir Vasilev 10:35-11:05 | 10:35-11:05 |
| 11:00 | | 11:20-11:35 | *<Invited>* Julia Bandow 11:20-11:50 | Jean-Michel Pouvesle | | *<Invited>* Georg Isbary 11:05-11:35 | *<Invited>* Cristina Canal 11:05-11:35 | 11:05-11:35 |
| 11:30 | | 11:35-11:50 | | Kim Rouven Liedtke | | *<Invited>* Jeniffer Shin 11:35-12:05 | *<Invited>* Fabio Palumbo 11:35-12:05 | 11:35-12:05 |
| | | 11:50-12:05 | Gregory Fridman | Mohammed Yousfi | | | | |
| 12:00 | | 12:05-12:20 | Uta Schnabel | Seth Norberg | | *<Invited>* Jing Fang 12:05-12:35 | *<Invited>* Sudhir Bhatt 12:05-12:35 | 12:05-12:35 |
| | | 12:20-12:35 | Kamonporn Panngom | Tomy Abuzairi | | | | |
| 13:00 | | 12:35-14:00 | Lunch Break 12:35-14:00 | | | Lunch Break 12:35-14:00 | BoD meeting 13:00- (MTG Room 1) | |
| 13:30 | | | | | | | | |
| 14:00 | | 14:00-15:45 | **Chair: Katsuhisa Kitano** | | | **Chair: Katsuhisa Kitano** | | 14:00-15:45 |
| | | | Poster Session 14:00-15:45 (Reception Hall) | | Tea Celemony | Poster Session 14:00-15:45 (Reception Hall) | | Tea Celemony |
| 15:00 | | | | | | | | |
| 15:30 | | 14:45-16:00 | Break (15min) | | | Break (15min) | | 14:45-16:00 |
| | | | **Chair: Richard Satava** | **Chair: Eric Robert** | | **Chair: Klaus-Dieter Weltmann** | **Chair: Pietro Favia** | |
| 16:00 | | 16:00-16:15 | Simon Schneider | Jong-Shinn Wu | | *<Invited>* Lars Ivo Partecke 16:00-16:30 | Yuichi Setsuhara | 16:00-16:15 |
| | | 16:15-16:30 | Endre J. Szili | Han S. Uhm | | | Yong Wang | 16:15-16:30 |
| | Registration 16:30- | 16:30-16:45 | Chanchai Chutsirimongkol | Yasushi Nishida | | *Special Session* "COST Activities Overview" 16:30-17:50 Miles Turner Deborah O'Connell Kai Masur Stephan Reuter | Robert D. Short | 16:30-16:45 |
| | | 16:45-18:05 | *Special Session* "Challenges in Industry" 16:45-18:05 Amnon Lam Miriam Mann Tetsuji Shimizu Lionel Duvillaret | Gyungsoon Park Hachiro Yasuda Daniela Boehm Masafumi Ito Taichi Miura | | | Anchu Viswan Farazaneh Arefi-Khonsari Beate Haertel Yoko Yamanishi Kaori Sano | 16:45-17:00 17:00-17:15 17:15-17:30 17:30-17:45 17:45-18:00 |
| 17:00 | Welcome Reception | | | | | | | |
| 18:00 | | | | | | | BoD meeting 18:00- (MTG Room 1) | 18:00- |
| 19:00 | | | | | | | | |
| 20:00 | | | | | | | | |

## Wed (May 21)

| | Room A (Noh Theatre) | Room B (Conference Room) |
|---|---|---|
| | **Chair: William Graham** | |
| 09:00-09:45 | *<Plenary>* Michael Keidar 9:00-9:45 (Room A) | |
| 9:45-10:05 | Break (20min) | |
| | **Chair: Georg Isbary** | **Chair: Katsuhisa Kitano** |
| 10:05-10:35 | *<Invited>* Jürgen Schlegel 10:05-10:35 | *<Invited>* Vittorio Colombo 10:05-10:35 |
| 10:35-11:05 | *<Invited>* Hiroaki Kajiyama 10:35-11:05 | *<Invited>* Xinpei Lu 10:35-11:05 |
| 11:05-11:35 | *<Invited>* Kiwon Song 11:05-11:35 | *<Invited>* Eric Robert 11:05-11:35 |
| 11:35-13:00 | Lunch Break 11:35-13:00 | |
| | Conference Excursion | |

## Thu (May 22)

| | Room A (Noh Theatre) | Room B (Conference Room) |
|---|---|---|
| | **Chair: Jean-Michel Pouvesle** | |
| 9:00-9:45 | *<Plenary>* Jürgen Lademann 9:00-9:45 (Room A) | |
| 9:45-10:15 | *<Tutorial>* Klaus-Dieter Weltmann 9:45-10:15 (Room A) | |
| 10:15-10:35 | Break (20min) | |
| | **Chair: Alexander Fridman** | **Chair: Timo Gans** |
| 10:35-11:05 | *<Invited>* Yuzuru Ikehara 10:35-11:05 | *<Invited>* Mark Kushner 10:35-11:05 |
| 11:05-11:35 | *<Invited>* William Graham 11:05-11:35 | *<Invited>* Anne Bourdon 11:05-11:35 |
| 11:35-12:05 | *<Invited>* Theresa Freeman 11:35-12:05 | *<Invited>* Zdenko Machala 11:35-12:05 |
| 12:05-12:35 | General Assembly 12:05-12:35 (Room A) | |
| 12:35-14:00 | | BoD meeting if needed |
| | Lunch Break 12:35-14:00 | |
| | **Chair: Stephan Reuter** | **Chair: Zdenko Machala** |
| 14:00-14:15 | Jörn Winter | Rene Bussiahn |
| 14:15-14:30 | João Santos Sousa | Oleg Petrov |
| 14:30-14:45 | Toshiro Kaneko | Xiao Tan |
| 14:45-15:00 | Kristian Wende | Takehiko Sato |
| 15:00-15:15 | Jue Zhang | Norimitsu Takamura |
| 15:15-15:30 | Svetlana A. Ermolaeva | Tomoko Ito |
| 15:30-15:45 | Matteo Gherardi | David B. Graves |
| 15:45-16:00 | Jan-Wilm Lackmann | Tomoyuki Murakami |
| 16:00-16:30 | Break (30min) | |
| | **Chair: Tomoyuki Murakami** | **Chair: Deborah O'Connell** |
| 16:30-16:45 | Katsuhisa Kitano | Miles Turner |
| 16:45-17:00 | Kai Masur | Christof C. W. Verlackt |
| 17:00-17:15 | Atsushi Tani | Tatsuru Shirafuji |
| 17:15-17:30 | Awards Ceremony | |
| | move to Hotel Nikko | |
| 18:45-21:30 | Conference Banquet (Hotel Nikko) | |

## Fri (May 23)

| | Room A (Noh Theatre) | Room B (Conference Room) |
|---|---|---|
| | **Chair: David Graves** | **Chair: Toshiro Kaneko** |
| 9:00-9:30 | *<Invited>* Sophie Lerouge 9:00-9:30 | *<Invited>* Peter Bruggeman 9:00-9:30 |
| 9:30-10:00 | *<Invited>* Sarah Cousty 9:30-10:00 | *<Invited>* Valery A. Titov **(*)** 9:30-10:00 |
| 10:00-10:20 | Break (20min) | |
| 10:20-10:35 | Kenji Ishikawa | Stephan Reuter |
| 10:35-10:50 | Kohei Soga | Timo Gans |
| 10:50-11:05 | Hiromasa Tanaka | Helena Tresp |
| 11:05-11:20 | Michael V. Autieri | Petr Lukes |
| 11:20-11:35 | Nozomi Takeuchi | Sybille Hasse |
| 11:35-12:10 | Closing 11:35-12:10 (Room A) | |

**(*)** Presented by Svetlana A. Ermolaeva

# Program （Oral）

| Venue | Time | Abstract No. | Presenting Author (Affiliation, Country) | Title |
|---|---|---|---|---|
| | | | | **CHAIR: Masaharu Shiratani** |
| **Plenary** | | | | |
| Room A | 09:15-10:00 | 19-PL01 | Masaru Hori (Nagoya University, Japan) | Bridge the Gap between Plasma and Medical Sciences towards Future Medical Care |
| | | | | **CHAIR: Kai Mazur** |
| **Invited** | | | | |
| Room A | 10:20-10:50 | 19-AI01 | Hans-Robert Metelmann (Greifswald University, Germany) | Plasma-Jet supported surgery of advanced head and neck cancer - Requirements and first steps for proof of concept |
| | 10:50-11:20 | 19-AI02 | Tomoko Ohshima (Tsurumi University, Japan) | Possible dental applications of plasma-based sterilization using the reduced pH method : treatment of dental caries and root canal infection |
| | 11:20-11:50 | 19-AI03 | Julia Bandow (Ruhr University Bochum, Germany) | Modulation of protein activity by atmospheric pressure plasmas |
| **Oral (Contributed)** | | | | |
| Room A | 11:50-12:05 | 19-AO01 | Gregory Fridman (Drexel Plasma Institute, USA) | Effect of Reactive Nitrogen Species Produced in Water by Reverse Vortex Gliding Arc Plasmatron on Plant Germination and Growth Rate |
| | 12:05-12:20 | 19-AO02 | Uta Schnabel (INP Greifswald e.V., Germany) | Non-thermal atmospheric pressure plasmas for food decontamination |
| | 12:20-12:35 | 19-AO03 | Kamonporn Panngom (Plasma Bioscience Research Center, Kwangwoon University, Republic of Korea) | Induction of Fungal Cell Death and Enhancement of Host Resistance by Non-thermal Dielectric Barrier Discharge (DBD) Plasma |
| | | | | **CHAIR: Richard Satava** |
| **Oral (Contributed)** | | | | |
| Room A | 16:00-16:15 | 19-AO04 | Simon Schneider (Ruhr-University Bochum, Germany) | Detailed Study of Plasma-Surface Interactions with an Atmospheric Pressure Plasma Jet (APPJ) as Selective Source for O, $O_3$ and N |
| | 16:15-16:30 | 19-AO05 | Endre J. Szili (University of South Australia, Australia) | Synthetic biological sensors and their role in unraveling mechanisms of plasma medicine |
| | 16:30-16:45 | 19-AO06 | Chanchai Chutsirimongkol (Thailand Center of Excellent for Life Science, Thailand) | Non-Thermal Plasma for Acne and Aesthetic Skin Improvement |

Monday, May 19

| Venue | Time | Abstract No. | Presenting Author (Affiliation, Country) | Title |
|---|---|---|---|---|
| **Special Session: "Challenges in Industry"** | | | | |
| Room A | 16:45-17:05 | 19-AO07 | Ammon Lam (IonMed LTD., Israel) | Preliminary Evaluation of Novel Skin Closure of Pfannenstiel Incisions Using Cold Helium Plasma and Chitosan Films |
| | 17:05-17:25 | 19-AO08 | Miriam Mann (Leibniz Institute for Plasma Science and Technology (INP Greifswald), Germany) | Standards in Plasma Medicine: Development, Contents and Importance of the first German DIN Specification. |
| Room A | 17:25-17:45 | 19-AO09 | Tetsuji Shimizu (terraplasma GmbH, Germany) | Activities of terraplasma GmbH |
| | 17:34-18:05 | 19-AO10 | Lionel Duvillaret (Kapteos, France) | Cold Plasma Diagnostic Using Vectorial Electrooptic Probe |

| Venue | Time | Abstract No. | Presenting Author (Affiliation, Country) | Title |
|---|---|---|---|---|
| | | | | **CHAIR: Victor Vasilets** |
| **Invited** | | | | |
| Room B | 10:20-10:50 | 19-BI01 | Masaharu Shiratani (Kyushu University, Japan) | Enhancement of food energy efficiency using plasmas |
| | 10:50-11:20 | 19-BI02 | Steven Shannon (North Carolina State University, U.S.A.) | Large scale low operating cost plasma sources for agricultural water treatment |
| **Oral (Contributed)** | | | | |
| Room B | 11:20-11:35 | 19-BO01 | Jean-Michel Pouvesle (Université d'Orléans, France) | *In vivo* tissue oxygenation triggered through Plasma Gun treatment |
| | 11:50-12:05 | 19-BO02 | Kim Rouven Liedtke (University of Greifswald, Germany) | The effect of plasma activated medium on pancreatic cancer cells |
| | 11:35-11:50 | 19-BO03 | Mohammed Yousfi (CNRS, Toulouse University, France) | Genotoxic and cytotoxic effects on multi cellular tumor spheroids exposed to low temperature plasmas |
| | 12:05-12:20 | 19-BO04 | Seth Norberg (University of Michigan, USA) | Controlling Plasma Jets with Gas Shields and Their Interactions with Water Covered Tissue |
| | 12:20-12:35 | 19-BO05 | Tomy Abuzairi (Shizuoka University, Japan) | Surface Modification of Dot-arrayed Carbon Nanotubes for Multifunctional Bio-chip Sensors Using Atmospheric Pressure Plasma Jet |
| | | | | **CHAIR: Eric Robert** |
| **Oral (Contributed)** | | | | |
| Room B | 16:00-16:15 | 19-BO06 | Jong-Shinn Wu (National Chiao Tung University, Taiwan) | Hybrid Plasma Fluid Modeling and Gas Flow Simulation of Atmospheric-Pressure Plasmas |
| | 16:15-16:30 | 19-BO07 | Han S. Uhm (Kwangwoon University, Republic of Korea) | Mass decontamination of biological warfare agents by plasmas |
| | 16:30-16:45 | 19-BO08 | Yasushi Nishida (National Cheng Kung University, Taiwan) | Air Cleaning System with Use of High Electric Field Plasma without Discharges |
| | 16:45-17:00 | 19-BO09 | Gyungsoon Park (Kwangwoon University, Republic of Korea) | Ionic strength of solutions can modulate the anti-microbial effects of non thermal atmospheric pressure plasma |
| | 17:00-17:15 | 19-BO10 | Hachiro Yasuda (Toyohashi University of Technology, Japan) | Analysis of Plasma-Decontamination Process in Solution Using Bacterial Spores Differentially Labeled with GFP |
| | 17:15-17:30 | 19-BO11 | Daniela Boehm (Dublin Institute of Technology, Ireland) | In-package dielectric barrier discharge atmospheric cold plasma (DBD ACP) for inactivation of *Pseudomonas aeruginosa* biofilms |

Monday, May 19

| Venue | Time | Abstract No. | Presenting Author (Affiliation, Country) | Title |
|---|---|---|---|---|
| Room B | 17:30-17:45 | 19-BO12 | Masafumi Ito (Meijo University, Japan) | Inactivation process of *P. digitatum* spores evaluated by dose of ground-state atomic oxygen |
| | 17:45-18:00 | 19-BO13 | Taichi Miura (Soka University, Japan) | Effects of Low-Temperature Atmospheric-Pressure Plasma Irradiation on the Differentiation of Mouse Embryonic Stem Cells |

| Venue | Time | Abstract No. | Presenting Author (Affiliation, Country) | Title |
|---|---|---|---|---|
| | | | | **CHAIR: Mark Kushner** |
| **Plenary** | | | | |
| Room A | 09:00-09:45 | 20-PL01 | Eun Ha Choi (Kwangwoon University, Korea) | Plasma Physics and Chemistry for Biological Cell Interactions and Healing Diseases by Nonthermal Atmospheric Pressure Plasma |
| **Tutorial** | | | | |
| Room A | 09:45-10:15 | 20-AT01 | David Graves (University of California at Berkeley, USA) | Mechanisms of plasma biomedicine: what do we know? |
| | | | | **CHAIR: Svetlana Ermolaeva** |
| **Invited** | | | | |
| Room A | 10:35-11:05 | 20-AI01 | Akira Myoui (Osaka University Hospital, Japan) | Biological Effect of Plasma Processing on Ceramics Artificial Bone |
| | 11:05-11:35 | 20-AI02 | Georg Isbary (Department of Dermatology, Hospital Schwabing, Germany) | Cold atmospheric plasmas for dermatologic and oncologic purposes |
| | 11:35-12:05 | 20-AI03 | Jennifer Shin (KAIST, KOREA) | HEALING OF WOUNDS BY ATMOSPHERIC PRESSURE PLASMA |
| | 12:05-12:35 | 20-AI04 | Jing Fang (Peking University, China) | Researches on Applying Atmospheric-Pressure Non-thermal Plasmas to Dental Medicine |
| | | | | **CHAIR: Klaus-Dieter Weltmann** |
| **Invited** | | | | |
| Room A | 16:00-16:30 | 20-AI05 | Lars Ivo Partecke (University of Greifswald, Germany) | Treatment options of atmospheric pressure plasma in GI-Cancer |
| **Special Session: "COST Activities Overview"** | | | | |
| Room A | 16:30-16:50 | 20-AO01 | Miles Turner (Dublin City University, Ireland) | COST Action MP1101: Biomedical Applications of Atmospheric Pressure Plasmas |
| | 16:50-17:10 | 20-AO02 | Deborah O'Connell (University of York, UK) | An atmospheric pressure plasma reference source and protocols for biomedical applications |
| | 17:10-17:30 | 20-AO03 | Kai Masur (INP Greifswald, Germany) | Biological Standard Tests for an Evaluation of Different Plasma Sources and Treatment Regimes |
| | 17:30-17:50 | 20-AO04 | Stephan Reuter (ZIK plasmatis at the INP Greifswald, Germany) | Introduction to the EU COST Action TD1208 - Electrical discharges with liquids for future applications |

Tuesday, May 20

| Venue | Time | Abstract No. | Presenting Author (Affiliation, Country) | Title |
|---|---|---|---|---|
| | | | | **CHAIR: Farzaneh Arefi-Khonsari** |
| **Invited** | | | | |
| Room B | 10:35-11:05 | 20-BI01 | Krasimir Vasilev (University of South Australia, Australia) | Nanoengineered plasma polymer films for antibacterial coatings |
| | 11:05-11:35 | 20-BI02 | Cristina Canal (Universitat Politècnica de Catalunya, Spain) | Plasma modification of biomaterials for hard and soft tissue repair: relevance for drug delivery |
| | 11:35-12:05 | 20-BI03 | Fabio Palumbo (CNR-IMIP Bari, Italy) | Atmospheric plasma deposition of biocomposite coatings |
| | 12:05-12:35 | 20-BI04 | Sudhir Bhatt (University Pierre and Marie CURIE, France) | Nanometric thick copolymers elaborated by low and atmospheric pressure non-equilibrium plasmas for biomedical applications |
| | | | | **CHAIR: Pietro Favia** |
| **Oral (Contributed)** | | | | |
| Room B | 16:00-16:15 | 20-BO01 | Yuichi Setsuhara (Osaka University, Japan) | Behaviors of Atmospheric-Pressure Discharge and its Interaction with Soft Materials as a Basis for Plasma Medicine |
| | 16:15-16:30 | 20-BO02 | Yong Wang (University of Missouri, USA) | Non-thermal Atmospheric Plasmas in Dental Restoration: Improved Resin Adhesive Penetration |
| | 16:30-16:45 | 20-BO03 | Robert D. Short (University of South Australia, Australia) | A biological “tissue model” to study the plasma delivery of reactive oxygen species |
| | 16:45-17:00 | 20-BO04 | Anchu Viswan (Shizuoka University, Japan) | Simulation Study of Virus Concentration Using Plasma-functionalized Graphite-encapsulated Magnetic Nanoparticles with Biotin-Avidin System |
| | 17:00-17:15 | 20-BO05 | Farazaneh Arefi-Khonsari (University Pierre and Marie Curie, France) | Biodegradable copolymer coatings deposited by low pressure plasma polymerization for controlled drug delivery - first *in vivo* results |
| | 17:15-17:30 | 20-BO06 | Beate Haertel (University of Greifswald. Institute of Pharmacy, Germany) | Plasma-based stimulation of biotechnological processes in medicinal mushroom mycelia |
| | 17:30-17:45 | 20-BO07 | Yoko Yamanishi (Shibaura Institute of Technology, Japan) | Electrically-driven micro-bubbles assisted protein crystallization |
| | 17:45-18:00 | 20-BO08 | Kaori Sano (Department of Environmental and Life Sciences, Toyohashi University of Technology, Japan) | Measurement of reactive oxygen species in plasma-treated water |

| Venue | Time | Abstract No. | Presenting Author (Affiliation, Country) | Title |
|---|---|---|---|---|
| | | | | **CHAIR: William Graham** |
| **Plenary** | | | | |
| Room A | 09:00-09:45 | 21-PL01 | Michael Keidar (George Washington University, USA) | Towards understanding mechanism of cold atmospheric plasma in cancer treatment |
| | | | | **CHAIR: Georg Isbary** |
| **Invited** | | | | |
| Room A | 10:05-10:35 | 21-AI01 | Jürgen Schlegel (Technische Universtität M ünchen, Germany) | Plasma Cancer Therapy - state of the art and path forward |
| | 10:35-11:05 | 21-AI02 | Hiroaki Kajiyama (Department of Obstetrics and Gynecology, Nagoya University Graduate School of Medicine, Japan) | New strategic plasma therapy for advanced and/or refractory epithelial ovarian cancer |
| | 11:05-11:35 | 21-AI03 | Kiwon Song (Yonsei University, South Korea) | Non-thermal atmospheric pressure plasma preferentially induces apoptosis in p53-mutated cancer cells by activating ROS-responsive pathways |
| | | | | **CHAIR: Katsuhisa Kitano** |
| **Invited** | | | | |
| Room B | 10:05-10:35 | 21-BI01 | Vittorio Colombo (Alma Mater Studiorum - University of Bologna, Italy) | Investigation of the effectiveness of a low power inductively coupled plasma source for biomedical applications |
| | 10:35-11:05 | 21-BI02 | XinPei Lu (HuaZhong University of Science and Technology, P.R. China) | Room Temperature Plasma Jets and Active Species Diagnostics |
| | 11:05-11:35 | 21-BI03 | Eric Robert (University of Orleans, France) | Understanding of gas flow, plasma and target interplay: a key prerequisite for the optimization of plasma jet treatments |

Thursday, May 22

| Venue | Time | Abstract No. | Presenting Author (Affiliation, Country) | Title |
|---|---|---|---|---|
| | | | | **CHAIR: Jean-Michel Pouvesle** |
| **Plenary** | | | | |
| Room A | 09:00-09:45 | 22-PL01 | Jürgen Lademann (Charité-University Medicine Berlin, Germany) | Application of tissue-tolerable plasma in dermatology: Risk assessment and prospects |
| **Tutorial** | | | | |
| Room A | 09:45-10:15 | 22-AT01 | Klaus-Dieter Weltmann (Leibniz Institute for Plasma Science and Technology (INP), Germany) | Plasmas sources for medical use |
| | | | | **CHAIR: Alexander Fridman** |
| **Invited** | | | | |
| Room A | 10:35-11:05 | 22-AI01 | Yuzuru Ikehara (National Institute for Advanced Industrial Science and Technology (AIST), Japan) | An application of low temperature plasma to achieve minimal invasive surgery |
| | 11:05-11:35 | 22-AI02 | William Graham (Queen's University Belfast, UK) | Collaborative studies of a helium-based kHz jet. |
| | 11:35-12:05 | 22-AI03 | Theresa Freeman (Thomas Jefferson University, USA) | Microsecond DBD Plasma for Differentiation, Development and Regeneration |
| | | | | **CHAIR: Stephan Reuter** |
| **Oral (Contributed)** | | | | |
| Room A | 14:00-14:15 | 22-AO01 | Jörn Winter (Centre for Innovation Competence (ZIK) plasmatis at the INP Greifswald, Germany) | Tracking plasma generated $H_2O_2$ from gas into liquid phase and revealing its dominant effect on human skin cells |
| | 14:15-14:30 | 22-AO02 | João Santos Sousa (Laboratoire de Physique des Gaz et des Plasmas, CNRS and Univ. Paris-Sud, France) | Degradation of DNA and Proteins Induced by Microplasma Jets |
| | 14:30-14:45 | 22-AO03 | Toshiro Kaneko (Tohoku University, Japan) | Minimally-Invasive Gene Transfection Using Atmospheric Pressure Plasma |
| | 14:45-15:00 | 22-AO04 | Kristian Wende (INP Greifswald, ZIK plasmatis, Germany) | Differential protein expression and thiol oxidation pattern in human keratinocytes in response to non-thermal plasma to reveal activation route |

| Venue | Time | Abstract No. | Presenting Author (Affiliation, Country) | Title |
|---|---|---|---|---|
| Room A | 15:00-15:15 | 22-AO05 | Jue Zhang (Peking University, China) | A genome-wide profiling of response genes in eukaryotic cells to non-thermal atmospheric pressure plasma treatment |
| | 15:15-15:30 | 22-AO06 | Svetlana A. Ermolaeva (Gamaleya Institute of Epidemiology and Microbiology, Russia) | Effects of microwave argon plasma on cell-wall-lacking *Mollicutes* bacteria |
| | 15:30-15:45 | 22-AO07 | Matteo Gherardi (Alma Mater Studiorum - University of Bologna, Italy) | Non-Thermal Plasma Promotes Apoptosis and Cell-Cycle Arrest in a Lymphoma Cell Line |
| | 15:45-16:00 | 22-AO08 | Jan-Wilm Lackmann (Ruhr University Bochum, Germany) | RNase A is Permanently Inactivated by a Dielectric Barrier Discharge by Chemical Modifications |
| | | | | **CHAIR: Tomoyuki Murakami** |
| | 16:30-16:45 | 22-AO09 | Katsuhisa Kitano (Osaka University, Japan) | Cryopreservation of plasma treated water (PTW) for disinfection |
| | 16:45-17:00 | 22-AO10 | Kai Masur (INP Greifswald, Germany) | Modulation of Cell Activities by Changing the Plasma Composition |
| | 17:00-17:15 | 22-AO11 | Atsushi Tani (Osaka University, Japan) | Selective Supply of Active Species using Plasma Treated Water (PTW) for Effective and Safety Disinfection |

Thursday, May 22

| Venue | Time | Abstract No. | Presenting Author (Affiliation, Country) | Title |
|---|---|---|---|---|
| | | | | **CHAIR: Timo Gans** |
| **Invited** | | | | |
| Room B | 10:35-11:05 | 22-BI01 | Mark Kushner (University of Michigan, USA) | Progress and Needs in Modeling of Plasma Interactions with Tissue: Wet, Dry, Direct and Indirect |
| | 11:05-11:35 | 22-BI02 | Anne Bourdon (Ecole Centrale Paris, France) | Simulation of atmospheric pressure helium discharges in capillary tubes and in plasma jets |
| | 11:35-12:05 | 22-BI03 | Zdenko Machala (Comenius University, Slovakia) | Identification of RONS in water induced by air plasmas and their biomedical effects |
| | | | | **CHAIR: Zdenko Machala** |
| **Oral (Contributed)** | | | | |
| Room B | 14:00-14:15 | 22-BO01 | Rene Bussiahn (Leibniz Institute for Plasma Science and Technology (INP Greifswald), Germany) | Plasma therapy for large-scale wound treatments: development of a flexible plasma source |
| | 14:15-14:30 | 22-BO02 | Oleg Petrov (Joint Institute for High temperatures, RAS, Russia) | Cold atmospheric plasma sources, plasma diagnostics and plasma factors at medical applications |
| | 14:30-14:45 | 22-BO03 | Xiao Tan (Huazhong University of Science & Technology, China) | Single-cell-level Mobile Microplasma Jet For Cancer Cell Apoptosis |
| | 14:45-15:00 | 22-BO04 | Takehiko Sato (Tohoku University, Japan) | Generation of micro plasma in water for biomedical applications |
| | 15:30-15:45 | 22-BO05 | Norimitsu Takamura (Kumamoto University, Japan) | Propagation Difference of Atmospheric-pressure Helium Plasma jets Using Different Dielectric Materials |
| | 15:45-16:00 | 22-BO06 | Tomoko Ito (Osaka University, Japan) | Mass spectrometry of ions formed in atmospheric-pressure plasma jets |
| | 15:00-15:15 | 22-BO07 | David B. Graves (University of California at Berkeley, USA) | Atmospheric Pressure Dielectric Barrier Discharges in Air: Chemistry and Antimicrobial Effects |
| | 15:15-15:30 | 22-BO08 | Tomoyuki Murakami (Tokyo Institute of Technology, Japan) | Biologically Relevant Species in Atmospheric Pressure Helium-Oxygen Plasmas Operated in Ambient Air |

| Venue | Time | Abstract No. | Presenting Author (Affiliation, Country) | Title |
|---|---|---|---|---|
| | | | | **CHAIR: Deborah O'Connell** |
| | 16:30-16:45 | 22-BO09 | Miles Turner (Dublin City University, Ireland) | Plasma chemistry modelling in atmospheric pressure plasmas: Errors and uncertainty |
| | 16:45-17:00 | 22-BO10 | Christof C. W. Verlackt (University of Antwerp, Belgium) | Reactive Molecular Dynamics Simulations for the Interaction of Reactive Oxygen Species with Biomolecules |
| | 17:00-17:15 | 22-BO11 | Tatsuru Shirafuji (Osaka City University, Japan) | Numerical Simulation of Electric Double Layer in Contact with DBD - Effects of Mobility and Diffusion Coefficient of Liquid Ions - |

Friday, May 23

| Venue | Time | Abstract No. | Presenting Author (Affiliation, Country) | Title |
|---|---|---|---|---|
| | | | | **CHAIR: David Graves** |
| **Invited** | | | | |
| Room A | 09:00-09:30 | 23-AI01 | Sophie Lerouge (Ecole de technologie superieure (ETS), Canada) | Primary-amine rich coatings to enhance the biocompatibility of cardiovascular implants |
| | 09:30-10:00 | 23-AI02 | Sarah Cousty (Centre hospitalier universitaire de Toulouse, France) | Medical applications of plasma technology: welcome to the future |
| **Oral (Contributed)** | | | | |
| Room A | 10:20-10:35 | 23-AO01 | Kenji Ishikawa (Nagoya University, Japan) | Electron Spin Resonance Study of Plasma-Biological Surface Interactions under Atmospheric Pressure Plasmas |
| | 10:35-10:50 | 23-AO02 | Kohei Soga (Tokyo University of Science, Japan) | Atmospheric Plasma Processing to Form Organic Coating on Ceramic Nanoparticles for Biomedical Imaging |
| | 10:50-11:05 | 23-AO03 | Hiromasa Tanaka (Nagoya University, Japan) | Diagnostics of intracellular signaling systems of glioblastoma brain tumor cells treated with plasma-activated medium |
| | 11:05-11:20 | 23-AO04 | Michael V. Autieri (AJ Drexel Plasma Institute, Drexel University, USA) | Plasma Stimulates Angiogenesis |
| | 11:20-11:35 | 23-AO05 | Nozomi Takeuchi (Tokyo Institute of Technology, Japan) | Two-Dimensional Numerical Simulation of Mass Transfer of Reactive Species through Plasma-Liquid Interface |

| Venue | Time | Abstract No. | Presenting Author (Affiliation, Country) | Title |
|---|---|---|---|---|
| | | | | **CHAIR: Toshiro Kaneko** |
| **Invited** | | | | |
| Room B | 09:00-09:30 | 23-BI01 | Peter Bruggeman (University of Minnesota, United States) | Gas phase diagnostics of plasma jets and their induced liquid phase chemistry in the context of interactions with prokaryotic and eukaryotic cells |
| | 09:30-10:00 | 23-BI02 | Valeriy Titov (G.A. Krestov Institute of Solution Chemistry RAS, Russia) | Properties and Some Possible Applications of Gas Discharges Contacting with Liquids |
| | | Program Change | | |
| | | (23-BI02) | Svetlana A. Ermolaeva (Gamaleya Institute of Epidemiology and Microbiology, Russia) | Effects of the non-thermal argon plasma on intracellular bacteria: biological mechanisms and feasible applications |
| **Oral (Contributed)** | | | | |
| Room B | 10:20-10:35 | 23-BO01 | Stephan Reuter (ZIK plasmatis at the INP Greifswald, Germany) | Tailored Reactive Oxygen Species and their generation mechanisms from the plasma, the gas and the liquid phase to human cells |
| | 10:35-11:50 | 23-BO02 | Timo Gans (York Plasma Institute, University of York, UK) | Key reactive species in cold atmospheric pressure plasmas: absolute measurements |
| | 10:50-11:05 | 23-BO03 | Helena Tresp (Center for Innovation Competence plasmatis at INP Greifswald e.V., Germany) | Plasma Jet (V)UV-Radiation Impact on Biorelevant Liquids and Cell Suspension |
| | 11:05-11:20 | 23-BO04 | Petr Lukes (Institute of Plasma Physics AS CR, Czech Republic) | Evidence about Formation of Peroxynitrite in Air Plasma-Treated Water through a Second-Order Post-Discharge Reaction of $H_2O_2$ and $HNO_2$ |
| | 11:20-11:35 | 23-BO05 | Sybille Hasse (ZIK plasmatis, INP Greifswald e.V., Germany) | PLASMA TREATMENT OF HUMAN SKIN TISSUE |

# Program （Poster）

Monday, May 19

CHAIR: Katsuhisa Kitano [ 2:00p.m. - 3:45p.m. ]

| Poster No. | Presenting Author (Affiliation, Country) | Title of Paper |
|---|---|---|
| **01. medical treatment with discharge plasmas** | | |
| 19-P01-01 | Vandana Miller (AJ Drexel Plasma Institute, Drexel University, USA) | Plasma-Tissue Interactions |
| 19-P01-02 | Adam M. Hirst (University of York, UK) | Mapping the Effects of Low Temperature Plasma Treatment of Prostate Cancer Cell Lines and Primary Cells: Along the Path to Cell Death |
| 19-P01-03 | Takamichi Hirata (Tokyo City University, Japan) | Healing Burns Using Atmospheric Pressure Plasma Irradiation |
| 19-P01-04 | Genu Takahashi (Tokyo City University, Japan) | Relationship of Nitric Oxide Concentration in the Blood Pressure Lowering in Rats Following Atmospheric Pressure Plasma Inhalation |
| 19-P01-05 | Dehui Xu (Xi'an Jiaotong University, China) | OH radical as a major factor for cell adhesion by cold atmospheric plasma |
| 19-P01-06 | Keisuke Hirasawa (Cambwick Healthcare KK, Japan) | Possible Clinical Application of Electron Discharge at Extremly Low Energy Level for Suppression of Oxidative Stress |
| 19-P01-07 | Sung Kil Kang (Pohang University of Science and Technology, Republic of Korea) | Compact microwave atmospheric plasma devices for biomedical applications |
| 19-P01-08 | Augusto Stancampiano (alma Mater Studiorum - University of Bologna, Italy) | A novel plasma based teeth whitening process |
| 19-P01-09 | Satoshi Kaiho (Tokyo city university, Japan) | Diagnostic Imaging of Plasma-Treated Rat Hypoxic Ischemic Encephalopathy Model Using X-ray CT |
| **02. biological reactions to gas plasmas or plasma-treated media/surfaces** | | |
| 19-P02-01 | Franck Clément (Pau University, France) | Analyses of Reactive Oxygen and Nitrogen Species induced by atmospheric pressure guided streamers in a physiological liquid medium |
| 19-P02-02 | Shunsuke Yoshizawa (University of Tsukuba, Japan) | Molecular Mechanism of Plasma-Induced Chemical Reaction on Protein and Amino Acid in Aqueous solution |
| 19-P02-03 | Carly E. Anderson (University of California Berkeley, USA) | Interaction of Ambient Air Corona Discharges with Aqueous Solutions and Simple Biomolecules |
| 19-P02-05 | Kathrin Duske (University Medical Center Rostock, Germany) | A comparative in vitro study of different non-thermal atmospheric pressure plasma-jets concerning cell adhesion capacity on rough titanium alloys |

Monday, May 19
[ 2:00p.m. - 3:45p.m. ]

| Poster No. | Presenting Author (Affiliation, Country) | Title of Paper |
|---|---|---|
| 19-P02-06 | Hirofumi Kurita (Toyohashi University of Technology, Japan) | Estimation of Radical Intensity and Apoptosis Induction Activity of Aqueous Media using Single-Molecule DNA Measurement |
| 19-P02-07 | Kodai Sakuramoto (Kochi University of Technology, Japan) | UV absorption of water induced by APPJ irradiation |
| 19-P02-08 | Angela Privat-Maldonado (University of York, UK) | Determining the effect of plasma on bacterial DNA at the single cell level |
| 19-P02-09 | Jean-Philippe Sarrette (Toulouse University / CNRS, France) | Degradation of fatty acids by nitrogen flowing afterglows at reduced pressure |
| 19-P02-10 | Elena V. Sysolyatina (Gamaleya Research Institute of Epidemiology and Microbiology, Russia) | Bactericidal and wound healing properties of the air plasma generated by the ferroelectric generator |
| 19-P02-11 | Malte U. Hammer (ZIK plasmatis @INP Greifswald, Germany) | Influence of plasma-treated liquids on structure and function of lipid membranes |
| 19-P02-12 | Abraham Lin (Drexel Plasma Institute, USA) | Stimulation of Intracellular Reactive Oxygen Species in Uniform and Non-Uniform Regimes of Nanosecond Pulsed Dielectric Barrier Discharge Treatment |
| 19-P02-13 | Gai Ohashi (University of Tsukuba, Japan) | Degeneration of amyloid-β fibrils in aqueous solution by low-temperature atmospheric-pressure plasma |
| 19-P02-14 | Satoshi Ikawa (University of Tsukuba, Japan) | Evaluation of oxidative stress inside cell membrane by the penetration of HOO radical with the reduced pH method for plasma disinfection |
| 19-P02-15 | Julia van der Linde (Greifswald University, Germany) | Analysis of intraperitoneal application of TTP on murine small bowel |
| 19-P02-16 | Caitlin Heslin (Dublin Institute of Technology, Ireland) | Efficacy and Safety Considerations for the Use of Atmospheric Cold Plasma in Wound Treatment |
| 19-P02-17 | Hiroshi Hashizume (Meijo University, Japan) | Proliferation mechanism of budding yeast cells with oxygen radical treatment |
| **03. plasma-based sterilization/decontamination** | | |
| 19-P03-01 | Bulteau Anne-Laure (Pau University - UMR CNRS, France) | Oxydative stress responses induced by atmospheric pressure guided streamers on bacteria *Escherichia coli* |
| 19-P03-02 | Zdenko Machala (University of California, Berkeley and Comenius University, USA, Slovakia) | Frugal Air Spark-like Plasma for Antimicrobial $NO_x$ Generation |

| Poster No. | Presenting Author (Affiliation, Country) | Title of Paper |
|---|---|---|
| 19-P03-03 | Toshihiro Takamatsu (Tokyo Institute of Technology, Japan) | Investigation of biological effect and toxin degradation using temperature controllable multi-gas plasma jet |
| 19-P03-04 | Nid'a H. Alshraiedeh (Queen's University Belfast, UK) | Atmospheric Pressure, Non-Thermal Plasma for Control of *P. aeruginosa* Biofilms: Effect of Biofilm Components on Phenotypic Resistance |
| 19-P03-05 | Joanna Abigael Daseco (University of the Philippines, Philippines) | Comparative Study on the Use of Different Metal Electrodes in Low Pressure Glow Discharge Plasma Sterilization |
| 19-P03-06 | Zuzana Kovaľová (Supelec, France) | Decontamination of the inner walls of a narrow tube at atmospheric pressure using long distance propagation discharge in argon |
| 19-P03-07 | Takaya Oshita (Tokyo Institute of Technology, Japan) | Influence Investigation of Gas Temperature on Inactivation of Oral Bacteria using Temperature-controllable Plasma Jet |
| 19-P03-09 | Guanyang Tang (Tohoku University, Japan) | Water Sterilization by a Nano-second-pulsed Plasma Discharge in Gas Bubbles |
| 19-P03-10 | Yosuke Watanabe (Tokyo Institute of Technology, Japan) | Effect of gas species on plasma-bubbling sterilization |
| 19-P03-11 | Xiaoli Yang (Shizuoka University, Japan) | Roles of Oxygen and Nitrogen Atoms $N_2/O_2$ Plasma on Inactivation of Spore-forming Microorganisms |
| 19-P03-12 | Eva Dolezalova (Institute of Plasma Physics AS CR, Czech Republic) | Detection of membrane damages in *Escherichia coli* after plasma treatment |
| 19-P03-13 | Karol Hensel (Comenius University, Slovakia) | Inactivation of bacteria and cells by DC transient spark discharge |
| 19-P03-14 | Kun Qien (Gunma University, Japan) | Enhancement of the Sterilization Efficiency of Argon Plasma Jet by Addition of $O_2$ and $H_2O_2$ |
| 19-P03-15 | Hiroto Matsuura (Osaka Prefecture University, Japan) | The Effect of Active Radical Production on the Plasma Degradation of Phorbol Esters in Bio-diesel Fuel industry |
| 19-P03-16 | Joey Kim T. Soriano (University of the Philippines, Philippines) | Mold sterilization of contaminated oil-on-canvas paintings via microwave atmospheric plasma pencil (MAPP) |
| 19-P03-17 | Siti Khadijah binti Za'aba (UNIVERSITI MALAYSIA PERLIS, Malaysia) | Inactivation Acinetobacter Bacteria by Atmospheric Plasma |
| **04. agricultural applications of plasma technologies** | | |
| 19-P04-01 | Takaaki Amano (Kyushu University, Japan) | Preservation of Growth Enhancement of Plants after Atmospheric Pressure DBD Plasma Irradiation |

Monday, May 19
[ 2:00p.m. - 3:45p.m. ]

| Poster No. | Presenting Author (Affiliation, Country) | Title of Paper |
|---|---|---|
| 19-P04-02 | Thapanut Sarinont (Kyushu University, Japan) | Effects of ambient gas species for plasma irradiation to seeds on plant growth promotion |
| 19-P04-03 | Kohei Takano (Iwate University, Japan) | Improvement of Growth Rate of Plants using Bubble Discharge in Water |
| 19-P04-04 | Taesoo Kim (Kwangwoon University, Republic of Korea) | Effects of Dielectric Barrier Discharge (DBD) Plasma on Seed Germination and Plant Growth |
| **06. plasma-based surface modification for medical/biological applications** | | |
| 19-P06-01 | Maria Letizia Focarete (alma Mater Studiorum - University of Bologna, Italy) | Atmospheric pressure non-equilibrium plasma for the production of composite materials |
| 19-P06-02 | Andreas Heilmann (Fraunhofer Institute for Mechanics of Materials IWM, Halle (Saale), Germany) | Aerosol-Assisted Atmospheric Pressure Dielectric Barrier Discharges on Polymer Surfaces for anti-microbial properties |
| 19-P06-03 | Andrey Choukourov (Charles University in Prague, Faculty of Mathematics and Physics, Czech Republic) | Direct covalent coupling of biomolecules to nanostructured plasma polymers |
| 19-P06-04 | Yoshihiro Akimoto (Kyorin University School of Medicine, Japan) | Molecular Morphological Analysis of the Effect of Low Temperature Plasma on the Wound Healing of Skin |
| 19-P06-05 | So-Hyoun Jeon (Sungkyunkwan University, Republic of Korea) | Flow manipulation in thread-based microfluidics by plasma treatment of wool with various gas |
| 19-P06-06 | Mei-Chen Liu (Ming Chi University of Techinology, Taiwan) | Surface-Modification Techniques of Thin Film Transistors and Capacitances by Plasma Deposition $SnO_xC_y$ for Improve Electric Conductivity of Biomedical Applications |
| 19-P06-07 | Kullachard Ozawa (The Petroluem and Petrochemical college Chulalongkorn University, Thailand) | Preparation of Nylon/Chitin Membranes by Solution Casting and DBD Plasma Treatment for Wound Care Application |
| 19-P06-08 | Kentaro Hayashida (Organization for Innovation and Social Collaboration, Shizuoka University, Japan) | Observation of Skin Changes by Atmospheric Plasma Jet Irradiation |
| 19-P06-09 | Chia-Ti Chang (Tatung University, Taiwan) | Micro-arc Oxidation Titanium and Post Treatment by Cold Plasma and Graft Polymer for Improving Biocompatibility |
| 19-P06-10 | Anna Liguori (Alma Mater Studiorum - University of Bologna, Italy) | Atmospheric pressure plasma patterning of biocompatible substrates: comparison of localized treatment effectiveness with different plasma sources |

| Poster No. | Presenting Author (Affiliation, Country) | Title of Paper |
|---|---|---|
| 19-P06-11 | Paolo Baldissara (Alma Mater Studiorum - University of Bologna, Italy) | Plasma as a new odontoiatric tool to improve implants adhesion |
| **07. biochemical/biomolecular engineering with plasmas** | | |
| 19-P07-01 | Naresh Kumar (Kwangwoon University, Republic of Korea) | Influence of deuterium oxide generated through non thermal $D_2O$ plasma jet on biomolecule |
| 19-P07-02 | Yohei Ikeda (Ehime University, Japan) | Minimization and Localization of Cell Damage under Microplasma Irradiation for Gene Transfection |
| 19-P07-03 | Seiryu Shibakawa (Ehime University, Japan) | Evaluation of DNA Damage Irradiated by Plasmas for Gene Transfection |
| 19-P07-04 | Chihiro Tsutsui (Tokyo City University, Japan) | Cell Activation using Micro-Spot Atmospheric Pressure Plasma Derived FGF-2/VEGF |
| 19-P07-05 | Masaru Yoshioka (Ehime University, Japan) | Plasma Gene Transfection with Surface Discharge |
| **08. fundamentals of atmospheric-pressure plasmas** | | |
| 19-P08-01 | Young J. Hong (Kwangwoon university, Republic of Korea) | Measurement of electron temperature and 1s excited atom density by using collisional radiative model in nonthermal atmospheric Ar plasma jet |
| 19-P08-02 | Kanako Sekimoto (Yokohama City University, Japan) | Mass spectrometric analysis of negative ion formation in atmospheric pressure corona discharges with point-to-plane electrodes |
| 19-P08-03 | Marguerite Dang Van Sung Mussard (LPP, Ecole Polytechnique, France) | Experimental study of a discharge propagating in a dielectric capillary - Interaction of a plasma jet with a surface |
| 19-P08-04 | Youbin Seol (Korea Advanced Institute of Science and Technology, Republic of Korea) | Study on the radical production in atmospheric pressure pulsed DBD plasma jets |
| 19-P08-05 | Hikaru Nozaki ( Nagaoka University of Technology, Japan) | Study on Coloring Effect for Metal Surface using Atmospheric Pressure Plasmas |
| 19-P08-06 | Sandra Richter (Fraunhofer Institute for Mechanics of Materials IWM, Halle, Germany) | Correlations of in-line analytical investigations of atmospheric pressure plasma processes with surface analysis |
| 19-P08-07 | Camille Faith P. Romero (Doshisha University, Japan) | Development of ECR Microwave Antenna for the production of streaming atmospheric-pressure plasma |

Monday, May 19
[ 2:00p.m. - 3:45p.m. ]

| Poster No. | Presenting Author (Affiliation, Country) | Title of Paper |
|---|---|---|
| 19-P08-08 | Yubin Xian (Huazhong University of Science and Technology, China) | He Plasma Plumes in Different Surrounding Gases |
| 19-P08-09 | Giichiro Uchida (Osaka University, Japan) | Characteristics of Reactive Particle Production in Atmospheric Pressure DBD Plasma Jet |
| **09. plasma-surface interactions relevant for medical/biological applications** | | |
| 19-P09-01 | Jan Benedikt (Ruhr-University Bochum, Germany) | Atmospheric pressure plasma jet for separated and combined treatment with plasma generated reactive species and photons |
| 19-P09-02 | Dai Itsuki (Osaka University, Japan) | Modification of hydroxyapatite and polystyrene surface for cell culture by low-pressure plasmas |
| 19-P09-03 | Yoshiyuki Suda (Toyohashi University of Technology, Japan) | Defect formation of lipid bilayer membrane by dielectric barrier discharge irradiation and comparison with chemical treatment |
| 19-P09-04 | Kosuke Takenaka (Osaka University, Japan) | Interactions of Atmospheric Pressure Non-equilibrium-Plasma with Organic Materials through Gas/Liquid Interface |
| 19-P09-05 | Naoyuki Kurake (Nagoya University, Japan) | Electron Spin Resonance Study of Plasma-Activated-Medium |
| 19-P09-06 | Yui Hayashi (Nagoya University, Japan) | Reaction of Amino Acid and Protein in Water Induced by Electric Discharge at Argon / Aqueous Solution Interface |
| 19-P09-07 | Hiromasa Yamada (Tsukuba University, Japan) | Characteristic measurements of a plasma flare of medical equipment using a low temperature plasma |
| **10. plasma sources for medical/biological applications** | | |
| 19-P10-01 | Anser Ali (Kwangwoon University, Republic of Korea) | Role of non-thermal dielectric barrier discharge (DBD) plasma for wound healing application |
| 19-P10-02 | Andreas Helmke (Fraunhofer Institute for Surface Engineering and Thin Films, Germany) | Ozone concentrations in the plasma volume and the surrounding of a plasmamedical dielectric barrier discharge source operated in ambient air |
| 19-P10-03 | Mohammed Yousfi (CNRS, Toulouse University, France) | Tuning of low temperature plasmas ejected in open air for biomedical applications from diagnostic and modeling tools |
| 19-P10-04 | Chae bok Lee (Department of Plasma Bioscience and Display , Republic of Korea) | Visualization of OH radical interactions in living cells by adding $D_2O$ in non-thermal plasma jet |
| 19-P10-05 | Suk Hwal Ma (Ajou university, Republic of Korea) | An atmospheric-pressure cold plasma jet device with a multi-microchannel structure |

| Poster No. | Presenting Author (Affiliation, Country) | Title of Paper |
|---|---|---|
| 19-P10-06 | Yasumasa Okazaki (Nagoya University, Graduate School of Medicine, Department of Pathology and Biological Responses, Japan) | Non-equilibrium atmospheric pressure plasma (NEAPP) generates oxidative injury |
| 19-P10-07 | Henryka Stryczewska (Lublin University of Technology, Poland) | Power Supply in Non-Thermal Plasma Generators for Biological Applications |
| 19-P10-08 | Victor N. Vasilets (Institute for Energy Problems of Chemical Physics, RAS, Russia) | Therapeutic effects of gases formed in hot air plasmas and medical applications of graphene-based polymer materials. |
| 19-P10-09 | Max Engelhardt (Ruhr-University Bochum, Germany) | Characterization of propagating ionization waves in atmospheric plasma discharges |
| 19-P10-10 | Jaeho Kim (National Institute of Advanced Industrial Science and Technology (AIST), Japan) | Discharge characteristics of an atmospheric pressure cold plasma jet for medical applications |
| 19-P10-11 | Yota Sasaki (Tokyo Institute of Technology University, Japan) | Investigation of Singlet Oxygen ($^1O_2$) and OH radical in Bacterial Sterilization |
| 19-P10-12 | Thibault Darny (GREMI UMR7344 CNRS/ University of Orleans, France) | Selective reactive species production in a μs helium plasma gun discharge |
| 19-P10-13 | Hea Min Joh (Dong-A University, Republic of Korea) | The study of atmospheric pressure plasma to induce p53-mediated apoptosis through ROS generation in human lung cancer cells |
| 19-P10-14 | Xiaoqian Cheng (The George Washington University, USA) | The Effect of Differing Cold Plasma Composition on Glioblastoma Cell Viability |
| **11. plasma and/or liquid diagnostics and sensors** | | |
| 19-P11-01 | Shusuke Nishiyama (Hokkaido University, Japan) | LIF Imaging of Sodium Atoms in Atmospheric-Pressure Miniature Gas Flow DC Glow Discharge in Contact with Sodium Chloride Solution |
| 19-P11-02 | Xuekai Pei (Huazhong University of Science & Technology, China) | Measurement of OH radicals in RT-APPJ using laser-induced fluorescence |
| 19-P11-03 | Kentaro Tomita (Kyushu University, Japan) | Thomson Scattering Measurements of Atmospheric Plasmas Contacting with Ionic Liquids |
| 19-P11-04 | Tatsuo Ishijima (Kanazawa University, Japan) | Investigation of Chemical Species Production Rates in Aqueous Solution Irradiated by Non-equilibrium Atmospheric Pressure Jet |

Monday, May 19
[ 2:00p.m. - 3:45p.m. ]

| Poster No. | Presenting Author (Affiliation, Country) | Title of Paper |
|---|---|---|
| 19-P11-05 | Takayuki Ohta (Meijo University, Japan) | Molecular structure of microorganisms measured by multiplex coherent anti-Stokes Raman scattering microspectroscopy |
| **12. modeling and numerical simulation** | | |
| 19-P12-01 | Kazumasa Ikuse (Osaka University, Japan) | Numerical simulation of Fenton reactions in water exposed to an atmospheric-pressure plasma |
| 19-P12-02 | Maksudbek Yusupov (University of Antwerp, Belgium) | Modeling of the behavior of reactive oxygen species in a liquid water layer of interest for plasma medicine |
| **13. others** | | |
| 19-P13-01 | Akiyo Tanaka (Kyushu University, Japan) | Tissue Distribution of Indium After Repeated Intratracheal Instillations of Indium-Tin Oxide in Hamsters |
| 19-P13-02 | Masato Kiuchi (National Institute of Advanced Industrial Science and Technology (AIST), Japan) | Atmospheric Chemical Reaction by Air Activation Apparatus Using Corona Discharge and UV Lamp |

Tuesday, May 20

CHAIR: Katsuhisa Kitano [ 2:00p.m. - 3:45p.m. ]

| Poster No. | Presenting Author (Affiliation, Country) | Title of Paper |
|---|---|---|
| **01. medical treatment with discharge plasmas** | | |
| 20-P01-01 | Nasruddin (Department of Clinical Nursing, Graduate School of Medical Science, Kanazawa University, Japan) | Visually non-contact argon plasma jet on microliter water-dropped wound accelerates wound healing |
| 20-P01-02 | Zilan Xiong (University of California at Berkeley, USA) | Atmospheric Pressure Plasma for Nail Fungus Treatment |
| 20-P01-03 | Minjoo Lee (Tokyo City University, Japan) | Treatment of Cardiac Disease by of Atmospheric Pressure Plasma Inhalation |
| 20-P01-04 | Konstantin Sobyanin (Gamaleya Institute of Epidemiology and Microbiology, Russia) | Plasma inactivation of biofilms formed ex vivo within a root chanal by the causative agent of pulpitis |
| 20-P01-05 | Chiharu Tokita (Tokyo City University, Japan) | Research for Regenerative Medicine Using Micro-spot Atmospheric Pressure Plasma Source |
| 20-P01-06 | Shiyu Zhong (Xi'an Jiaotong University, China) | Cell death and cytokine release induced by surface plasma in immortalized human keratinocytes (HaCaT) |
| 20-P01-07 | Jun-ichiro Ikeda (Osaka University, Japan) | Effect of non-equilibrium atmospheric pressure plasma in cancer initiating cells |
| 20-P01-08 | Maty Tzukerman (Rambam Medical Center, Israel) | The Effect of Cold Plasma Treatment on Cancer Stem Cells |
| 20-P01-09 | Yan-Ren Lin (Changhua Christian Hospital, Taiwan) | Pediatric skin inflammatory reactions (urticaria) increases the risk of developing new-onset depression - a database study |
| 20-P01-10 | Guillaume Collet (Université d'Orléans, France) | NTP Antitumor Soft Treatment: Evidence of a Triggering Effect? |
| **02. biological reactions to gas plasmas or plasma-treated media/surfaces** | | |
| 20-P02-01 | Ryo Ono (The University of Tokyo, Japan) | Role of Radicals on Cell Viability |
| 20-P02-02 | Roger Martin Agustin (Toyohashi University of Technology, Japan) | Development of method for analyzing eukaryotic cellular responses to atmospheric pressure non-thermal plasma using yeast knockdown collection |
| 20-P02-03 | Francesca Cavrini (Alma Mater Studiorum - University of Bologna, Italy) | Antimicrobial activity of a low power inductively coupled plasma source at safe levels for eukaryotic cells |
| 20-P02-04 | Jeongeong -Hae Choi (Pusan National University, Republic of Korea) | Treatment with low temperature atmospheric pressure plasma enhances cutaneous delivery of epidermal growth factor by regulating E-cadherin-mediated cell junctions |

Tuesday, May 20
[ 2:00p.m. - 3:45p.m. ]

| Poster No. | Presenting Author (Affiliation, Country) | Title of Paper |
|---|---|---|
| 20-P02-05 | Sung Un Kang (Ajou university school of medicine, Republic of Korea) | Non-thermal atmospheric pressure plasma inhibits invasion of thyroid cancer cells : Involvement of cytoskeletal modulation and MMP change |
| 20-P02-06 | Uroš Cvelbar (Jožef Stefan Institute, Serbia) | Effects of Plasma on Lens Epithelial Cells |
| 20-P02-07 | Ji Hoon Park (Department of Electrical and biological Physics, Kwangwoon University, Republic of Korea) | A New Generation of Biocompatible Pulse-discharged Plasma by Marx Generator and its Application on the Biomolecules |
| 20-P02-08 | Shota Sasaki (Tohoku University, Japan) | Effective Region of Atmospheric Pressure Plasma on Transfection |
| 20-P02-09 | Sander Bekeschus (Leibniz Institute for Plasma Science and Technology (INP Greifswald), Germany) | The High Significance of Hydrogen Peroxide in Cold Atmospheric Pressure Plasma treated Human Blood Immune Cells |
| 20-P02-10 | Mareike A. Ch. Hänsch (Leibniz Institute for Plasma Science and Technology, INP-Greifswald e.V., Germany) | Bacteria show increased susceptibility against common available antibiotics and no resistance by repetitive atmospheric pressure plasma application |
| 20-P02-11 | Pei-Lin Shao (National Cheng Kung University, Taiwan) | Second degree burn wound healing on mice stimulated by $N_2$/Ar micro-plasma exposure |
| 20-P02-12 | Nathaniel D. Taylor (Drexel University, USA) | Energy Source Effects of Non-thermal Plasma Jet on Skin Cancer Cells in Artificial Tissue Scaffold |
| 20-P02-13 | Anne Mai-Prochnow (CSIRO Materials Science and Engineering, Australia) | Bacterial biofilm response to argon plasma treatment |
| 20-P02-14 | Julius Andrew P. Nunez (University of the Philippines, Philippines) | Antibacterial performance of magnetron sputtered $TiO_2$ thin films deposited at varying discharge current and deposition time |
| 20-P02-15 | Anke Schmidt (Centre for Innovation Competence plasmatis, Leibniz Institute for Plasma Science and Technology (INP), Germany) | Transcriptional profiling in human keratinocytes in response to non-thermal plasma and identification of transcription factor for regulating differential gene expression |
| 20-P02-16 | Paulien Smits (Eindhoven University of Technology, Netherlands) | Dielectric barrier discharge devices tailored to specific skin treatments |

| Poster No. | Presenting Author (Affiliation, Country) | Title of Paper |
|---|---|---|
| 20-P02-17 | Kiijung Kim (KAIST, Republic of Korea) | The effect of atmospheric pressure plasma to angiogenesis |
| 20-P02-18 | Matteo Zuin (Consorzio RFX, Associazione Euratom-ENEA sulla fusione, Italy) | Control of time-limited activation of human primary fibroblasts through ROS generation induced by cold atmospheric plasma treatment |
| **03. plasma-based sterilization/decontamination** | | |
| 20-P03-01 | Silvia Polverini (Alma Mater Studiorum - University of Bologna, Italy) | Plasma source for fast and continuous purification of water flows |
| 20-P03-02 | Hiroshi Okawa (Yamato Scientific Co.,Ltd., Japan) | Bactericidal Characteristics and Material Conformity of Atmospheric-Pressure Glow Discharge |
| 20-P03-03 | Takuya Towatari (Meijo University, Japan) | Inactivation of microorganism in liquid treated with neutral reactive oxygen species |
| 20-P03-05 | Tomomasa Itarashiki (Kyushu University, Japan) | Multi-torch type microwave air plasma designed for medical sterilization |
| 20-P03-06 | Toru Sasaki (Nagaoka University of Technology, Japan) | Inactivation effect of marine microorganisms on hydrogen mixed gas plasma generated by dielectric barrier discharges |
| 20-P03-07 | Yuichiro Takemura (Kinki University, Japan) | Sterilization treatment of bacterial spores contaminated spices by Atmospheric Pressure Plasma Jet |
| 20-P03-08 | Kohei Umeda (Kumamoto University, Japan) | Difference of Cell Death Ratio between using Atmospheric-pressure Dry- and Mist- Plasma Jets |
| 20-P03-09 | Zimu Xu (University of Science and Technology of China, China) | Sterilizing Effect of *Xanthomonas Campestris* pv. Campestris (*Xcc* ) by Corona-Discharge Nonthermal Plasma Exposure at Atmospheric Pressure |
| 20-P03-10 | Akira Yonesu (University of the Ryukyus, Japan) | Internal sterilization of a narrow tube by ECR plasma |
| 20-P03-11 | Utku Kursat Ercan (Izmir Katip Celebi University, Turkey) | Cellular Responses in *E. coli* upon Exposure to Non-Thermal DBD Plasma Treated N-Acetylcysteine (NAC) Solution |
| 20-P03-12 | Sarah Higginbotham (Queen's University BELFAST, UK) | EVALUATION OF THE BACTERICIDAL EFFECT OF A HELIUM BASED ATMOSPHERIC PRESSURE NON THERMAL PLASMA JET ON THE 'ESKAPE' PATHOGENS |
| 20-P03-13 | Romolo Laurita (Alma Mater Studiorum - University of Bologna, Italy) | Comparison of the growth inhibition potential of different dielectric barrier discharge operating regimes |

Tuesday, May 20
[ 2:00p.m. - 3:45p.m. ]

| Poster No. | Presenting Author (Affiliation, Country) | Title of Paper |
|---|---|---|
| 20-P03-14 | Cristina Muja (Toulouse University, CUFR J.F. Champollion, France) | Bacterial surface decontamination of different types of materials using an UV-C dielectric barrier discharge flat lamp |
| 20-P03-15 | Hayat Zerrouki (Toulouse University / CNRS, France) | Morphologic changes observed on *E. coli* bacteria submitted to nitrogen and air plasma jets and afterglows |
| 20-P03-16 | Lu Han (Dublin Institute of Technology, Ireland) | Inactivation Mechanism of Atomospheric Cold Plasma against *Escherichia coli* and *Staphylococcus aureus* in Liquid |
| **04. agricultural applications of plasma technologies** | | |
| 20-P04-01 | Min Ho Kang (KwangWoon University, Republic of Korea) | The application of $O_3$ and plasma generated by arc discharge in control of rice Bakanae disease caused by *Fusarium fujikuroi* |
| 20-P04-02 | Mohamed El Shaer (Faculty of Engineering, Zagazig University, Egypt) | Treatment of Microorganisms in Vegetables and Fruits by Gliding Arc |
| 20-P04-03 | Kohei Yoshida (Iwate University, Japan) | Effects of electrical stimulation by high voltage pulse on yield in sawdust-bed cultivation *Lentinula edodes* |
| **05. pharmaceutical applications of plasma technologies** | | |
| 20-P05-01 | Daiki Yamagami (Okayama University, Japan) | Histological comparison of the wound healing process between non-thermal plasma hemostasis and thermal coagulation hemostasis |
| **06. plasma-based surface modification for medical/biological applications** | | |
| 20-P06-01 | Katja Fricke (Leibniz Institute for Plasma Science and Technology (INP Greifswald e.V.), Germany) | Generation of locally deposited Bioactive Thin Films using Atmospheric Pressure Plasma Jets |
| 20-P06-02 | Nichapat Boonyeun (Chulalongkorn University, Thailand) | Preparation of Bacterial Cellulose Composites with the aid of Dielectric Barrier Discharge (DBD) Plasma Treatment |
| 20-P06-03 | Sophie Lerouge (Ecole de technologie superieure (ETS), Canada) | Plasma polymer coatings for biomedical applications: effect of aqueous media |
| 20-P06-04 | Hitoshi Muguruma (Shibaura Institute of Technology, Japan) | Patterning of Endothelial Cells and Hepatic Stellate Cells with Two Step Plasma-polymerized Processes |
| 20-P06-05 | Joanna Pawłat (Lublin University of Technology, Poland) | Treatment of Polymer Surface in APPJ |
| 20-P06-06 | Chia-Hsuan Tseng (Graduate Institute of Biomedical Materials and Tissue Engineering, Taipei Medical University, Taiwan) | Cell adhesion enhancement of electrospun microtube array membrane (MTAM) by acetic acid (AA) plasma treatment for hollow fiber assay |

| Poster No. | Presenting Author (Affiliation, Country) | Title of Paper |
|---|---|---|
| 20-P06-07 | Jang-Hsing Hsieh (Ming Chi University of Technology, Taiwan) | Mechanical and biocompatibility of tunable TaOxNy thin films |
| 20-P06-08 | Ayako Oyane (National Institute of Advanced Industrial Science and Technology, Japan) | Laser-assisted biomimetic process for calcium phosphate deposition on a titanium metal |
| 20-P06-09 | Mohammad Jellur Rahman (Graduate School of Science and Technology, Shizuoka University, Japan, Bangladesh) | Surfactant-Free Green Approach to Obtain Water-Dispersible Carbon Nanotubes by RF Plasma Treatment |
| 20-P06-10 | Nina Recek (Jozef Stefan Institute, Slovenia) | Influence of polymer surface on cell proliferation and cell oxidative homeostasis |
| 20-P06-11 | Kiyoshi Ohnuma (Nagaoka University of Technology, Japan) | Plasma-patterned PDMS Coated with Vitronectin and γ-globulin Enables Patterning of Human iPS Cells |
| **07. biochemical/biomolecular engineering with plasmas** | | |
| 20-P07-01 | Mana Oga (Tokyo City University, Japan) | Research for Tissue Regeneration Using Micro-Spot Atmospheric Pressure Plasma Source |
| 20-P07-02 | Kuntinee Somboonying (The Petroleum and Petrochemical College, Chulalongkorn University, Thailand) | Deacetylation and Depolymerization of Chitin Hydrogel via Solution Plasma Process |
| 20-P07-03 | Takuya Yamasaki (Ehime University, Japan) | Gene Transfection to Human Skin Cells by Microplasma Irradiation Using Microcapillary Electrode |
| 20-P07-04 | Amel Zerrouki (Ehime University, Japan) | Analysis of Plasma Irradiation Effect on Cell Membrane Using Artificial Cells |
| **08. fundamentals of atmospheric-pressure plasmas** | | |
| 20-P08-01 | Su-Jeong Kim (Seoul National Univerisity, Republic of Korea) | How to Improve the Reproducibility of Treatment Using Helium Atmospheric Pressure Plasma Jet (He-APPJ) |
| 20-P08-02 | Seiya Yonemori (The University of Tokyo, Japan) | Effect of voltage polarity and surface condition on active species production by an atmospheric-pressure helium plasma jet |
| 20-P08-03 | Alexey Shashurin (The George Washington University, USA) | Physical processes in the low-frequency nonequilibrium atmospheric plasma jets |
| 20-P08-04 | Atsushi Komuro (The University of Tokyo, Japan) | Effects of humidity on gas heating in atmospheric-pressure streamer discharge |

Tuesday, May 20
[ 2:00p.m. - 3:45p.m. ]

| Poster No. | Presenting Author (Affiliation, Country) | Title of Paper |
|---|---|---|
| 20-P08-05 | Jeong-beom Lee (Korea Advanced Institute of Science and Technology, Republic of Korea) | On the microwave plasma jet characteristics by impedance analysis |
| 20-P08-06 | Hidefumi Uchiyama (TATEYAMA MACHINE CO.,LTD, Japan) | Free Radical Generation by Cold Atmospheric Argon Plasma in Aqueous Solutions. An ESR Spin Trapping Study. |
| 20-P08-07 | Shinsuke Mori (Tokyo Institute of Technology, Japan) | Influence of Spore Deposition onto the Dielectric Surface on the Mode of Dielectric Barrier Discharge |
| 20-P08-08 | Kiyoyuki Yambe (Niigata University, Japan) | Relation between Plasma Plume Density and Helium Gas Flow in Atmospheric Pressure Plasma |
| **09. plasma-surface interactions relevant for medical/biological applications** | | |
| 20-P09-01 | Kensaku Goto (Osaka University, Japan) | Generation of reactive species in water exposed to low-temperature atmospheric-pressure plasma jets |
| 20-P09-02 | Hironobu Hojo (Osaka University, Japan) | Effect of Plasma Jet on Carbohydrate Derivatives |
| 20-P09-03 | Andrea Friedmann (Fraunhofer Institute for Mechanics of Materials IWM, Halle, Germany) | Evaluation of Cell Growth on Nanostructured and Functionalized Polystyrene |
| 20-P09-04 | Sou Takasawa (Shibaura Institute of Technology, Japan) | Local Injection using Reagent-laden Micro-bubbles |
| 20-P09-05 | Enbo Yang (Shizuoka University, Japan) | Plasma Surface Functionalization of Graphite-Encapsulated Gold Nanoparticles for Bio-medical Application |
| 20-P09-06 | Han Chou (Shizuoka University, Japan) | Surface Functionalization of Graphite-encapsulated Magnetic Nanoparticles with Amino Groups Using RF Excited Ar/$NH_3$ Plasma |
| **10. plasma sources for medical/biological applications** | | |
| 20-P10-01 | Simone Bianconi (Alma Mater Studiorum - University of Bologna, Italy) | Investigation of the effectiveness of a *Gatling machine gun* -like plasma source for biomedical and materials treatment applications |
| 20-P10-02 | Philippe Guillot (Toulouse University, CUJR JFC, France) | Experimental characterization of a coaxial microwave plasma source and efficiency on microbial surface decontamination |
| 20-P10-03 | Satoru Hida (Nagaoka University of Technology, Japan) | Sterilization of *Escherichia coli* by atmospheric pressure plasma irradiati on using superimposed waveform pulsed-power generator |
| 20-P10-04 | Sun Ja Kim (Dong-A University, Republic of Korea) | Generation of Multiple Plasma Plumes and Biomedical Applications in an Atmospheric Pressure Plasma Jet Array |

| Poster No. | Presenting Author (Affiliation, Country) | Title of Paper |
|---|---|---|
| 20-P10-05 | Han Seul Lee (Kwangwoon University, Republic of Korea) | Study on Low Temperature Plasma by using Pulse Mode SSPA |
| 20-P10-06 | Seung-Ju Lim (Kwangwoon university, Republic of Korea) | Characteristics of Nonthermal Plasma source in Various Liquids |
| 20-P10-07 | GeonBO Sim (Kwangwoon University, Republic of Korea) | Characteristics of Bovine Teeth Whitening in Accordance with Gas Environments of Atmospheric Pressure Nonthermal Plasma Jet |
| 20-P10-08 | Masaya Sugimoto (Akita Prefectural University, Japan) | Investigation of Sterilization Effect with Low Pressure RF Oxygen Plasma |
| 20-P10-09 | Charles C. Bailey (Drexel University, USA) | Development of a hand sanitizer employing non-thermal plasma activated mist |
| 20-P10-10 | Youngmin Kim (Hong Ik University, Republic of Korea) | Low voltage Plasma-on-a-Chip for Infection Treatment |
| 20-P10-11 | Jun-Seok Oh (Kochi University of Technology, Japan) | Bladder cancer cell lines cultured by plasma treated cell culture medium |
| 20-P10-12 | Paolo Sanibondi (Alma Mater Studiorum - University of Bologna, Italy) | Diagnostics of a low power inductively coupled plasma source for potential biomedical applications |
| 20-P10-13 | Dongping Liu (Dalian Nationalities University, China) | Atmospheric-pressure microplasmas for medical/biological applications |
| 20-P10-14 | Chang Min Lee (Ajou University, Republic of Korea) | An atmospheric-pressure microplasma jet device with Ni-Co alloy electrode and glass insulator |
| **11. plasma and/or liquid diagnostics and sensors** | | |
| 20-P11-02 | Ma. Camille C. Lacdan (University of the Philippines Diliman, Philippines) | Spectroscopic Investigation of Nitrogen Radical Transport in Atmospheric Jet Plasma |
| 20-P11-03 | Mikhail Vasiliev (Joint Institute for High Temperatures of the Russian Academy of Sciences (JIHT RAS), Russia) | Diagnostics of cold atmospheric pressure plasma generated by various plasma sources |
| 20-P11-04 | Keigo Takeda (Nagoya University, Japan) | Characteristics of AC excited Non-equilibrium Atmospheric Pressure Helium Plasma Jet for Medical Application |
| 20-P11-05 | Delphine Riès (CNRS, France) | OH LIF for *in situ* plasma jet diagnostics |
| 20-P11-06 | Mario Dünnbier (INP Greifswald e.V. plasmatis, Germany) | Ion measurements of a cold atmospheric pressure plasma jet: The influence of ambient air humidity |

Tuesday, May 20
[ 2:00p.m. - 3:45p.m. ]

| Poster No. | Presenting Author (Affiliation, Country) | Title of Paper |
|---|---|---|
| **12. modeling and numerical simulation** | | |
| 20-P12-01 | Wouter Van Gaens (University of Antwerp, Belgium) | Revealing the NO generation mechanism in a needle type plasma jet |
| 20-P12-02 | Satoshi Uchida (Tokyo Metropolitan University, Japan) | Reactive Molecular Dynamics between Oxygen Radial and Phosphatidylcholine by Plasma Irradiation |
| 20-P12-03 | Dingxin Liu (Xi'an Jiaotong University, China) | The penetration process of gaseous reactive species into aqueous solution: A modeling study |
| **13. others** | | |
| 20-P13-01 | Takanori Ito (Iwate University, Japan) | Preservation of Fresh Food Using AC Electric Field |
| 20-P13-02 | Hiromasa Tanaka (Nagoya University, Japan) | Signaling circuits that are affected by plasma-activated medium in brain tumor cells |

# Plenary Presentations

# Bridge the Gap between Plasma and Medical Sciences towards Future Medical Care

Masaru Hori

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Plasma medicine, a new region has made a sudden raise in plsma science and technology[1]. This emerging field of plasma medicine will establish a new interdiciplenary science and open newly enourmos industries. Such an epoch making area is considered to be realized by the great progress in the development of non-equilibrium atmospheric pressure plasam and in-liquid plasma. It is necessary for the establish of plasma medicine to diagnose plasma induced chemical, physical and biological reactiosn in interfaces among gas, liquid, solid and eventually control their reactions by the internal parameter in the equipment. In the case of semiconductor processing, plasma scientists have made great efforts to get the friend-ship with many different fields, such as the device, the materail, the chemistry and so on. As a result, the plasma science and technology has been developed with involving many sciences. There is, however, great problems standing on the way of research of the plasma medicine, that is, a big gap between plasma and medical sciences. In Japan, the national project on the plasma medicine has started in 2013 and now there have been fruitful results, especially by the expose of low temperature atmopheric pressure plasmas with a relative low density to the organism. Additionally, the ultrahigh density atmospheric pressure plasma [2] was exposed to the medium where cancer cells are installed, and the plsma activated medium killed cancers selectively to normal ones [3,4]. Recently, the effectiveness of the plasma activated medium to the selective killing of cancers was confirmed in vitro[5]. The project also includes the arrest of bleeding, the gene delivery and regeneration and so on. The variety of results obtained by their own methods according to their own objectives should be systematized. In this article, recently outstanding results are reviewed from view points of plasma diagnostics, plasma processing, plasma induced medical phenomena, medical care, safety and thus the great benefit from collabolations among differents scientists from the plasma, the medicine and the molecular biology is introduced.

**Acknowledgments**

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**References**

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# Plasma Physics and Chemistry for Biological Cell Interactions and Healing Diseases by Nonthermal Atmospheric Pressure Plasma

E. H. Choi[1], Y. H. Kim[1], Y. J. Hong[1], K. Y. Baik[1], G. Park[1], P. Attri[1], B. J. Park[1], G. J. Lee[1], N. K.Kaushik[1], B. Park[1], K. N. Kim[2], G. S. Cho[1], and H. S. Uhm[1]

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Current research activities of plasma bioscience and medicines in Plasma Bioscience Research Center (PBRC), Korea, will be introduced along with the plasma physics and chemistry for biological cell interactions and healing diseases. Basic diagnostics for electron temperature and ion density have been introduced and measured to be ~ 1 eV and ~ $1\times10^{13}$ $cm^{-3}$ , respectively, for the nonthermal atmospheric pressure dielectric barrier discharge (DBD) plasma [1] and soft plasma jet [2] by atmospheric collisional radiative model and wave-packet model[3].

Herein, we have also investigated the basic generation mechanism of reactive oxygen species (ROS), especially for hydroxyl radical OH and hydrogen peroxide $H_2O_2$ species by ultraviolet absorption spectroscopy [4], and their interactions with microbial [5] and mammalian cells resulting in apoptotic cell death in accordance with the absolute densities of OH and $H_2O_2$ radical species in biological solutions. Especially, differential selectivity of cell death for epithelial human lung cancer cell H460 has been observed to be higher than normal lung cell L132 in higher density of radical species, which is caused by mitochondrial membrane potential decrease and enzymatic dysfunction [1]. Similar effects of selective apoptosis were also observed with oral squamous cell carcinoma, which the mechanism is suspected to be linked with epidermal growth factor receptor (EGFR). Additionally, applications of non-thermal plasma treatment on hard tissue regeneration have been considered with regard to osteoblasts and mesenchymal stem cells. Recently, we also apply DBD plasma and nitric oxide (NO) producing microwave plasma to enhance differentiation and activation of microbial and mammalian cells. Furthermore, we provide the possibility that nonthermal plasma jet could be developed as a potential anti-diabetic therapy via ROS and NO dependent signaling pathway for glucose uptake and insulin secretion [6].

## References

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# Towards Understanding Mechanism of Cold Atmospheric Plasma in Cancer Treatment

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The efficacy of cold plasma in a pre-clinical model of various cancer types (lung, bladder, breast, head, neck, brain and skin) was demonstrated. Both *in-vitro* and *in-vivo* studies revealed that cold plasmas selectively kill cancer cells. It was shown that: (a) cold plasma application selectively eradicates cancer cells in vitro without damaging normal cells. (b) Significantly reduced tumor size *in vivo*. Cold plasma treatment led to tumor ablation with neighbouring tumors unaffected. These experiments were performed on more than 10 mice with the same outcome. It was found that tumors of about 5mm in diameter were ablated after 2 min of single time plasma treatment. The two best known cold plasma effects, plasma-induced apoptosis and the decrease of cell migration velocity can have important implications in cancer treatment by localizing the affected area of the tissue and by decreasing metastasic development. In addition, cold plasma treatment has affected the cell cycle of cancer cells. In particular, cold plasma induces a 2-fold increase in cells at the G2/M-checkpoint in both papilloma and carcinoma cells at ~24 hours after treatment, while normal epithelial cells (WTK) did not show significant differences. It was shown that reactive oxygen species metabolism and oxidative stress responsive genes are deregulated. We investigated the production of reactive oxygen species (ROS) with cold plasma treatment as a potential mechanism for the tumor ablation observed. Simulations of the cold plasma interaction with tumor performed showed reasonable agreement with experimental evidence.

# Application of tissue-tolerable plasma in dermatology: Risk assessment and prospects

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The application of tissue-tolerable plasma (TTP) is a promising method for wound healing. It has antibacterial and antimicrobial effects and can stimulate fibroblast cells towards faster attachment and proliferation. In the present paper a risk assessment of TTP and of in vivo and in vitro results of TTP application in wound healing are given.
Three properties of the TTP were evaluated for safety aspects. The investigations were carried out using an Argon plasma-based plasma jet kINPen med® that was developed by the Leibniz Institute for Plasma Science and Technology in cooperation with Neoplas tools GmbH, both of Greifswald, Germany.
1. As the result of a gaseous discharge, radicals are produced on the skin surface. The human skin is continuously exposed to free radicals that are produced by environmental factors like solar UV radiation. The human skin has developed a protection system against the destructive action of these highly reactive molecules in form of the antioxidative potential. The effects of the plasma on the antioxidative potential of the skin were investigated. It was found that during the plasma treatment of tissue, the antioxidative potential is reduced only in the upper part of the stratum corneum but not in deeper cell layers.
2. During the plasma formation, also UV radiation is produced. The spectrum of the plasma on the skin surface and at different depths of porcine ear model skin was investigated depending on different physical parameters influencing the formation of the plasma. If the optimum parameters are selected for plasma formation, the skin is exposed to less UV radiation than if it were exposed to 10 minutes of solar UV radiation on a summer day at noon.
3. In the third series of experiments, the influence of the duration of the plasma treatment on the temperature of the skin surface was analyzed depending on different physical properties influencing the plasma parameters. If the duration of the plasma treatment of the skin was in the optimal range for wound healing, no thermal damage had been observed.
Recently, TTP had been reported to be highly efficient in reducing the bacterial load of the skin surface. However, these studies were mostly performed using either cell culture assays or animal skin in vitro. We aimed to compare the antiseptic efficacy of TTP and an octenidine dihydrochloride (ODC)-based wound antiseptic on chronic wounds. Sixteen patients suffering from chronic ulcers were treated with either TTP or ODC 3 times a week for 2 weeks. The wound healing rate during the study period was monitored. Also, the bacterial colonization of the wound surface was investigated by determining the density of colony forming units in the bacterial culture. TTP showed a wound healing rate superior to that of ODC although the tip of the plasma beam was only 2 mm in diameter.
TTP has been shown to be a novel antiseptic approach for the treatment of chronic ulcers with an antiseptic efficiency comparable to one of the most efficient and biocompatible liquid antiseptics.

# Invited Presentations

# Plasma-Jet supported surgery of advanced head and neck cancer – Requirements and first steps for proof of concept

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Patients suffering from advanced head and neck cancer have to face a poor prognosis, whenever cancer cells are reaching and infiltrating anatomical structures like main arteries, that cannot be resected. Plasma medicine in the clinical setting of head and neck surgery introduces the potential for a less invasive removal of these tumor reminents via apoptosis. The concept is to reduce tumor bulk conventionally by surgery as far as possible and then treat any remaining small layers of cancer cells by plasma-jet cleaning. There are four requirements [1] for proof of concept: (A) head and neck cancer cells are selectively sensitive to a plasma-jet, (B) a plasma-jet is practical in a clinical setting, (C) a plasma-jet as an adjunct to surgery is crucial for total tumor resection, (D) long term follow up reveals improvement of healing rate by plasma-jet supported surgery. Cell culture based studies have indicated that plasma may be a useful tool to fight cancer cells [2]. In this study we applied cold atmospheric pressure plasma from the argon plasma jet kinpenMed® (neoplas tools, Greifswald/Germany) to treat and compare solid tumor tissue of oral cavity carcinoma patients with surrounding healthy tissue. Skin, mucosa and cancer samples were analyzed for markers of cell death and DNA-damage (γH2A.X, TUNEL, cytochrome C), proliferation (Ki76) and differentiation (keratin 1 and 14) by employing immune-fluorescence staining. Also the secretion of different cytokines or growth factors was analyzed 24 hours after the plasma treatment. Especially the comparison of healthy skin samples and their respective cancerous counterparts will help to understand how a plasma treatment could be adapted in order to selectively kill tumor cells without harming the healthy surrounding tissue. The aim of this study is to start proof of concept for plasma-jet supported surgery of advanced head and neck cancer, i.e. (A) to find a treatment procedure which selectively reduces cancer patient's cells while healthy cells still remain unaffected and (B) to make use of plasma-jet practical in a clinical setting.

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# Possible dental applications of plasma-based sterilization using the reduced pH method : treatment of dental caries and root canal infection

Tomoko Ohshima[1,3], Hiromitsu Yamazaki[1], Satoshi Ikawa[2], Emi Usui[1], Yasuko Momoi[1], Kaname Yamamoto[1], Noriyasu Hosoya[1], Nobuko Maeda[1]& Katsuhisa Kitano[1,3]

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**Introduction:** In dental treatment, the control of infectious microorganisms is very important. However, effective sterilization methods for infected dentine have yet to be established. We have been interested in the bactericidal effect of low frequency atmospheric pressure plasma jets (LF jet), which is expected to have excellent sterilizing properties and osmotic strength without residual toxicity in comparison with conventional disinfectants. We have noted that sterilization of liquids with the plasma jet is difficult, but with concurrent pH reduction (the reduced pH method) [1] increased effectiveness against oral pathogens is expected.

**The sterilization test against oral pathogens using the *in vitro* infection models:** In order to evaluate the sterilizing properties of LF jet against oral pathogens, particularly *Streptococcus mutans*, *Enterococcus faecalis* and *Candida albicans*, all which contribute to dental caries and resistant root-canal infections, sterilization experiments accompanied by CFU assays were carried out. The results showed that the LF jet irradiation had drastic bactericidal effects in liquid suspension when the pH was less than 4.5 (Fig.1)[2]. Significant bactericidal effects were shown within three minutes of irradiation in the caries models. However, the effect of the LF jet in infected root-canals which were modeled using extracted teeth, was not successful. Then we prepared plasma-treated water and applied it to the infected root models and the result showed a sterilization rate of 97.4% (n=39).

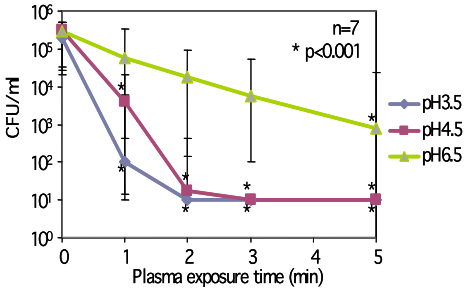

Fig. 1 CFU assay of *E. faecalis* in various pH solutions.

**Conclusion:** As relatively high levels of sterilization were obtained *in vitro* using infected tooth models, it indicates the possibility of applying plasma-based sterilization techniques in clinical dentistry. For verification of the effectiveness *in vivo*, the further trials using animal models should be conducted.

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# Modulation of protein activity by atmospheric pressure plasmas

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While composed of only 20 different building blocks (the proteinogenic amino acids), proteins fulfill cellular functions as diverse as performing enzymatic reactions, acting as sensors or regulators, and determining structure and motility. The diversity in protein structure and function is sheer endless and while some proteins are essential for life, others can be a nuisance in biotechnological research or even harmful to humans.

We investigate the impact of cold atmospheric pressure plasmas on the structure and activity of different proteins. Using protein and peptide mass spectrometry, we link changes in structure and activity to chemical modifications [1]. We could show that protein inactivation mechanisms depend on the plasma employed, the protein under investigation, and the treatment conditions. We provide insights on how plasmas can inactivate even the most resilient proteins. Not all proteins, however, are inactivated by plasma. We identified a stress response protein that is activated by plasma treatment and whose function it is to protect other proteins from aggregation.

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# Enhancement of food energy efficiency using plasmas

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The demand for food will continue to increase towards 2050 as a result of population growth by an additional 2.7 billion people, increased incomes and growing consumption of meat [1]. The combined effects of climate change, land degradation, cropland losses, water scarcity and species infestations may cause projected yields to be 5–25% short of demand by 2050. This would require new ways to increase food supply. However, rather than focussing solely on increasing production, food security can be increased by enhancing supply through optimizing food energy efficiency. Increasing food energy efficiency provides a critical path for significant growth in food supply without compromising environmental sustainability. One way to enhance agricultural production with low additional energy consumption is plasma induced growth enhancement of plants. We have studied effects of plasma irradiation to seeds on plant growth using a combinatorial plasma irradiation method with a scalable atmospheric air DBD device [2-6]. The main results are summarized as follows.

1) 3 min. plasma irradiation to seeds enhances plant growth in a long term more than 7 days.
2) The response of plants to the plasma irradiation becomes gradually weak with time, and the ratio of plant length with plasma irradiation to control decreases from 3.7 at the first day to 1.3 at 7 day.
3) Temperature rise during plasma irradiation has little effect on the growth enhancement, because the maximum ratio of plant length with heat shock to control is 1.4 whereas that the maximum ratio with plasma irradiation to control is 3.7.
4) Radicals play a key role in the growth enhancement, whereas photons and ions do not.
5) Key radicals of the growth enhancement have rather short lifetime in water, because plasma irradiation to seeds in shallow water becomes more effective.
6) Plasma activated water as well as PH of water has little effects on the growth enhancement.
7) The response of plant growth to plasma irradiation depends on species of plants, while several plants show plasma irradiation induced growth enhancement.
8) Plasma etching of seed coat has little contribution to the growth enhancement, because nearly the same growth enhancement is obtained for seeds with and without seed coat.
9) Plasma irradiation to plants after germination shows adverse effects of their growth, probably due to $NO_x$ generated by plasma.
10) Plasma irradiation shows plasma growth enhancement in multi-generations.

**Acknowledgment**

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# Large scale low operating cost plasma sources for agricultural water treatment

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Low temperature atmospheric pressure plasmas have found use in a plurality of applications due to their efficient formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [1]. Two engineering barriers for many applications of atmospheric plasma discharges are scalability and cost of operation. Most systems are smaller in scale; many also have relatively low charged particle densities from which these reactive species are derived. Additionally the cost of operation, particularly for systems that require noble gas dilution for stable operation, can be very high.

A novel plasma source has been developed that utilizes ambient air as the feed gas. [2] VHF ballasting is utilized to provide negative feedback to ion overheating instability and arc formation [3]. The result is a volume warm glow at atmosphere with an approximate power density of 5-10 W/cm$^3$ with no streamer or arc formation. Significant ROS and RNS formation has been observed via optical emission spectroscopy and interaction with a plurality of materials, particularly water.

This source is scalable. The unique electrical characteristics of the VHF tuning circuit, coupled with the VHF ballasting influence on impedance with respect to electron density provides passive load balancing for parallel source operation. Similar methods have previously been utilized in high frequency driven microdischarges [4]. This enables source scale up with reduced capital cost.

Demonstrations of water modification for a variety of applications have been made. Fixation of RNS in water with air species dominant plasma configurations yield nitrate concentrations comparable to commercial fertilizer; seedling irrigation with treated water has increased plant dry weight 80% over the control. Generation of ROS in spiked water samples have demonstrated efficient removal of common contaminants including 1,4-Dioxane and PFC's. A review of these experiments with emphasis on scalability, cost, and energy intensity will be presented.

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http://research.ncsu.edu/ott/for-inventors/chancellors-innovation-fund/

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# Biological Effect of Plasma Processing on Ceramics Artificial Bone

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In orthopedic surgeries, autologous bone grafting has been the golden standard for the treatment of bone defects. However, recent advances in synthetic calcium phosphate ceramics have altered the picture and ceramic bone substitutes have become as a standard along with autografts. Interconnected porous ceramics is also useful as a scaffold for bone tissue engineering. However, surface property of hydroxyapatite (HA) ceramics is hydrophilic. Recently, we found that He-based dielectric barrier discharge plasma treatment was able to improve the hydrophilicity. Our data suggest that plasma treatment may facilitate bone regeneration in the pores and improve the integration of ceramics to host bone.

The material used in this study is interconnected porous calcium hydroxyapatite (IP-CHA) with a porosity of 75% in volume, an average pore diameter of 150 μm and an average interpore connection window diameter of 40 μm. IP-CHAs have been approved for clinical use by PMDA as a bone substitute and are also useful as a scaffold material for bone tissue engineering [1] [2]. IP-CHA discs (φ5mm x h2mm) were placed in the discharge chamber and plasma treatment at a sub-atmospheric pressure was applied. After plasma treatment, water infiltrated easily into the pores, indicating plasma treatment improved the hydrophilicity of inner pore surface [3]. We have also examined the effect of plasma surface treatment on the biocompatibility of IP-CHA in two different *in vivo* bone defect models, rabbit femoral condyle intramedullary bone defect model and rat calvaria full thickness bone defect model. In both *in vivo* models, we found a tendency of better biocompatibility in plasma-treated group. Surface property alteration may also affect the performance of HA ceramics as a scaffold for bone tissue engineering. Mesenchymal stem cells obtained from rat femur were introduced into pores of IP-CHA discs and were cultured in osteogenic medium for 2 weeks. Alkaline phosphatase activity, a marker of osteoblastic differentiation, was significantly enhanced in plasma-treated IP-CHA compared with non-treated one.

In conclusion, plasma treatment of porous HA ceramics could enhance osteoconductivity and bone regeneration in the pores via increased hydrophilicity and potential favorable changes in the surface for osteogenic differentiation.

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# Cold atmospheric plasmas for dermatologic and oncologic purposes

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Cold atmospheric plasmas (CAPs) have gained a lot of attention in the scientific community. Initially, CAPs demonstrated very broad antimicrobial properties in several *in vitro* studies and showed that they can safely be applied in humans. It is not surprising that subsequent clinical trials and case reports confirmed these features in patients suffering from chronic infected wounds and other secondary infected skin diseases [1-3]. Independently to these promising antimicrobial attributes, CAPs revealed that they could directly contribute to healing processes and enhance wound healing, as well [4-5].

Recently, CAPs showed that another target might be the pain reduction. A case report with a patient suffering from Hailey-Hailey disease profited by CAP application and subsequent amelioration of pain [3]. Another report showed in a patient following surgery of cholesteatoma and subsequent chronic infections of the external auditory tract, which resulted in severe pain, that a plasma treatment could be applied [6]. The CAP exposure lead to a significant reduction in pain and clearance of bacterial carriage, allowing antibiotics and analgesics to be ceased. Ongoing studies are investigating the effect of CAPs in patients suffering from shingles/zoster, a *Varicella zoster virus* (VZV) related disease. First results are promising concerning pain reduction in plasma treated patients.

Oncology is another possible medical field of indication for CAPs. Several studies demonstrated in *in vitro* and in animal trials *in vivo* that various cancer cell lines and cancers respond positively to a plasma application [7].

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# HEALING OF WOUNDS BY ATMOSPHERIC PRESSURE PLASMA

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The therapeutic use of atmospheric pressure plasma (APP) has been proposed in dermatological wound healing, but the fundamental mechanism underlying the effects of APP on the physiology of the involved cells remains unclear. In this study, we report novel findings regarding the induction of dramatic changes in the cellular phenotypes of human dermal fibroblasts (HDFs) caused by non-thermal helium jet plasma. Interestingly, APP induces a MET (mesenchymal to epithelial transition)-like response in HDFs where mesenchymal fibroblasts transform into cells with epithelial characteristics in terms of both morphology and gene/protein expression. Moreover, the plasma treated cells feature down-regulation of fibrosis related genes, which supports the potential use of APP to treat the wounds to promote healing and suppress fibrosis. These observed phenomena were found to be mediated by intracellular reactive oxygen species and cytoskeleton dependent signaling pathways.

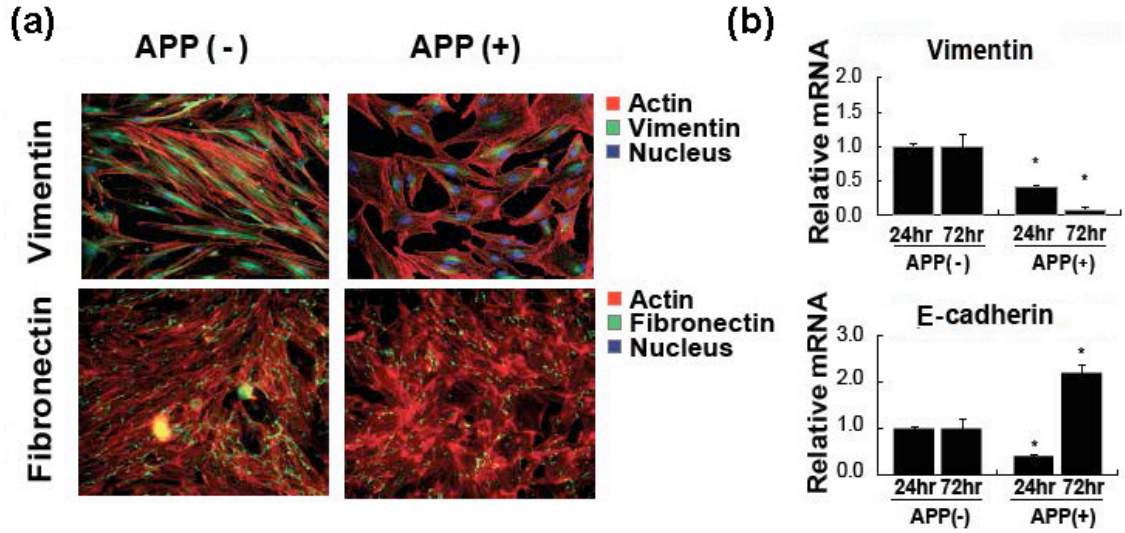


**Figure 1:** *Plasma treated cells exhibit MET-like traits: (a) Immunofluorescence images of vimentin, fibronectin, and actin on dermal fibroblasts with or without plasma treatment show down-regulation of mesenchymal proteins along with pronounced morphological changes of cells from a spindle-like shape to a rounded shape by APP treatment. (b) APP treatment induces down- and up-regulation of mesenchymal (vimentin) and epithelial (E-cadherin) genes, respectively.*

**Acknowledgments**
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# Researches on Applying Atmospheric-Pressure Non-thermal Plasmas to Dental Medicine

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This report presents recent progresses of our group on research of applying low-temperature atmospheric-pressure plasmas to various dental medicine[1]. The fields include plasma-induced and plasma-assisted teeth bleaching; surface modification and functionalization of selected dental materials, dental bacteria and bacterial biofilm inactivation in root canals, and plasma activated water for dental applications.

In the context of plasma assisted teeth bleaching, we report an effective bleaching of extracted teeth with different concentrations of dental whitening gel (6%, 15%, 25%, and 35% H2O2), assisted by a PMJ source operated in compressed air as the working gas. To quantify the results, the images of the tooth specimens with pure whiting gel and plasma assisted were analyzed by a spectrophotometer with a standard color classification scheme used for evaluating tooth whitening in clinics. Statistical analysis showed a significant difference between the plasma treated groups and the control group. The average colorimetric values increase about 2.5–3.0 times for the plasma assistance group.

For the inactivation of bacteria and bacterial biofilms of the dental materials, we report here a series of experiments aimed at elucidating the effect of non-thermal plasma interacting with Ti and Y-TZP surfaces of the biomaterials. The results show the changes in the surface energy, the properties detected from optical interferometry and X-ray photoelectron spectroscopy, and the changes in the contact angle on the surfaces. Those indicate that the non-thermal plasma schemes could be an attractive alternative for increasing the surface energy and wettability of the two widely utilized biomaterials.

With the devices of Plasma Micro-Jet and Plasma Pipette sources, we performed the experiments on the in vitro treatment of root canals in extracted human teeth, which are inoculated with planktonic *E. faecalis* and *E. faecalis* bacterial biofilm. For the two halves of one root specimen, the untreated half of the sample indicated that the bacteria were alive by confocal scanning microscopic analysis. In another half part treated with plasma, on the other hand, the bacteria were all dead, especially in dentinal tubules with smaller sizes. Those results propose potential clinic routes to inactivate bacteria and bacterial biofilms in root canals.

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# Treatment options of atmospheric pressure plasma in GI-Cancer

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**Background:** The relative new field of plasma medicine and the use of cold atmospheric plasma in the treatment of tumour cells especial in the use in gastrointestinal cancer has attracted a great deal of attention. The rate of microscopic incomplete resections of gastrointestinal cancers including pancreatic cancer, gastric cancer and stomach cancer and liver metastasis has not changed considerably over the past years. This is due to the fact, that the surgical technique has improved in the past decades and more and more technical demanding resections are performed. Future intra-operative applications of tissue tolerable plasmas (TTP) could help to address this problem. The development of non-thermal atmospheric plasmas displaying spectra of temperature within or just above physiological ranges allows biological or medical applications of plasmas with great options for surgeons in the future.
**Methods:** We give an overview of the current literature on the treatment of GI-Cancer with TTP *in vitro* and *in vivo* and present the recent developments in this field of plasma research. In our lab we have investigated the effects of TTP on human and murine pancreatic cancer cell lines *in vitro* (Annexin-V-FITC/DAPI-Assay and propidium iodide DNA staining assay) as well as in the *in vivo* tumour chorio-allantoic membrane (TUM-CAM) assay. In our recent investigations we concentrated on the indirect effects of TTP *in vitro* and also in a murine model of peritoneal carcinosis.
**Results:** TTP of 20 seconds induced only a mild elevation of an experimental surface temperature of 23.7 degree Celsius up to 26.63+/−0.40 degree Celsius. *In vitro* TTP significantly ($p=0.0003$) decreased cell viability showing the strongest effects after 20s TTP in our experimental setting. Also, TTP effects increased over time levelling off after 72 hours (30.1+/−4.4% of dead cells (untreated control) versus 78.0+/−9.6% (20s TTP)). However, analyzing these cells for apoptosis 10s TTP revealed the largest proportion of apoptotic cells (34.8+/−7.2%, $p=0.0009$ versus 12.3+/−6.6%, 20s TTP) suggesting non-apoptotic cell death in the majority of cells after 20s TTP. Using solid pancreatic tumours in the TUM-CAM model TUNEL-staining showed TTP-induced apoptosis up to a depth of tissue penetration (DETiP) of 48.8 +/−12.3µm (20s TTP, $p<0.0001$). This was mirrored by a significant ($p<0.0001$) reduction of Ki-67+ proliferating cells (80.9+/−13.2% versus 37.7+/−14.6%, $p<0.0001$) in the top cell layers as well as typical changes on HE specimens. Even though the difference in reduction of cell viability between direct and indirect plasma treatment was significant ($p<0.01$), there was a significant effect of indirect plasma treatment on tumour cells *in vitro*.
**Conclusions:** The records in the literature and our own data suggest very promising possible future intra-operative applications of TTP to reduce microscopic residual disease in GI-cancer resections. Further promising applications include other malignancies (central liver/lung tumours) as well as synergistic effects combining TTP with chemotherapies. Yet, adaptations of plasma sources as well as of the composition of effective components of TTP are required to optimize their synergistic apoptotic actions. The treatment of patient with disseminated peritoneal disease with plasma treated liquids could offer further treatment options at least in palliative situations of advanced GI tumour disease. Further studies, especially *in vivo* tumour models are necessary to define optimal TTP-consistence and treatment plans.

# Nanoengineered plasma polymer films for antibacterial coatings

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Medical device associated infections are a serious problem for medical professionals, patients and healthcare systems worldwide. These infections are mainly caused by bacterial attachment to and colonization of the device surface. For this reason, it has been well accepted that preventing bacterial adhesion to the device surface through the application of an antibacterial coating can be a potential solution.[1]

In my talk, I will discuss the problem of medical device associated infections and strategies for development of antibacterial surfaces. I will also outline recent approaches developed in our group for generation of antibacterial coatings which can be placed on the surface of various medical devices. These approaches are based on plasma polymers and facilitated by nanoparticles or nanostructure.

Several of our approaches involve silver nanoparticles which are either in situ synthesized into amine reach plasma polymer films through first loading with silver ions and subsequent reduction[2] or electrostatically adsorbed using appropriate surface functionalities of both plasma polymer films and nanoparticles [3]. The uniqueness of the first strategy is that the release rate of silver ions from the coatings can be controlled in a manner to allow healthy growth of mammalian cells without any compromise on the antibacterial properties. In addition to examining for potential cytotoxicity, we also test potential inflammatorily complication that the coatings may have. We have also developed strategies for controlled release of “convention” antibiotics from surfaces. In one case the antibiotics are loaded into electrochemically etched nano-porous alumina utilizing the pores as reservoirs. A plasma polymer layer of controlled thickness is used to control the release rate of the loaded drug through regulating the pores opening at the surface.[4] A second, much more facile strategy involves antibiotic particles deposited on plasma polymer films. Control over the release rate of the antibiotic is achieved via a plasma polymer overlayer of predetermined thickness.[5] I will also present more recent strategies that have not be published yet. These include plasma polymers that intrinsically inhibit bacterial growth and surface based on immobilized antibacterial agents.

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# Plasma modification of biomaterials for hard and soft tissue repair: relevance for drug delivery

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A **biomaterial** can be defined as a substance that has been designed to direct, by controlling interactions with components of living systems, the development of any diagnostic or therapeutic method in human or veterinary medicine [1]. Low temperature plasma modification of different kinds of materials has been widely studied with views on modifying properties such as wettability, topography, etc. However, its potential influence on drug delivery has not received great attention yet.

In this presentation, as hard and soft tissue repair and regeneration is sought, two main kinds of biomaterials will be retained: a) calcium phosphate bioceramics, and b) textile-based polymers (fibers, yarns, meshes). In both kinds of applications, **achieving local drug delivery** for an adequate period of time raises great interest and would provide benefits to the patient, such as reducing the number of drug administrations, thus conferring high added value to the materials [2]. Both atmospheric and low pressure plasmas are of interest with views on different medical applications and in the design of advanced biomaterials with controlled drug release properties. Different strategies are considered with that aim, such as using either plasma functionalization alone or in combination with plasma polymerization, and their effects are discussed.

Some of the examples discussed include Hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) as the most clinically used **calcium phosphate** (CaP) ceramics for bone regeneration. Its surface modification with a He atmospheric pressure plasma jet led to stronger interactions of a particular antibiotic with the surface and allowed for slower release of the drug from the plasma treated ceramics. Other works evaluated plasma polymerization on the surface of both HA and β-TCP which was able to act as barrier layer and delay the diffusion of drugs with angiogenic activity.

When dealing with soft tissue therapies such as wound healing or hernia repair, **Medical Textiles** are of relevance. The effects of low pressure and atmospheric plasmas were evaluated on the incorporation and release of different drugs [3], and plasma polymerization was used on polypropylene implants to tune simultaneously drug incorporation and cell response.

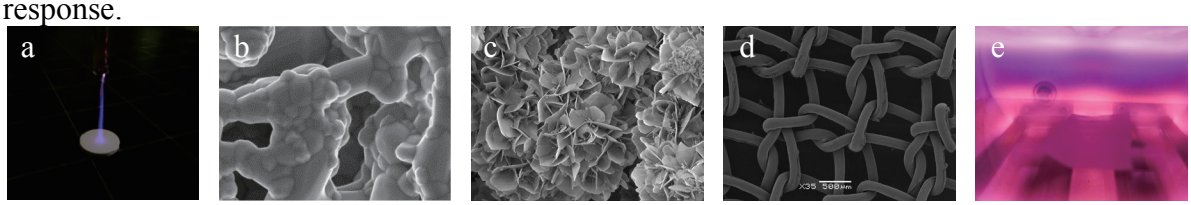


**Figure 1:** *(a) Atmospheric plasma jet. Different biomaterials: (b) sintered β-TCP ceramics, (c) hydroxyapatite cements, (d) polypropylene meshes and (e) low pressure plasma.*

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# Atmospheric plasma deposition of biocomposite coatings

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In the present work we report on a simple method for the deposition of biomolecule containing composite coatings by means of Atmospheric-Pressure Dielectric Barrier Discharge (AP-DBD). A biomolecule aqueous solution can be easily injected in a atmospheric pressure plasma reactor and a monomer such as ethylene added to form an organic matrix in which the macromolecule is dispersed. Biomolecules such as Lysozyme (Lyz), an antibacterial protein, and RGD peptide, a typical cell attachment enhancer, have been tested. The use of mild plasma condition allows to not affect the biomolecule structure hence the composite materials can have a correct bioactivity. A deposition rate as high as 60 nm/min can be achieved.
X-ray photoelectron spectroscopy and FT-Infrared analysis confirm the structure retention of the biomolecule, and scanning electron microscopy indicate a fairly morphological homogeneous coating.
This plasma-polymerization technique could be a powerful and versatile tool expecially in drug delivering systems.

**Acknowledgements**

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# Nanometric thick copolymers elaborated by low and atmospheric pressure non-equilibrium plasmas for biomedical applications

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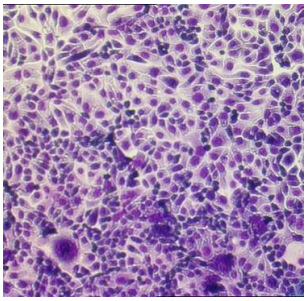
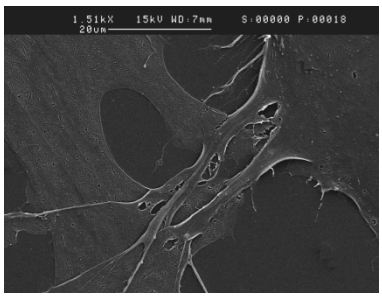
The **plasma copolymerization** of organic precursors for surface modification of a variety of substrates in order to tailor the physico-chemical properties of the substrates will be discussed for tunable biomolecule-surface interactions. The research approach includes: (a) the study of copolymerization of amphiphilic and antifouling coatings in low pressure systems, (b) the strategy for tuning the chemistry of cell adhesive layers for cell culturing and for non-fouling properties, (c) the realization of multiple copolymer layer devices for encapsulating anti-cancer drugs with a regulated release, (d) and the extension to atmospheric pressure deposition.

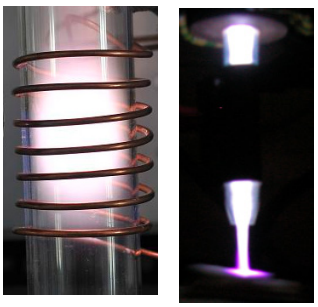
The examples to be addressed in this talk will start with deposition of surface tailored PFDA-PEG coatings to optimize the **protein repellence** of the surfaces. Then will discuss on the development of catalyst and solvent free chemical synthesis for the preparation of nano thick, **biodegradable and biocompatible** PCL-PEG copolymer coatings [2]. The copolymers deposited by low pressure rf plasma were characterized by a good retention of monomers functionalities and the biological response to the plasma deposited PCL-PEG copolymer surfaces was investigated *in vitro* using Human ovarian carcinoma cells (NIH:OVCAR-3), Human bone marrow endothelial cells (HBMEC) and Embryologic Fibroblast. The results show that by gradually varying the ε-CL/DEGME partial pressure ratio of the monomers, the **cell adhesion** on the PCL-PEG surface can be tailored [3]. In order to understand the release behavior of plasma copolymers for **anti-cancer applications**, Cisplatin loaded PCL-PEG multilayers were deposited in a RF low pressure plasma reactor. The cell death was quantified by performing WST-1 cell proliferation assay. Investigations of the role of each layer in the multilayer PCL-PEG coatings for the **drug release** were demonstrated *in vitro* [4]. Preliminary *In vivo* study of anticancer drug loaded cellophane and other biocompatible substrates were performed for hepatic and interparitonial implantation for different duration. Finally, PEG coatings were deposited by an home-made open air **atmospheric pressure plasma jet** system and the results obtained in terms of cell repellence were compared with the low pressure ones.



*NIH:OVCAR-3 on PCL-PEG*




*Fibroblast on PCL-PEG*



*LP ICP and APPJ reactors*

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# Plasma Cancer Therapy - state of the art and path forward

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The use of cold atmospheric plasma (CAP) for the treatment of cancer is a rapidly developing field in plasma medicine. It has been shown that CAP is effective against tumour cells both *in vitro* and *in vivo*. In low concentration plasma is able to stop tumour cell growth, and to induce cell death in higher concentrations. Moreover, plasma appears to be more effective than standard therapies including radiotherapy and chemotherapy. First results also indicate that CAP seems to be selective for cancer cells since treatment is more successful in tumour cells than in normal non-neoplastic cells. The current developments in this field show the prospects of this new approach.

Several open questions exist, and their answers will be the prerequisite for the further development of plasma treatment as a genuine medical method in tumour therapy. A standardized measurement of the capacities of the different plasma devices and the resulting plasma chemistry would be the basis for a better comparability of the different approaches. It also will be necessary to develop orthotopic experimental protocols since *in vitro* models are highly artificial, including the unresolved question of the importance of the liquid phase for the plasma effects. Surface tumours including skin cancer or colon tumours seem to be good candidates for medical CAP application in oncology. However, other tumour types should not be excluded from further experiments because the scientific field is in an early phase and further developments in the field might open new approaches to the treatment of so far "untypical" tumours. A very important issue is the development of appropriate devices for medical use including endoscopic devices.

To date, it is most likely that plasma could enter a place in a combination therapy with established tumour treatment schedules. Plasma could mediate drug delivery and drug uptake by tumour cells, and thereby enhance the effects of standard chemotherapy regimens and may restore chemosensitivity in so far therapy resistant tumour types.

CAP is an interesting new therapeutic approach in the treatment of cancer. Novel applications are imaginable highlighting the importance of a close interdisciplinary cooperation between oncologists, biologists, physicists, chemists and engineers.

# New strategic plasma therapy for advanced and/or refractory epithelial ovarian cancer

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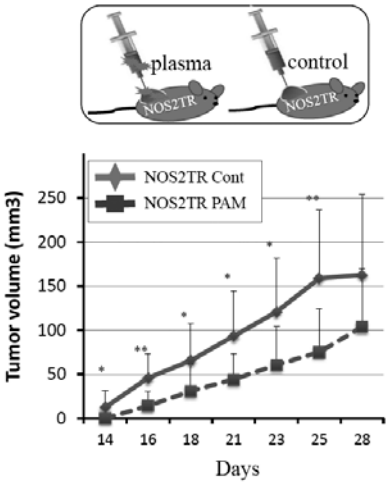
It is well-known that epithelial ovarian cancer (EOC) is one of the main life-threatening female genital tract malignancies throughout the world. Despite the fact that complete clinical remission can be achieved in approximately 80% of these patients owing to cytoreductive surgery, followed by systematic front-line chemotherapy, the majority of those clinical complete responders develop recurrent disease. Once patients experience recurrence, complete cure is almost impossible.

Nonequilibrium atmospheric pressure plasma therapy has recently been focused on as a novel medical practice. We previously demonstrated the direct growth-inhibitory effect of plasma in EOC cells, compared to human fibroblast [1]. Furthermore, we have elucidated the anti-tumor potential of indirect plasma, particularly in plasma-activated medium (PAM). Simultaneously, we examined the role of reactive oxygen species (ROS) or their scavengers in chronic antineoplastic-resistant EOC cells. We demonstrated that incubation with PAM led to an anti-proliferative effect in parallel with intracellular ROS up-regulation on chemo-resistant cells as well as parental cells. In addition, we confirmed that most of its effects were attributable to ROS from plasma and PAM with NAC, which is widely used as an anti-oxidant, reversed the anti-tumor effects. The inhibition of glutathione synthesis by BSO enhanced the in vitro anti-tumor efficacy of PAM. Using murine s.c. xenograft model, we confirmed the effect of PAM against chemoresistant EOC cells *in vivo* [2].

Considering the intraperitoneal treatment for numerous micrometastatic disseminations of EOCs, direct plasma irradiation therapy cannot target all the numerous tumors throughout the peritoneal cavity. In this context, intraperitoneal (IP) treatment may be more practical and desirable from a clinical view. In the current presentation, we would like to provide the perspective of indirect plasma IP therapy as a promising treatment option for EOC.

Figure 1
The Effect of PAM on tumor growth *in vivo*

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# Non-thermal atmospheric pressure plasma preferentially induces apoptosis in p53-mutated cancer cells by activating ROS-responsive pathways

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Non-thermal atmospheric pressure plasma (NTAPP) is an ionized gas at room temperature and has potential as a new apoptosis-promoting cancer therapy that acts by generating reactive oxygen species (ROS) [1]. However, it is imperative to determine its selectivity and to standardize the composition of NTAPP. Here, we designed an NTAPP-generating apparatus combined with a He gas feeding system and demonstrated its high selectivity toward p53-mutated cancer cells. We first determined the proper conditions for NTAPP exposure to selectively induce apoptosis in cancer cells. The apoptotic effect of NTAPP was greater for p53-mutated cancer cells; artificial p53 expression in p53-negative HT29 cells decreased the pro-apoptotic effect of NTAPP. We also examined extra- and intracellular ROS levels in NTAPP-treated cells to deduce the mechanism of NTAPP action. While NTAPP-mediated increases in extracellular nitric oxide (NO) did not affect cell viability, intracellular ROS increased under NTAPP exposure and induced apoptotic cell death. This effect was dose-dependently reduced following treatment with ROS scavenger. NTAPP induced apoptosis even in doxorubicin-resistant cancer cell lines, demonstrating the feasibility of NTAPP as a potent cancer therapy. Collectively, these results strongly support the potential of NTAPP as a selective anticancer treatment, especially for p53-mutated cancer cells.

**Figure 1**

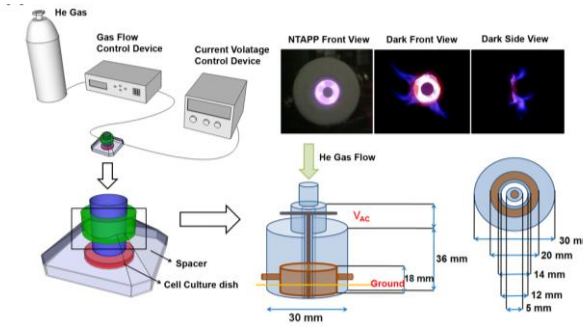


**Figure 2**

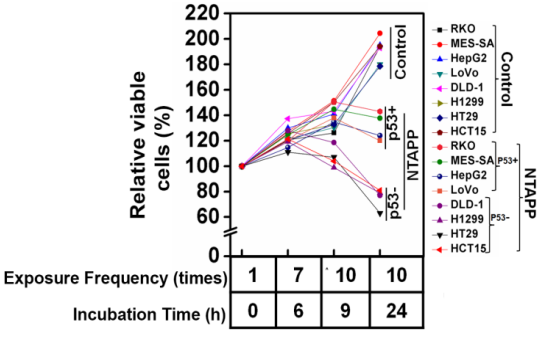


**Figure 1:** Schematic description of the NTAPP-generating device used to treat living cells.
**Figure 2:** Highly preferential anti-proliferative effect of NTAPP on cancer cells without functional p53

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# Investigation of the effectiveness of a low power inductively coupled plasma source for biomedical applications

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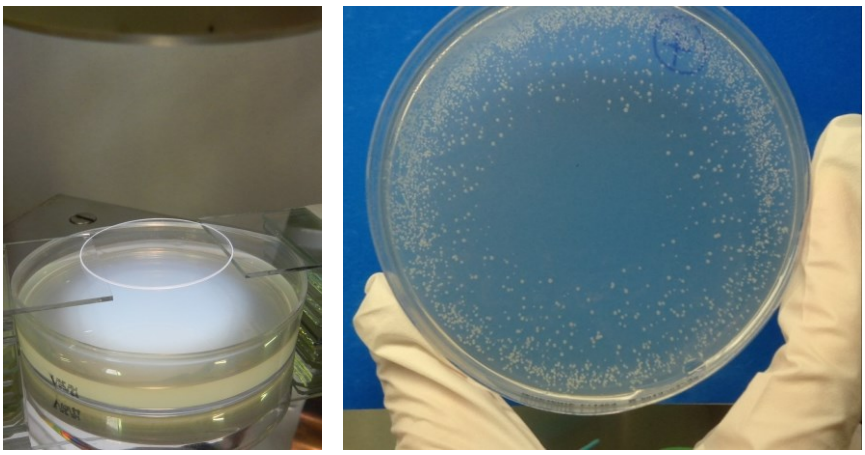
Low power inductively coupled plasma (ICP) sources integrated with a quenching device for the efficient production of reactive species at atmospheric pressure have been recently developed for potential biomedical applications [1-2]. In this work, an ICP torch supplied by a 1 kW-13.56 MHz power generator and operated with argon/air mixtures is presented; as a quenching device, a dielectric tube with suitably designed air injection ports and an exit orifice for the gaseous effluent is placed at the torch outlet.

A thorough characterization of the plasma source was performed to garner fundamental insights to be correlated with biological experiments; results will be presented regarding discharge behaviour, effluent temperature and fluid-dynamics, reactive species and UV radiation production, with and without quenching device. Attention will be dedicated also to the production of chemical species in treated liquid mediums. Moreover, in-vitro experiment to assess the decontamination potential of the ICP source were carried out on bacteria typically associated with chronic wounds and designed to represent a realistic wound environment; similar experiments were performed on fibroblasts and epithelial cells to evaluate the eventual cytotoxicity of the treatments.



LEFT: ICP torch with quenching device during operation for bacterial inactivation tests
RIGHT: Growth inhibition area of *B. atrophaeus* after plasma treatment

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# Room Temperature Plasma Jets and Active Species Diagnostics

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Due to several urgent applications, room temperature atmospheric pressure plasma jets (RT-APPJs) have attracted lots of interest worldwide in the past decade and many different types of RT-APPJs have been reported. One of the most interesting phenomena of RT-APPJs, i.e. “plasma bullet” behavior has been studied by many different groups. We know much more about the “plasma bullet” now than in the beginning. In this paper, an overview of the research on the “plasma bullet” behavior will be presented, which including the effect of the photoionization, seed electrons, Penning ionization, gas flow rate, polarity of the applied voltage, pulse rising time, pulse repetition frequency, and pulse width. In addition, some newly discovered “plasma bullet” phenomena, including the multiple bullets for a single voltage pulse, dragon shape propagation path, and virtual electrode propagation of the plasma plume are reported[1-5]. Finally, two of the most important reactive species, i.e. spatial and temporal resolved OH and O absolute concentrations are measured and the effects of working gas composition, electrical parameters on their absolute concentrations are presented.

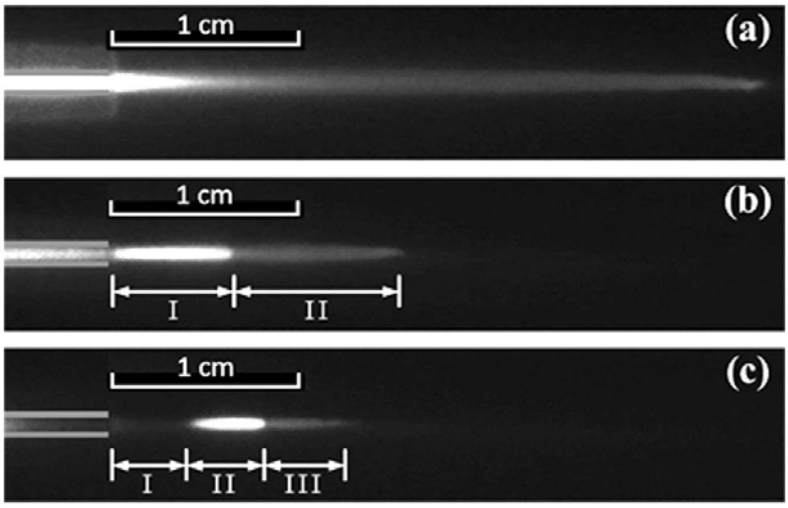


**Figure 1:** *Photographs of three distinctive plasma plume types generated using (a) 2 μs pulse; mostly a dim (typical to a plasma bullet) area is seen; (b) 998 μs pulse; bright (I) and dim (II) areas are seen; and (c) 999.1 μs pulse; dark (I), bright (II), and dim (III) areas are seen. It should be emphasize that they look like these by naked eyes.*

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# Understanding of gas flow, plasma and target interplay: a key prerequisite for the optimization of plasma jet treatments

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Plasma jets delivered at atmospheric pressure have been recently successfully used in an impressive number of experimental works including plasma diagnostics, biomedical treatments and material processing. The generation of plasma occurs in a simple discharge reactor and is then delivered either as a plasma effluent or propagates towards the target through fast ionization wave or streamers mechanisms. While the plasma source are very simple, it has been evidenced that many parameters may significantly influence the plasma characteristics offering at the same time a large versatility for plasma delivery but also requiring a special attention to match and characterize the plasma features for any specific application.

In this work, emphasis will be given on two critical topics necessarily involved in any plasma jet biomedical applications. The first consists in the major influence of the target over which plasma jet impingement occurs. It has been shown that depending on the conductivity of the target, secondary plasma generation occurs from the target, leading to a critical modification of the reactive species (RS) generation. Preliminary results obtained during *in situ* OH radical LIF diagnostics and ICCD plasma imaging experiments confirm the influence of the properties of different water targets. The second main issue concerns the strong interplay between the rare gas flow (in most case helium, argon or neon) and the plasma species generated during plasma jet ionization wave propagation. Drastic modification of the rare gas flow features have been recently evidenced and characterized through Schlieren visualization, pressure measurements and ICCD imaging [1].

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# An application of low temperature plasma to achieve minimal invasive surgery

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By recent progression in plasma technologies, we have come to be able to apply plasma generated in atmosphere on various situations and aspects in medical practices. One of low temperature glow-like plasma, we call “SAKAKITA plasma”, which has been developed by us to achieve blood coagulation without thermal damages [1, 2]. The equipment to produce SAKAKITA plasma has the fully coated three kinds of electrodes with dielectric, and employed a dielectric barrier discharge with a peak-to-peak voltage of ~8 kV and frequency of ~60 kHz with small displacement current. The generated plasma prompted blood coagulation keeping thermal elevation less than 40°C without arc-like plasma formation [1].

From the view point of minimally invasiveness, our plasma equipment to produce low temperature plasma is not only a new device for bleeding control, but also play a role to change a basic concept for invasiveness connected with surgical procedure. In oncology area, the word, invasiveness, is used to describe the ability of tumor cells to infiltrate or destroy surrounding tissue. Whereas in surgery, the word describes a property of surgical procedures carried out on patients. Indeed, minimal invasiveness means one of the ideal conditions that surgeons endeavor to achieve. So far as we know, because the thermal damages are inevitable by use of current methods for electrocoagulation such as high-frequency electrical coagulator, ultrasonic wave equipment, or laser, minimal invasive surgery has not been achieved. Thus, electrical coagulator without thermal damages to reduce scar tissue formation is needed.

Use of low temperature plasma-coagulation effectuated to reduce thermal injury, and that the subsequent inflammatory response was significantly less than use of high-frequency electro-coagulator. In this symposium, we will provide an overview of current attempts above and the possible underlined mechanism. Moreover, we would like to discuss the feasibility of using this technology to realize for minimal invasiveness surgery and overcome pancreatic cancer.

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# Collaborative studies of a helium-based kHz jet.

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Results of studies to explore the underlying physics and chemistry of a helium-based kHz plasma jets, the effect of the plasma effluent on biological material and the development of models and techniques relevant to medical applications in general will be presented. One aim is assess the potential of helium-based plasma jets in high value, specialist medical procedures. The work reported is part of a collaboration between physicists and pharmacists at Queen's University Belfast,

The source used in these studies is a kHz driven atmospheric helium plasma jet, based on earlier designs [1,2] and consisting of a 1mm thick quartz tube, with an inner diameter of 4 mm. Two copper electrodes (2 mm wide) encircle the tube, separated by 25 mm. The common operating condition is either helium or helium and 0.5% oxygen flowed through the tube at 2 slm, a 6 kV pulse applied at 20 kHz to the downstream electrode, which is 5 mm from the end of the plasma tube, and the upstream electrode grounded. These stated parameters are of course varied in studies of biological material to optimise jet and plume behaviour or plasma produced species.

Our space and time resolved emission images confirm that the present system is driven by a streamer-type discharge in the inter-electrode region producing an ionization front or "plasma bullet" which creates the emitting plume. Time and space resolved Thomson scattering measurements indicate electron densities and temperatures of ~$10^{14}$ $cm^{-3}$ and 0.25 eV in the outer regions of the plume ~$10^{13}$ $cm^{-3}$ and 0.17 eV in the centre when operating in helium. Emission spectra reveal the production of OH, O and H from the surrounding air while other reactive species such as ozone and singlet detection oxygen have been detected in absorption measurements [4].

Recent modifications of a global model for rf driven plasmas [3] indicate that a wide variety of atomic, molecular, ionic and neutral species are created in the plume.

The efficacy of the plasma system in the inactivation of both gram-positive and gram-negative bacterial biofilms has been explored determined [4,5,6]. Measurements suggest that membrane damage through lipid peroxidation and membrane perturbation is most likely the primary factor in the bacterial cell death but other cellular components including DNA and proteinaceous enzymes were also damaged or inactivated by plasma exposure. This confirms that this plasma mediates a non-selective, multiple-target mechanism of action, suggesting that the emergence of microbial resistance toward it may be unlikely.

The author acknowledges the major contributions to this work from Qias Algwari, Mahmoud Alkawareek, Ni'da Alshraiedeh, Wameedh Adress, Brendan Gilmore, Tomo Murakami and Deborah O'Connell.

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# Microsecond DBD Plasma for Differentiation, Development and Regeneration

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Differentiation of mesenchymal cells into chondrocytes or osteoblasts is of paramount importance in tissue engineering, fracture healing, bone fusion and regenerative therapies. We hypothesized that mesenchymal cell stimulation by non-thermal dielectric barrier discharge (NT) plasma, which produces and induces ROS, would both promote skeletal cell differentiation and limb autopod development. In our recent paper, we show NT-plasma treatment enhanced both osteo- and chondrogenesis (1). More recently, we have shown dramatically accelerated development of limb autopods in response to NT-plasma treatment. Noticeable changes included increased survival, enhanced development of digit length and definition of digit separation. This work, coupled with NT-plasma's ability to stimulate wound healing, led us to ask if NT-plasma treatment may also have the potential to enhance tissue regeneration in vivo.

Compared to lower species regenerative capacity in mammals is limited. However, recently certain strains of mice have shown the ability to regenerate the tissue lost due to complete removal of tissue using a 2mm ear punch. To investigate the regenerative potential of NT-plasma, in this model a 2mm punch was created in the ear of a nonregenerative mouse strain (C57BL6) and treated with low dose microsecond NT-plasma once a day for a 5-day period. Closure of the ear hole was measured and percent closure was calculated for area and diameter. Additionally, the ear punch site was analyzed for changes in blood flow and oxygen concentration using the Vevo Lazr photoacoustic imaging system with ultrasound and Doppler. Results from this study indicate that NT-plasma increases the regenerative ability of the mouse ear. Additionally, tissue oxygenation is significantly increased immediately after ear punch and up to Day 4, when compared to control. Further work in this area could extend this technology to clinical applications for enhanced trauma repair, fracture healing, plastic surgery and bone fusions.

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# Progress and Needs in Modeling of Plasma Interactions with Tissue: Wet, Dry, Direct and Indirect*

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The use of plasmas in treatment of tissue and biological surfaces has significantly progressed both in the development of plasma sources and in our understanding of the consequences of plasma exposure of living tissue. From a plasma perspective, plasma medicine shares many features of other materials processing applications, including the need to control the flux of ions and radicals to surfaces. Plasma medicine is both more constrained and more challenged in developing the plasma sources producing the desired reactivity. In other applications, pressure and temperature are nearly free parameters, whereas in plasma medicine, the vast majority of applications are constrained to 1 atm and ambient temperature. In other applications, the fluxes produced by the plasma source directly and unambiguously interact with the surface to be modified. In plasma medicine, the fluxes are either modified by, for example, liquid layers, or the catalyzing reaction for the biological outcome is the end result of a chain of reactions far removed from the plasma. Modeling and simulation has clearly benefited our understanding of plasma materials processing of inorganic materials, for example, semiconductor fabrication. This benefit has accrued from our ability to optimize plasma sources and predict resulting material properties. Similar benefit from modeling and simulation in plasma medicine is now beginning to be realized.

In this talk, a brief review and current status will be presented on modeling of plasma sources and plasma surface interactions in the context of plasma treatment of biological materials. Examples will be drawn from global, multidimensional and molecular dynamics models. The challenges and potential, and linkages to biochemistry, for plasma modeling to achieve a similar level of benefit as in other fields of materials processing will be discussed.

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# Simulation of atmospheric pressure helium discharges in capillary tubes and in plasma jets

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In recent years, the interest for atmospheric pressure plasma microjets has increased due to the possibility of propagating low temperature plasma discharges on several centimeters in open air. These discharges are currently studied for a large number of applications such as decontamination and biomedical applications.
It is now clear from experimental (see for example the recent review of [1]) and modeling studies ([2, 3], [4], [5] and [6]) that the plasma bullets are ionization waves, propagating in the easily ionized helium column. At this point, comparisons of results from modeling and experiments are mainly qualitative. Recently, many experimental efforts have been done to carry out detailed measurements in plasma jets as the radial profiles of the densities of excited He atoms at different axial positions from the tube exit in [7]. Then there is a need to go a step further and to carry out quantitative comparisons between modeling and experiments.
First, in this work, we propose to study the discharge ignition and dynamics inside the dielectric tubes for different electrode geometries and applied voltage pulses and to compare with optical measurements recorded in [8]. Second, we propose to study the influence of various concentrations of nitrogen admixtures in helium on the discharge dynamics in the tube and in the plasma jet. Results will be compared with experiments carried out in [7]. Finally, the dynamics of interaction of two discharge fronts with the same polarity and its comparison with experiments [9] will be presented.

**Acknowledgments**
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# Identification of RONS in water induced by air plasmas and their biomedical effects

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Recent advances of biomedical applications of non-thermal atmospheric pressure plasmas show that the biomedical effects are mostly due to reactive oxygen and nitrogen species (RONS) [1]. RONS are very important in biological systems, e.g. in antimicrobial strategies, immune response, or cell signaling pathways [1-3]. Identification of plasma-induced RONS in aqueous media and studying their effects on biomolecules and cell viability/functionality is one of the key directions in plasma medicine.

By controlling the dissipated power, air discharge plasmas can be operated to generate either primarily ozone ($O_3$) or nitrogen oxides ($NO_x$), among other species, such as $OH^\bullet$ radicals. $O_3$ transferred to the liquid, hydrogen peroxide ($H_2O_2$) formed dominantly from $OH^\bullet$ radicals, and nitrites ($NO_2^-$) and nitrates ($NO_3^-$) from $NO_x$ dissolving in water are relatively long-lived RONS in aqueous solutions. They are commonly measured by colorimetric methods using indigo dye for $O_3$, $TiOSO_4$ reagent for $H_2O_2$, and Griess reagent for $NO_2^-$. Low concentrations of $NO_3^-$/$NO_2^-$ can be precisely detected by ion chromatography [4], while high concentrations by direct UV-vis absorption in the liquid phase.

Unless the aqueous solution is buffered, air plasma treatment leads to its acidification. Under acidic conditions, depending on the actual pH, $NO_2^-$ disproportionate to $NO_3^-$, both $NO_2^-$ and $O_3$ react with $H_2O_2$, and dissolved $O_3$ naturally decays within minutes. Therefore, for the correct measurements of these RONS, the sampling time after plasma treatment is critical and immediate post-treatment stabilization is often needed to suppress their cross-interactions or decay.

Very short-lived RONS are also of great biomedical relevance. Peroxynitrites ($ONOO^-$/ONOOH) are formed mostly by the reaction of $H_2O_2$ and $NO_2^-$ under acidic conditions [4-5], however, they quickly dissociate to $NO^\bullet$+$O_2^{-\bullet}$ (or $NO_2^\bullet$+$OH^\bullet$) [6]. Their diagnostics by UV absorption or fluorescence spectroscopy is tricky due to their short life time, overlapping absorption with other RNS, and fluorescent probes cross-reactivity with other RONS [4-5].

$OH^\bullet$ radicals are considered the most active ROS but with extremely short life time (~ns). Electron paramagnetic resonance (EPR) using spin traps was shown as a perspective method for their diagnostics, although it is difficult to distinguish the EPR spectra of $OH^\bullet$ and $O_2^-$ [7]. Both $OH^\bullet$ and $O_2^-$ can be transferred from the gas to the liquid and formed by the aqueous reactions, such as ONOOH/$ONOO^-$ acidic dissociation, or Fenton reaction with $H_2O_2$.

Electrochemical probes are often employed in biochemistry, even on the microscale probing the RONS directly inside a cell [8]. Their potential use for plasma treated liquids is to be explored, accounting for the redox processes of solvated electrons and ions. Besides the specificity of diagnostic methods and aqueous cross-interactions of the measured RONS, mass transfer of the gas-phase RONS into the solution and the roles of specific RONS in the biomedical effects should be considered.

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# Primary-amine rich coatings to enhance the biocompatibility of cardiovascular implants

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Bioactive coatings are increasingly used to overcome the limitations of conventional biomaterials possessing good mechanical properties but insufficient biocompatibility. This is particularly true for poly(ethylene terephthalate) (PET, Dacron®) or expanded poly(tetrafluoroethylene) (ePTFE) used in vascular implants since the inertness of these materials prevents the stimulation of proper cell mechanisms implicated in vascular repair.
Our group used several different strategies using primary-amine rich plasma polymerized coatings to enhance biocompatibility around vascular implants, either directly or indirectly. Primary-amine rich coatings (called L-PPE:N) were prepared using a low-pressure radiofrequency (r.f.) glow discharge reactor and a mixture of anhydrous ammonia ($NH_3$) and ethylene ($C_2H_4$), as already reported and characterized extensively [1,2]. LPPEN was shown to strongly increase the adhesion of various cell types, including vascular smooth muscle cells, endothelial cells and mesenchymal stem cells, which are involved in tissue healing around vascular implants [3]. This non-specific effect is believed to be due to the positive charges created by the high density of primary amine groups when immersed in aqueous media (including bio-fluids) at physiological pH values. These enhance protein adsorption, as measured by quartz crystal microbalance with dissipation monitoring (QCM-D), and attract cells and platelets, leading to a cell-adhesive but also thrombogenic surface. LPPEN coatings can also be used to reach more specific cell-surface interactions, through the covalent immobilization of biomolecules on primary amine groups. We thus report interesting results of a bioactive coating specifically designed to enhance cell growth and resistance to apoptosis, using chondroitine sulfate as a sublayer on which growth factors are immobilized.

In both cases (specific/non-specific coatings), plasma polymers can be used as a versatile technique to create bioactive coatings on any kind of implants and 3D structure. Thus, L-PPE:N was deposited on flat polymeric films, but also on 3D electrospun nanofiber scaffolds designed for the replacement of blood vessels, in order to enhance the adhesion and retention of endothelial cells. *In vitro* cell-culture and perfusion tests showed a significant increase of cell growth, focal adhesion and retention under flow-induced shear stress. Moreover, cells were also shown to be limited to the top surface, thus enabling them to form a complete and stable monolayer, as required to prevent blood clot formation and thrombosis.

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# Medical applications of plasma technology: welcome to the future

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Recent advances in Physics have lead to the development of clinical applications of non thermal atmospheric pressure plasmas (NTP).

Although biological mechanisms between gaseous active species / electric field plasma-generated, and human tissues have yet to be deciphered, NTP are suggested to interact with basic cell features like cell proliferation, differentiation and apoptosis. Thus, plasma medicine fields now include cancer treatment [2], wound healing [3], tissue regeneration [4], antisepsis, sterilization, coagulation,…

This oral communication points out the potential application of NTP use through virtual clinical case of an oral lesion that could be more efficiently cured by plasma technology than current therapies.

Based on the medical literature (US National Library of Medicine National Institutes of Health), we will present a review of recent findings and advanced in plasma medicine science. Then, we will try to demonstrate that plasma technology would take crucial part in future alternative therapies.

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# Gas phase diagnostics of plasma jets and their induced liquid phase chemistry in the context of interactions with prokaryotic and eukaryotic cells

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Cold atmospheric pressure plasma jets (APPJs) have been studied insensitively in the context of biomedical applications. Nonetheless both plasma properties and reactive species densities and fluxes are not accurately known to date. In addition the presence of a liquid phase complicates the study of plasma-bio interaction significantly.

In this contribution, we will give an overview of the plasma chemistry [1,2] and electron kinetics [3,4] occurring in an radio frequency driven and pulsed APPJ. The capabilities and limitations of several diagnostics used to obtain these properties will be highlighted. In addition, the transfer from the plasma chemistry in the gas phase to the liquid phase and its relation with inactivation studies of prokaryotic and eukaryotic cells will be discussed [5,6].

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# Properties and Some Possible Applications of Gas Discharges Contacting with Liquids

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Atmospheric pressure gas discharges excited between metal and liquid electrolyte electrodes or in a liquid volume are the sources of non-equilibrium plasma for a lot of promising applications in chemistry, environmental protection, material treatment and medicine. At relatively low discharge current atmospheric pressure dc discharge with water (or electrolyte solution) cathode has a typical glow discharge structure and properties similar to those of a dc discharge with metal electrodes. At the same time, there are at least three important specific features of the discharges with liquid electrodes: (1) high voltage drop near a liquid cathode owing to low efficiency of electron emission from liquids, (2) non-equilibrium transfer of solvent and dissolved substances from a liquid cathode to gas phase caused by ion sputtering and influencing plasma properties, (3) formation of active species not only in a plasma volume but in a thin liquid layer.

Ion sputtering of liquid influences strongly plasma composition and properties: electric field strength and current density in positive column, electron energy distribution function, populations of vibrational and electronic excited states of plasma components, discharge emission spectra. Sputtering process can be characterized by a transfer coefficient – number of particles being sputtered from the liquid per one bombarding ion. According to experimental data and simulation by classical molecular dynamics method, values of transfer coefficients are about 50–500 particles/ion for water and $10^{-3}$ – 1 particles/ion for dissolved substances and depend on liquid composition and ion energy (cathode voltage drop). According to simulation results the ions of dissolved salts are sputtered both as ion pairs in the water clusters and as solvated cations and anions. The ratio between the various sputtered species depends on the ions energy and flux density. Calculations show the small concentration of alkaline metal atoms in plasma can change a balance of charge particles drastically. The channels of transfer processes influence on plasma properties and processes of active species formation will be discussed.

Now, growing interest is seen to various types of discharges with liquids as tools for water disinfection and destruction of pollutants, surface modification of polymers, biomedical applications and nanofabrication. Plasma activation combining with selectivity of reactions in aqueous medium leads to more selective treatment of natural and synthetic polymer materials without surface etching and formation of low molecular weight oxidized products. In this case material under treatment is immersed into a liquid being treated by plasma. Reactions of solvated macromolecules with primary active species and with products of their interactions with solution components results in a surface modification. Examples of plasma-solution treatment of polymers will be given in presentation. In particular, it has been shown the possibilities of polymer surface oxidation followed by immobilization of bioactive substances, catalysts, metal nano- and microparticles, initiation of polymerization processes in aqueous solutions of some organic monomers.

# Effects of the non-thermal argon plasma on intracellular bacteria: biological mechanisms and feasible applications

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Intracellular parasites represent a special problem among pathogenic microorganisms. Intracellular pathogens are less accessible to antibiotics. Their tight connections with human cells cause changes in cellular metabolism and functions that results in serious diseases. Besides the role in human pathology, intracellular pathogens are often contaminants of tissue cultures and biotechnological plants. Non-thermal plasma (NTP) proved its effectiveness as an antibacterial agent, which is low toxic for eukaryotic cells. This makes NTP an attractive candidate for decontamination of cells, cell cultures and tissues infected with intracellular and membrane-bound pathogens.

The aim of the work was to investigate effectiveness of NTP against human pathogens with different intracellular lifestyles. Obligate intracellular pathogen *Chlamydia trachomatis* multiplies within a host-derived replicative vacuole, termed an inclusion. A similar mechanism is used by many pathogenic bacteria including *Mycobacterium tuberculosis*, *Brucella* spp, *Salmonella* spp etc. Human pathogens belonging the *Mollicutes* class such as *Mycoplasma hominis* associate with host cell surface forming a membrane pocket similar in functions to a replicative vacuole. At last, some pathogens such as *Listeria monocytogenes, Shigella flexneri, Rickettsia spp* disrupt the phagosome to multiply within cytoplasm.

*C. trachomatis, M. hominis* and *L. monocytogenes* were used as model microorganisms to study effectiveness of non-thermal argon microwave plasma against bacteria with different cellular localization. The previously described microwave argon plasma source Microplaster β [1] was used to treat eukaryotic cell cultures infected with the bacteria. The ratio of bacteria amounts in treated and control untreated cells was used to evaluate the effectiveness of the treatment.

We demonstrated higher sensitivity of intraphagosomal *C. trachomatis* in comparison with intracytoplasmic *L. monocytogenes* while extracellular bacteria were similarly sensitive to NTP. Extracellular *M. hominis* was less sensitive to NTP than other bacteria while when intracellularly it demonstrated comparatively high sensitivity. De-regulation of host cell metabolism, and particularly of the host-derived replicative vacuole could be the cause of mortality among intracellular *C. trachomatis* and *M. hominis*. In contrast, upon phagosome escaping, *L. monocytogenes* is less dependent on the host cell. Obtained results suggested that indirect effects of NTP mediated by a host cell response might be a cause of the death of intracellular bacteria.

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# Tutorial Presentations

# Mechanisms of plasma biomedicine: what do we know?

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Low temperature, atmospheric pressure plasmas have been shown to be effective for a range of therapeutic applications: wound sterilization and healing; dermatology applications; dental and cosmetic care; stem cell differentiation and embryo development; bleeding suppression and tumor shrinkage, among others. It is generally acknowledged that various effects associated with these plasmas are, at least in principle, probably responsible for their effects. These include cellular effects of electric fields, charges, photons and reactive chemical species. In some cases, it is suspected that more than one mechanism might act synergistically with other effects to amplify biological efficacy.

In this talk, I will focus primarily on the role of reactive chemical species, especially reactive oxygen and nitrogen species, but other effects will also be addressed. [1] Oxidation-reduction biochemistry (or 'redox biochemistry') has received a great deal of attention in the last several decades in aerobic biology. [2] Initially, reactive radicals and other reactive species were thought of as a kind of 'molecular hoodlum,' to be suppressed if possible by 'antioxidants.' [3] However, research has shown that this perspective is far too simplistic, and that these species play many important roles in normal physiology, including in the immune system. It is perhaps no coincidence that innate immunity in the form of macrophages and neutrophils utilize reactive oxygen and nitrogen species in their role to attack microbial and parasitic invaders, as well as tumors. [4] Furthermore, it turns out that a number of important existing therapies are known or suspected to rely, at least in part, on redox chemistry. This key body of evidence provides a context within which to interpret plasma biomedical effects and also should help to suggest promising research strategies and techniques. Perhaps most importantly, study of this literature may help connect the field of plasma biomedicine to related fields in which considerable progress has already been made.

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# Plasmas sources for medical use

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Plasma Medicine as a new field includes not only the therapeutic applications. Already established applications of gas discharge plasmas for the antimicrobial treatment (decontamination and sterilization) of medical devices as well as the surface modification of implants (functionalization, coating) belong to this new research field. The origin of the discovery of plasma medicine is described very differently, but it must be noted that even before 100 years plasmas e.g. were used for improving blood circulation (Violet Rays, Zeileis method - 1912) and some today can still be found. Similarly, for several decades plasma is used directly in medicine, especially in the field of coagulation and later in the ablation e.g. of malignant tumors. Medically approved plasma sources on humans are generally used since years (eg, gastrointestinal tract, skin, mucous membranes), so what now happened ís a kind of advancement of technology.

The focus is now on the development of already clinically established and proven plasma sources and the construction of new plasma generators. For both purposes the "cocktail" of physical components (charged particles, the temperature, the free radicals, the radiation electromagnetic fields) is important. Depending on the medical application the assessment of individual components with their individual effects or risk has to be focused.

The development of suitable and reliable plasma sources for the different therapies requires an in-depth knowledge of their physics, chemistry and parameters. According to this, much basic research still needs to be conducted to minimize risk and to provide a scientific fundament for new plasma-based medical therapies. It is essential to perform a comprehensive assessment of physical and biological experiments to clarify minimum standards for plasma sources for applications in life science and for comparison of different sources. In this context tasks of standardization of medical plasma sources are of specific interest

This contribution intends to give an overview about different plasma sources for different therapeutic applications, and selected results in the field of dermatology, wound healing, veterinary medicine and dentistry.
After a general introduction, selected specific plasma sources which are used for the investigation of various biological effects are presented. Regarding the manageability in everyday medical life, atmospheric pressure plasma jets (APPJ) and dielectric barrier discharges (DBD) are of special interest.

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# Oral (Contributed) Presentations

# Effect of Reactive Nitrogen Species Produced in Water by Reverse Vortex Gliding Arc Plasmatron on Plant Germination and Growth Rate

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**Research aims:** Water quality, amount of minerals and salts, and chemical composition, especially pH and nitrogen content play a critical role in plant growth and development. Plants, in general, require certain amount of "free nitrogen" and prefer slightly reduced pH on the level of 5-6 for increased rate of germination, faster development, and improved nutritional value. Plasmas are well known for their ability to treat water (Fig.1) and open air plasmas, or plasmas generated in normal water (with some dissolved atmospheric nitrogen) would lower the pH and produce various nitrogen compounds in water which may be beneficial for plant treatment and other agricultural uses.

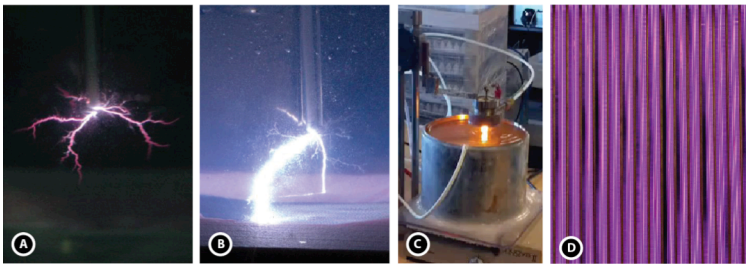


Fig.1. Photographs of four discharges used for water treatment: spark in water (A), pulsed arc in water (B), gliding arc plasmatron (C), and dielectric barrier discharge (D).

**Methodology:** We chose four plasmas (Fig.1) for treatment of different plants (sprouts, beans, strawberries, thyme, corn, roses, peppers, tomatoes, and hemp). In each case, plants were observed from germination to a mature plant. After maturation, the plants were cut and we analyzed root weight and length and stem weight and length along with BRIX measurement that gives total sugar content in the plant, indicative of overall nutritional value. Plasma parameters and treatment dose were recorded along with pH, $NO_2^-$, and $NO_3^-$ in the water.

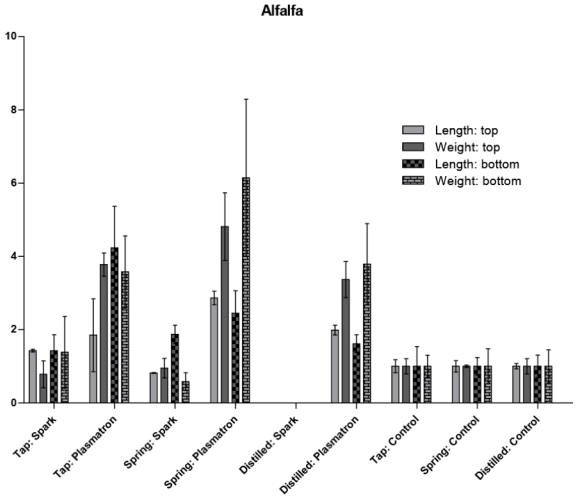


Fig.2. Results of length and weight of Alfalfa sprouts following treatment by plasma-treated water.

**Results:** We show significant improvement in germination and growth rate of the plants and attribute it to the nitrite and nitrate concentration increase in the water and lower pH.

**Conclusions:** While initial results are quite promising, many risks exist with plasma-treated water use in agriculture (for example increase in metal ion concentration). We plan to continue this research with detailed water mineralization analysis.

# Non-thermal atmospheric pressure plasmas for food decontamination

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Gentle sanitation of fresh fruits and vegetables is highly demanded since especially produce that is eaten raw increases the risk of food borne illnesses. Last noticed outbreaks in Western Europe concerned EHEC (enterohemorrhagic *Escherichia coli*) on seeds, *Listeria monocytogenes* on meat and Norovirus in frozen strawberries. Especially children, immunocompromised and elderly people have a higher risk for foodborne illnesses due to the consumption of humanpathogen-contaminated food. These populations are also a large clientele of hospitals. Currently used disinfection or sanitation methods for fresh fruits and vegetables lack antimicrobial effectiveness, but are high in costs, water consumption or chemicals. Non-thermal atmospheric pressure plasma offers a promising opportunity for the preservation of fresh food. The antimicrobial effects of plasma are well-known and investigated [1]. However, the diversity of plasma types and sources as well as the complexity of plasma chemistry and a variety of food (size, surface, and composition), each need is specific and requires individual adaptation. Depending on the used plasma source, treatment time, microorganism and specimen, reduction rates greater than 6 log were achieved [2-5]. The product safety must be increased without affecting the product quality. Sensory examinations showed only little influences on texture, appearance and odor. The advantages of plasma and the generated microbicidal compounds which led to high microbial inactivation on specimens offer a wide rage of possible uses along the whole value chain. Besides the scientific work, networking is essential for this kind of interdisciplinary research in order to include the industrial requirements successfully.

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# Induction of Fungal Cell Death and Enhancement of Host Resistance by Non-thermal Dielectric Barrier Discharge (DBD) Plasma

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In nature, microorganisms are associated with foods, host organisms, and non-living materials. Selective inactivation of microorganisms without damaging associated objects has been one of hot issues in sterilization by plasma [1]. In this study, we have evaluated the effectiveness of non-thermal dielectric barrier discharge (DBD) plasma in inducing apoptosis-like program cell death of a plant fungal pathogen, *Fussarium oxysporum f.sp. lycopersici* and enhancing resistance in host tomato plant. Fungal spores in different background solutions (PBS and saline) were treated with non-thermal DBD plasma by using argon and air as feeding gases. Tomato plant seedlings were treated with non-thermal DBD plasma by using argon as feeding gas.

The spore viability in saline solution was significantly reduced by argon plasma treatment whereas not much affected by air plasma. Spores in PBS were not much affected and air plasma did not influence significantly on spore germination. In Evans blue and propidium iodide (PI) staining, more cell death of fungal spores was observed in longer incubation after argon plasma treatment. Apoptosis-like cellular and molecular changes in fungal spores after argon plasma treatment were observed from Anexin V and TUNEL assay. In the experiments using tomato plant seedlings, mRNA expression of resistant genes was increasing after argon plasma treatment.

In summary, our study indicate that the argon plasma treated saline shows high efficacy in inducing fungal cell death during incubation and also enhancing the mRNA expression of resistant genes in tomato plant seedling. Further study is on going to elucidate the nature of plasma interaction with plant fungal spores and tomato plant seedlings.

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# Detailed Study of Plasma-Surface Interactions with an Atmospheric Pressure Plasma Jet (APPJ) as Selective Source for O, $O_3$ and N

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Atmospheric Pressure Glow Discharges (APGD) are effective sources of reactive particles, photons, ions or electric fields and can, therefore, be used for direct or remote treatment of biological substrates such as bacteria, biomolecules or tissues. It was shown many times that plasma treatment, induced by these discharges, can lead to local inactivation of microorganisms or biomolecules or improvements of the wound-healing process. However, due to the complexity of the plasma-substrate interaction, only limited and mostly qualitative understanding of this interaction is available. The challenge is to identify, which plasma components (e.g. reactive species, ions or photons) are dominating the treatment process and whether there are synergistic effects among them.

Here we present the result of the analysis of the remote APPJ and the results of treatment studies on bacteria and organic molecules. APPJ is a remote plasma source, as only reactive species and the plasma-generated photons in the plasma effluent are interacting with the substrate. This plasma source is chosen, because it can produce high densities of reactive species (O, $O_3$, N), which have been quantitatively determined by means of MBMS. Additionally, the APPJ can be modified in such a way that either only the reactive species or only the plasma generated photons can be used for the substrate treatment, allowing the separation of both effects [1,2]. The modification of the APPJ additionally allows to measure the full spectrum of the plasma emitted photons even below the cutting wavelength of ~115 nm of $MgF_2$ windows with the help of a helium-filled monochromator. The quantitative measurements of fluxes of reactive species and the qualitative changes in the emitted spectra provide us a better understanding of the interaction of these plasma components with biological substrates. Preliminary experiments with the APPJ have shown, that the reactive oxygen species O and $O_3$ are the most effective species to inactivate *E. coli* bacteria. Measurements of nitrogen species are important as well, as the nitrogen species influence the plasma radiation and can lead to additional effects on biological substrates due to plasma chemistry reactions. Furthermore, the influence of plasma treatment on organic molecules can be detected as changes in the FTIR or Raman spectrum. These data can be used to validate molecular dynamics simulations of biological molecules with reactive oxygen species [3].

We wish to acknowledge the cooperation with the group Biology of Microorganisms (Jun.-Prof. J. E. Bandow, Ruhr-University). Our work is supported by the German Research Foundation (grant no. PAK728, BE 4349/2-1).

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# Synthetic biological sensors and their role in unraveling mechanisms of plasma medicine

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Atmospheric-pressure plasma is an emerging medical technology with promising applications in healthcare [1]. Plasmas are now widely utilised in a variety of medical therapies including wound care, cancer therapy and dentistry. As the application of plasma in medicine advances, developing an understanding of the interaction of plasma with biological systems becomes increasingly important to address issues involved with the assessment of plasma medical device performance (e.g. mode of action) and the establishment of new safety guidelines. To this end new tools and methods are needed to facilitate the assessment of plasma with biological systems under controlled and repeatable conditions.

This talk will cover synthetic biological sensors that we employed to study plasma interactions with a soft, hydrated biological material [2]. Our system, comprising a gelatin gel and suite of (homogeneously) distributed biological and chemical reporters, allows us to monitor the spatial (surface) distribution and depth of penetration of reactive oxygen and nitrogen species (RONS) and to analyse their potential role in the plasma treatment of cell membranes within biological materials. Potentially, the use of synthetic biological sensors can be used to unravel the roles of different plasma species and the direct effect of whole plasma contact, from those of primary and secondary species — i.e. primary, those emanating directly from the plasma and secondary, those species created in the 'target' tissue. This type of insight could be useful in the future development of safe and effective plasma medical technologies.

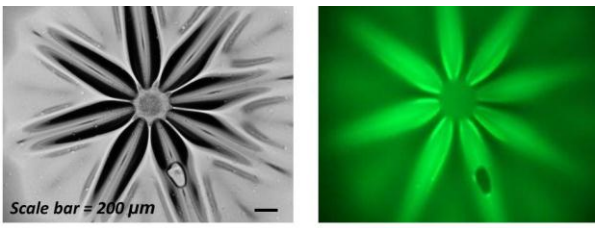


**Figure 1:** *Plasma jet treatment of the synthetic biological sensor resulting in a pattern of microchannels formed within the gelatin matrix (left bright-field image) enabling the direct plasma treatment of sensitive vesicles within the material (right fluorescence image).*

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# Non-Thermal Plasma for Acne and Aesthetic Skin Improvement

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Technology of Non-Thermal Plasma (NTP) that can generate low temperature plasma in normal atmosphere has been recognized widely as a new emerging tool with potential applications in life science. The applications and development of NTP was actually started in industrial fields after the discovery of nanotechnology and material science.

During last 10 years, there are many reports and publications of NTP confirming its safety and efficacy in health care and various medical applications. (1-6)

The promising future of NTP technology has aroused our interest to study in detail of a novel NTP device. The device generates Plasma by Dielectric –Barrier-Discharge (DBD) [7] with direct contact electrode. Micro Plasma beam is generated by ionizing surrounding air on electrode surface discharging directly to target tissue.

We have conducted clinical trials of the device: BIOPLASMA Cell Modulation device (developed by Photo Bio Care, Thailand) in acne and aesthetic skin improvement. The results will be discussed.

Furthermore, we are conducting clinical trials of Non-Contact NTP in chronic wound care. The initial results were satisfactory.

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# Preliminary Evaluation of Novel Skin Closure of Pfannenstiel Incisions Using Cold Helium Plasma and Chitosan Films

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**Objective**: To assess the safety and performance of a new energy-based skin closure system (BioWeld1™) for the surgical Pfannenstiel incision in patients scheduled for elective Cesarean section.

**Methods**: This prospective, single center, non-randomized study included 20 patients who were scheduled for elective cesarean section. The BioWeld1 system was performed after suturing the internal layers of the Cesarean section incision. A clinical evaluation of safety and efficacy was performed 1, 2, 4-7, 21 and 45 days after the procedure. The Vancouver Scar Scale was used to evaluate scarring.

**Results**: Up to 21 days after the procedure, no safety device-related adverse events were reported. All patients had full closure of the epidermis, a very low total Vancouver Scar Scale score, and no evidence of discharge, redness, edema, or thermal damage. None of the patients exhibited more than a mild degree of encrustation.

**Conclusion**: The BioWeld1 System has been shown to be safe and effective for skin closure in Cesarean section.

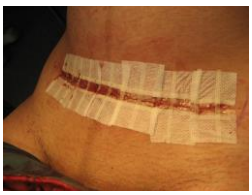

**Figure 1:** *Day of operation*

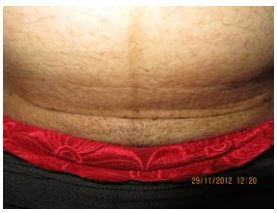

**Figure 2:** *Post-operative day 7*

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# Standards in Plasma Medicine: Development, Contents and Importance of the first German DIN Specification.

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An innovative field of plasma medicine is the application of atmospheric pressure plasma sources in dermatology. To establish innovative physical tools for dermatological applications, basic criteria for performance characterization *in vitro* should be helpful to pre-select useful devices. Comprehensive capture of plasma physical data is essential but insufficient. Additional biologically based test protocols are necessary. The DIN (German standards institute) specification entitled “General requirements for medical plasma sources” is already published. The specification named above describes obligatory basic criteria for the use of biomedical applications. Simple and generally applicable biological (inactivation of microorganisms, cytotoxicity and detection of chemical species in liquids) and physical test methods (temperature, thermal capacity, optical emission spectrometry, UV-irradiance, gas emission and leakage current) should give information about the effectiveness of medical plasma sources. These basic criteria should be helpful to identify plasma sources which are useful for further biomedical investigations with the aim of potential therapeutic applications. Furthermore, such pre-selection criteria will ensure the safety for investigators, patients and therapists. A generally known and applicable standard also guarantees identification of devices which are not useful as medical plasma sources.

Additionally, specifications and standards facilitate the introduction of products into the world market. With the help of standards, plasma sources achieve a higher acceptance and can be perfectly adjusted to dermatological and other medical applications. However, the creation of a standard is a long and educational process. The development process consists of different steps: applications, business plan, draft, public enquiry procedure and publication. For scientists, these steps are associated with new experiences, a lot of statutory provisions and different hurdles. Nevertheless the development process leads to an intensive and interdisciplinary scientific exchange and is helpful to consolidate application-oriented aspects of research.

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# Activities of terraplasma GmbH

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The company, terraplasma GmbH, was founded in May 2011 as a spin-off from the Max-Planck Society. Fundamental research, carried out within a technology transfer project at the Max Planck Institute for extraterrestrial physics, led to this development.

The company terraplasma GmbH offers a new and revolutionary technology - **cold atmospheric plasma -** for many aspects in hygiene, medicine and agriculture**.** We have a range of technologies available for plasma production at room temperature and atmospheric pressure including Microwave Argon plasma devices (tested in clinical trials with ~300 patients) [1], Surface Micro-Discharge plasmas [2], Venturi devices and Piezo powered devices. Our services include development, design and quality assurance of cold atmospheric pressure plasma products from customized demonstrators up to industrial prototype levels.

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# Cold Plasma Diagnostic Using Vectorial Electrooptic Probe

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The paper describes a pigtailed electrooptic (EO) probe as well which is used to characterize the transient evolution of the electric (E) field inside a cold plasma. The sensor consists in a fully dielectric optical arrangement including an isotropic EO crystal. This latter one acts as a transducer (via the Pockels effect) converting the E-field to be measured into an optical modulation of a laser beam [1]. The modulation, carried out via the polarization state of the probe beam, is then converted into a modulation of an electrical signal thanks to a polarizer and a photodiode. The optical sensor is pigtailed and connected to an instrument which ensures the reliability of the field measurement regardless the environment temperature. It allows to perform the simultaneous transient analysis of the two E-field vector transverse components [2,3]. The performances are: minimum detectable field lower than 1 V/m, measurement dynamics exceeding 130 dB, bandwidth spreading from 30 Hz up to more than 10 GHz and transverse spatial resolution better than 1mm.

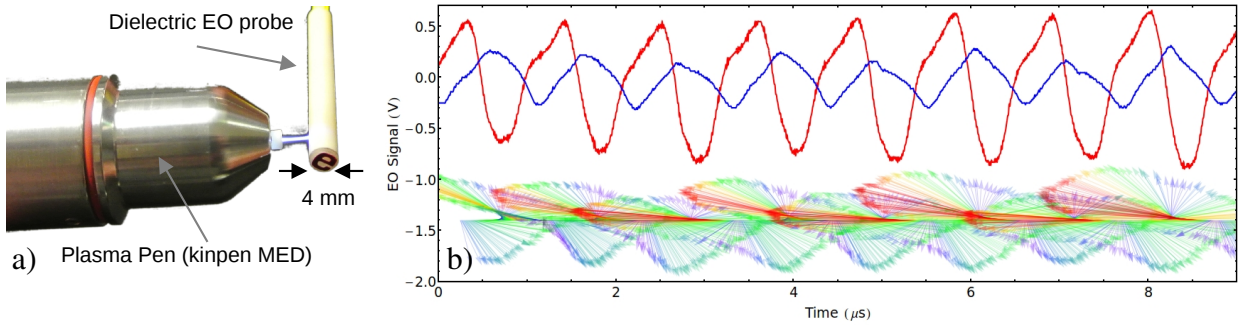


**Figure 1:** *Transient evolution of the E-field associated to the plasma jet. a) Photography of the experiment. b) Real time measurement of the two transverse components of the E-field vector. The reconstructed E-field vectors are given bellow the curves.*

An atmospheric pressure plasma jet source is here investigated. The so-called kinpen MED [4] operates at room temperature and is used in the field of plasma medicine [5]. The sensor is placed in front of the plasma jet and the real time measurement is presented in Fig. 1. This characterization clearly demonstrates that the field follows the plasma exitation (RF voltage of 2–3 kV peak-to-peak at ∼1 MHz). Furthermore, the field vector evolution exhibits an elliptical shape resulting from the plasma itself and from the igniting field. This result constitutes to our knowledge, the first vectorial assessment of a cold plasma induced E-field.

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# *In vivo* tissue oxygenation triggered through Plasma Gun treatment

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*In vivo* healthy tissue oxygenation was assessed following Plasma Gun (PG) application on the external surface of mouse skin. In situ, subcutaneous tissue oxygenation was evaluated from both oxygen partial pressure and blood flow measurements with an optical based oxygen sensing device (OxyLite/OxyFlo). Two low invasive optical probes were subcutaneously inserted in the left and right abdominal regions of anesthetized mice before plasma treatment. PG was operated at 2 kHz, 6 kV peak voltage amplitude. PG capillary was flushed with helium at 0.5 l/min, the gap between the PG capillary outlet and skin surface was set to 5 mm. It has been measured that PG application during periods of a few minutes doesn't lead to any observable skin ablation or burns while significant blood flow increase and oxygen partial pressure enhancement, up to a fourfold magnification, were measured during and after plasma delivery. The measurement resulting from the two probe protocol evidenced localized tissue activation in the region under plasma plume impingement. A rapid increase of oxygen and blood flow signals was measured right after the start of PG operation, leading to a steady state biological response about 5 min. after PG treatment whatever the duration of plasma application. Then following PG switch off, a slow decrease towards baseline levels of both blood flow and oxygen tension was recorded during typical time periods of a few tens of minutes. This first evidence for plasma triggered *in vivo* tissue oxygenation clearly emphasizes the potential impact for new therapeutic applications, including oncology, of non thermal plasmas [1]. It appears as a remarkable new mean to increase tissue oxygenation, which is an aim of anticancer strategies. As such, both radio therapy and chemotherapy require the best tissues oxygenation for either reactive species generation with ionizing radiations and ROS involving drugs. Tissue pO2 increase rules the efficacy of innovative angiogenesis-based treatments through "normalization" [2] of blood vessel structure and increasing drug delivery in chemotherapeutic protocols. Non thermal plasma thus appears as a potential loco regional, fast triggered and efficient alternative for such adjuvant therapies.

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# The effect of plasma activated medium on pancreatic cancer cells

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**Einführung:** Pancreatic cancer remains to be one of the most malignant tumour entities displaying a five year survival rate of 5% in the western hemisphere. Currently, the only hope for cure is a complete surgical resection. However, only less than 20% of patients are resectable and even in resected patients the five year survival rate does usually not exceed 30%. Furthermore, palliation therapy is not effective either [1]. Tissue tolerable plasma is a promising candidate employing highly reactive oxygen and nitrogen species [2] inducing apoptosis in tumour cells [3]. This effect can also be induced by indirect action of solutions formerly treated with plasma [4]. This could lead to treatment options in particular in advanced or even disseminated disease of the peritoneal cavity.

**Materials and methods:** The used tissue tolerable plasma source was the kINPen Med, employing argon as carrier gas at 4 slm. The murine pancreatic cancer cell line 6606PDA as well as murine fibroblasts of C57/Bl6 origin were analyzed for their metabolic activity following plasma therapy using the CellTiterBlue viability assay. The radical catcher N-acetyl-cystein was used in control experiments to asses the functional role of ROS/RNS.

**Results:** 6606PDA cells were sensitive to plasma treatment in a dose-dependent manner: direct plasma treatment of 60s lead to an 83% reduction of metabolic activity ($p<0,0001$) as did plasma activated medium after 60s leading to a 60% reduction ($p<0.0001$). The 15% difference in reduction between direct and indirect plasma was significant ($p<0.01$). Fibroblasts were less sensitive to plasma treatment leading to a reduction of 56% ($p<0.0001$) and 35% ($p<0.01$), respectively. The addition of NAC to 6606PDA induced a dose-pendent reduction of plasma sensitivity: 2mM NAC led to only a 32% ($p<0.0001$) and 32% ($p<0.001$) reduction of viability whereas 16mM NAC almost blocked the plasma effects to 9% and 7%, respectively ($p>0.05$). 2mM NAC treatment in fibroblasts almost abolished the plasma effects showing only 6% and 7% of cell viability decrease.

**Discussion and conclusions:** Our results support the hypothesis of López-Lázaro that the intracellular induction of ROS may induce apoptosis in cells. Malignant cells are more sensitive to ROS due to their higher basal intracellular level explaining a more selective action of plasma in tumour cells. This may lead to an application of higher plasma doses in clinical applications protecting the surrounding non-malignant tissue with radical catchers such as NAC. The treatment of patient with disseminated peritoneal of pleural disease could offer further treatment options in addition to applying local direct plasma in microscopic residual disease.

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# Genotoxic and cytotoxic effects on multi cellular tumor spheroids exposed to low temperature plasmas

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Biomedical applications of low temperature plasma jets are of growing interest. These plasma jets are an interesting source of active species (charged particles, radicals, long lived excited species, UV photons and even electric field) that can easily be launched on any prokaryote or eukaryote cells, living tissues, biomaterial surfaces, etc [1].
The present communication emphasizes the regionalized antiproliferative effects of low temperature plasmas on MultiCellular Tumor Spheroid (MCTS), a model that mimics the 3D organization and the regionalization of a microtumor region [2]. We report that helium flowing inside a glass tube DBD device ejecting low temperature plasma jet in open air (Fig. 1) inhibits HCT116 colon carcinoma MCTS growth in a dose dependent manner (Fig 2). This growth inhibition is associated with the loss of Ki67 and the regionalized accumulation of DNA damage detected by histone H2AX phosphorylation. This regionalized genotoxic effect leads to massive cell death and loss of the MCTS proliferative region. The use of reactive oxygen species (ROS) scavenger N-acetyl cysteine (NAC) and plasma pre-conditioned media demonstrate that gaseous plasma ROS generate genotoxic and cytotoxic aqueous species in the media culture that in turn play a major role in the observed effects on MCTS. These findings strengthen the major interest of the use of MCTS for the evaluation of antiproliferative strategies and open new perspectives for studies dedicated to demonstrate the potential of low temperature plasma in cancer therapy.

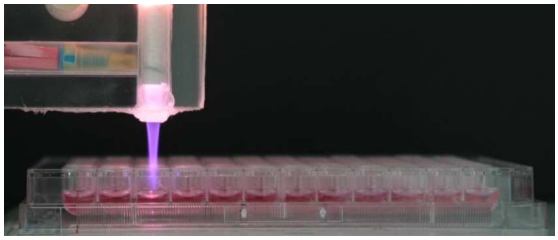

**Figure 1.** *Exposure to the He low temperature plasma jet of spheroid grown in 96-round bottom well plates*

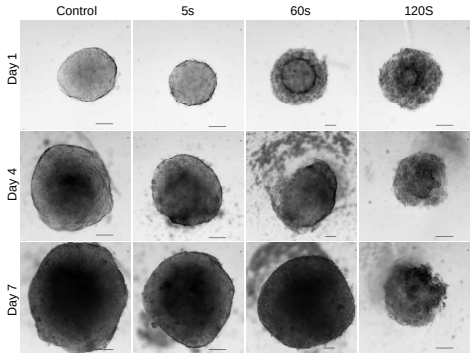


**Figure 2.** *Variation of HCT116 volume 1, 4 and 7 days after plasma exposure.*

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# Controlling Plasma Jets with Gas Shields and Their Interactions with Water Covered Tissue*

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Indirect treatment of biological surfaces with atmospheric pressure plasma jets are used in wound healing, sterilization and treatment of cancer cells. Shield gas curtains surrounding the effluent of the plasma jet provide additional control of reactive species production.[1] In many applications, there is a thin layer of a water dominated liquid covering the tissue. The liquid serves as a transfer function – the plasma produced radicals and ions are processed by the water prior to their reaching the tissue. The position of the plasma jet determines whether charged particles in addition to neutral radicals are delivered to the liquid covering the tissue. In this paper, we report on results from a computational investigation of a $He/O_2$ atmospheric pressure plasma jet with and without a shielding gas, with the plasma plume touching or not touching a water layer over tissue.

The model, *nonPDPSIM*, solves transport equations for charged, neutral species and electron energy, Poisson's equation for the electric potential, and Navier-Stokes equations for the neutral gas flow. Rate coefficients for the bulk plasma are obtained from local solutions of Boltzmann's equation. Radiation transport addresses photoionization of $O_2$ and $H_2O$ in the gas phase; and photoionization and photodissociation of $H_2O_{aq}$ in the liquid.

The transfer-function abilities of the water layer are demonstrated by the $He/O_2$ =99.8/0.2 plasma jet into humid air incident onto water covered tissue shown in Fig. 1. The 200 μm water layer is a buffer to convert the plasma produced active species to aqueous form. Electrons in the plume directly interact with the thin water layer which essentially converts all electrons to, for example, $HO_2^-{}_{aq}$ or $NO_x^-{}_{aq}$. OH fluxes are largely converted to $H_2O_{2aq}$. An $O_2$ shroud surrounding the effluent reduces the reactive nitrogen species produced in the gas phase and so reduces the flux of $HNO_{xaq}$ to the tissue below the water layer.

* Work supported by the DOE Office of Fusion Energy Sci. and the Natl. Science Foundation.

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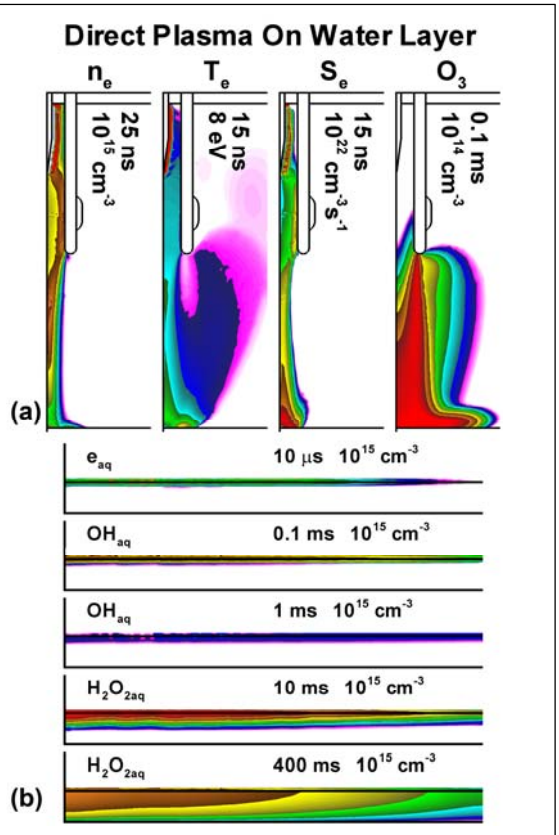


**Figure 1:** *$He/O_2$ plasma jet interacting with a water layer over tissue. (a) The effluent of the plasma jet. (b) Diffusion of species over time through the water layer.*

# Surface Modification of Dot-arrayed Carbon Nanotubes for Multifunctional Bio-chip Sensors Using Atmospheric Pressure Plasma Jet

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## 1. Introduction

Because of the unique properties of bio-chip sensor, it has been attracting interests as potential successor to a wide range of analytical technique [1]. Recently, biosensors based on carbon nanotubes (CNTs) have attracted considerable attention due to their high sensitivity [2, 3]. In successful realization of bio-chip sensors based on CNTs, it requires proper control of their CNTs fabrication and their surface immobilization. Compared to the conventional chemical modification techniques, plasma treatment has the advantages of shorter reaction time and non-polluting process. In this study, our objective is to develop multifunctional bio-chip sensors based on dot-arrayed CNTs and their surface immobilization using atmospheric pressure plasma jet (APPJ) [4], as illustrated in Fig.1.

## 2. Experimental

The fabrication of dot-arrayed CNTs was performed by electron beam lithography and plasma enhanced chemical vapour deposition. For the surface immobilization of dot-arrayed CNTs, an APPJ with a micro-capillary was used to immobilize functional groups. The success of the surface immobilization was confirmed by fluorescent dye which can be visualized by fluorescent microscope.

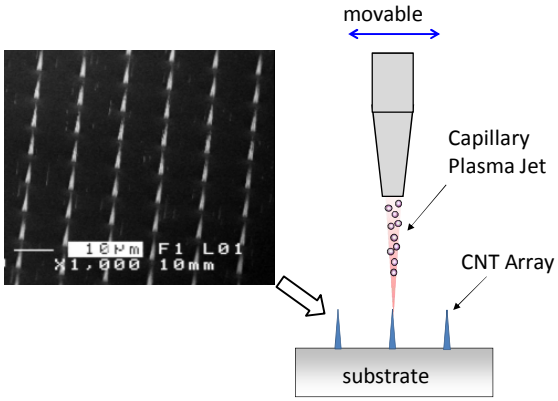


Figure 1. FE-SEM image of dot-arrayed CNTs for 1µm dot sizes and 10 µm dot intervals and schematic of surface treatment

## 3. Results and discussion

Surface functionalization of dot-arrayed CNTs was conducted by the capillary APPJ with $NH_3$ gas. Figure 2 shows a fluorescent microscope image of the surface aminated dot-arrayed CNTs using an APPJ with a larger aperture size. The fluorescent microscope image shows that the CNTs surfaces are uniformly immobilized with amine groups. The fabrication of dot-arrayed CNTs and their surface immobilization for bio-chip sensors have been successfully developed. The results show the feasibility of uniformly surface immobilization by the APPJ under atmosphere, indicating that dot-arrayed CNTs are good as bio-chip sensors.

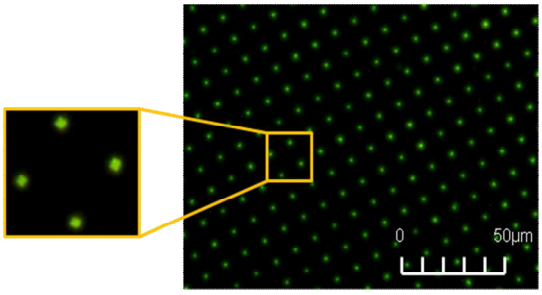


Figure 2. Fluorescent microscope image of the surface aminated dot-arrayed CNTs.

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# Hybrid Plasma Fluid Modeling and Gas Flow Simulation of Atmospheric-Pressure Plasmas

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Simulation considering direct coupling of discharge and gas flow has been considered a challenging task, mainly because of the wide disparity of time scales of light and heavy species, e.g., electron (~$10^{-10}$ s) and neutral species (~$10^{-3}$-1 s). Development of hybrid plasma fluid modeling and gas flow of atmospheric-pressure plasmas is presented in this paper. The fluid model we employed in the current study is basically the same as that presented by Lin *et al.* [1]. The fluid model includes the continuity equations for charged and neutral species, the momentum equations for charged and neutral species, the energy equation for electron and the Poisson's equation for electrostatic potential. In the gas flow solver [2], the governing equations include the mass, momentum, energy and species conservation equations. Both solvers are coupled using a temporal multiscale algorithm (TMA). The basic idea of the TMA is to integrate those species temporally which respond fast and slow to the driving voltage with a small (electron limited) and large (diffusion limited) time step, respectively. Parallel computing using domain decomposition with message passing interface (MPI) is applied to speed up the computation.

We employ a planar two-dimensional atmospheric-pressure dielectric barrier discharge jet (AP-DBD) (driven by 25 kHz and 6 kV) to demonstrate the effective coupling of both solvers to reach the quasi-steady state. Some typical results are presented in **Figure 1**. More details will be presented in the conference.

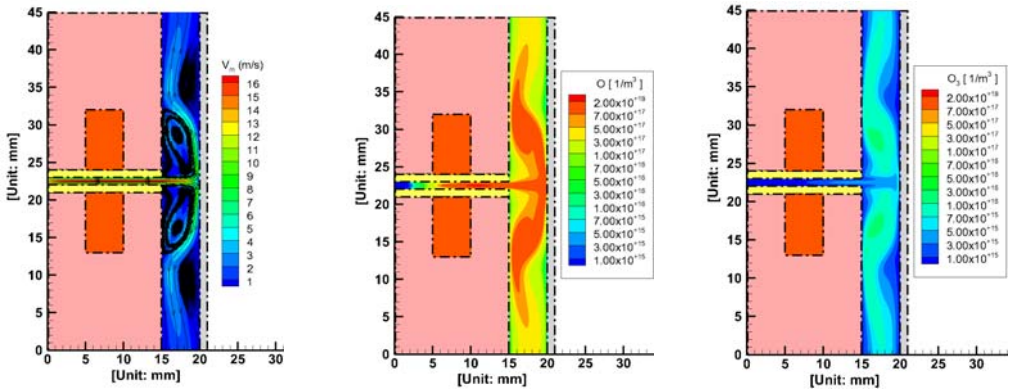


**Figure 1:** *Typical results of a planar DBD jet impinging on a flat surface.*

**Acknowledgement**: The authors would like to thank the financial support of National Science Council, Taiwan, through the grant NSC-103-2811-E-009 -004.

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# Mass decontamination of biological warfare agents by plasmas

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A comprehensive decontamination scheme of biological warfare agents, including airborne agents and surface contaminating agents, is presented. The plasma flame, presented here, provides a rapid and effective elimination of toxic substances in the interior air in isolated spaces. As an example, a reaction chamber, with a 22 cm diameter and 30 cm length, purifies air contaminated with toluene [1], which is the simulated chemical agent, with a flow rate of 5,000 liters per minute. This reaction chamber can also purify air contaminated with soot from a diesel engine, the simulated aerosol for biological agents. A simulated experiment also indicates that due to synergistic benefits derived from the combination of ozone from plasma discharge and acidic water, the acidic ozone water [2,3]very effectively kills endospores of *Bacillus atrophaeus* (ATCC 9372), thereby demonstrating the capability of cleaning a large surface-area in a very short time and reinstating the contaminated environment as free from toxic warfare agents. The acidic ozone water, after the decontamination process, disintegrates into ordinary water and oxygen without any trace of harmful materials to the environment. An experiment also indicates that the argon-oxygen plasma jet [4] can decontaminate the warfare agents on surfaces of sensitive equipments.

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# Air Cleaning System with Use of High Electric Field Plasma without Discharges

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Abstract

"High-Electric Field Plasma" (HEFP) technique in an atmospheric pressure has been developed to control and keep the environmental atmosphere in clean state; such as sterilization of air by killing the bacteria, much longer sustaining of fresh states of plants such as vegetables, fruits, flowers and so on, or changing the air into the fresh state by decomposing bad smell chemicals included and by catching a part of smog bad for environmental controlling. Here the "High-Electric Field Plasma" system means that the plasma has no apparent discharges and the electric field inside the plasma area is high to be $3-4\times10^6$ V/m in the atmospheric pressure. This field can decompose harmful or poisonous materials for the fresh plants and kill the bacteria. Also some of the chemicals can be captured by high electric field for charging the content materials [1-4].

In this paper, the sterilization in the air is demonstrated with use of *Aspergillus brasiliensis* (green mold) and *Staphylococcus aureus* (*S. aureus*) sputtered in the air. We also tried to sterilize *Escherichia coli (E. coli)* which is sputtered on the electrodes for applying the HEFP. Furthermore, we tried to clean up the bad smell air into fresh smell by decomposing the chemicals which are the origin of the unpleasant smell, with use of ammonia or toluene.

In our present air conditioner, a usage of harmful or poisonous materials such as ozone, OH radicals or UV light are suppressed not to work for these purposes. Therefore, this system is quite safe to the human bodies and fresh plants, even if they stay together within the cleaning system.

We appreciate Mr. M. Hirota for their collaboration for obtaining those data.

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# Ionic strength of solutions can modulate the anti-microbial effects of non thermal atmospheric pressure plasma

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Microenvironments surrounding microorganisms often modulate the effects of various anti-microbial agents. In the case of non-thermal plasma at atmospheric pressure, a potential means of microbial sterilization, increasing number of studies show that sterilization efficiency varies depending on the background environment surrounding microorganisms [1, 2]. Understanding the nature of interaction between plasma and environmental factors is essential for improving sterilization efficiency of plasma technology. In this study, we investigated the influence of NaCl in background media on anti-microbial effects of plasma using a model eukaryotic microbe, *Neurospora crassa* (filamentous fungus). Relative spore germination compared to control (Ar gas treated) was dramatically reduced in water but not in saline (0.85 % NaCl solution). Internal structure was less damaged and genomic DNA was less oxidized in spores treated with plasma in saline than water. During plasma treatment, spore germination was increased in response to the increased concentration of NaCl in solutions. Osmotic stability, pH, radicals, and chemical changes were not enough to explain the NaCl effects. Our further analysis show that ionic strength in the background solution may be the critical factor that can modulate the plasma effects.

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP), No. 2010-0027963 and No. NRF-2013R1A1A3011245.

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# Analysis of Plasma-Decontamination Process in Solution Using Bacterial Spores Differentially Labeled with GFP

Hachiro Yasuda[1], Yuki Tani[1], Roger M. Agustin[1], Daisuke Imamura[2], Hirofumi Kurita[1], Kazunori Takashima[1], Kazuhito Watabe[3], and Akira Mizuno[1]

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Inactivation of bacteria in solution by atmospheric pressure plasma is important from a viewpoint of plasma medicine. However, inactivation process of bacteria in solution during the plasma exposure to the liquid surface is not well understood. Protein in solution is damaged by DBD treatment without its degradation, and damage of cellular proteins seems to be closely related to the cell death as well as damages of DNA and/or cell membrane [1].

To analyze the plasma inactivation process, we have genetically constructed a series of *Bacillus subtilis* strains whose proteins of spore layer are tagged with GFP (Green Fluorescent Protein) [2]. Location of GFP fluorescence in each spore strain is limited to one of the layer of the spore (Figure 1). Using these spore collections, relation between cell death and inactivation of GFP was investigated.

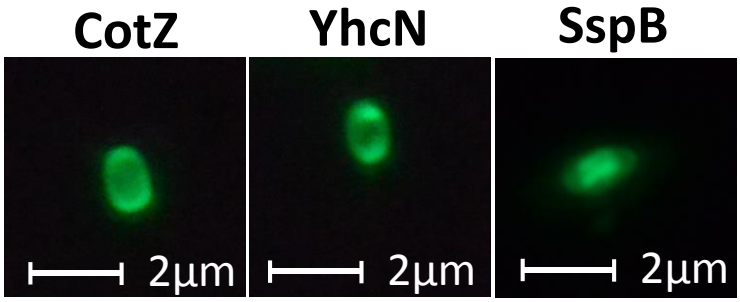


**Figure 1:** *Typical Bacillus subtilis spores differentially labeled with GFP*

Bleaching of the GFP initially occurred from outermost coat labeled GFP, and proceeded to inner positions of cortex, inner membrane, and finally reached to core. It is suggested that in plasma inactivation in solution, destruction of specific local position of spore is not dominant. The timing of cell death and bleaching of the fused GFP positioned in inner membrane was overlapped. The results suggest that the damage of inner membrane or germination receptor proteins located on the membrane is closely related to the plasma inactivation of *Bacillus subtilis* spores in liquids.

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# In-package dielectric barrier discharge atmospheric cold plasma (DBD ACP) for inactivation of *Pseudomonas aeruginosa* biofilms

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## Abstract

Due to increased resistance, biofilms represent a major challenge in biomedical industries [1]. In recent years, atmospheric cold plasma (ACP) has been widely investigated for potential applications as an alternative decontamination technology in industrial, food and healthcare sectors.

In this study the antimicrobial efficacy of DBD-ACP against *Pseudomonas aeruginosa* biofilms was investigated. The 48 h bacterial *P. aeruginosa* biofilms were grown in 96 well microtiter plates ($10^{7\text{-}8}$CFU $ml^{-1}$) and biofilm formation was monitored using CV assay. Samples were placed inside rigid polypropylene containers prior to ACP treatment, sealed within high barrier polypropylene and placed between two circular aluminum electrodes where a high voltage plasma discharge (80 $kV_{RMS}$) was generated. Following ACP exposure, samples were left unopened for 24 h post treatment storage time. The effects of mode of plasma exposure (direct/indirect) were also evaluated for this system. The percentage of surviving bacterial populations was estimated by colony count and XTT assay. The ability of ACP reactive species to penetrate through the biofilms complex structures was observed using confocal scanning laser microscopy (CSLM) followed by staining prepared samples with LIVE/DEAD bacterial viability kit.

Exposure to either direct or indirect plasma effectively reduced *P. aeruginosa* survival in biofilms. Using 80 $kV_{RMS}$, treating for 60 s with direct plasma exposure reduced bacterial biofilms by an average of 5.4 log cycles from initial 6.6 $\log_{10}$ CFU $ml^{-1}$. Increasing the treatment time to 120 s and 300 s reduced biofilms to undetectable levels. According to XTT assay, an extended treatment time of 300 s was necessary to reduce metabolic activity of cells in biofilms by an average of 70%. Further investigation of biofilm viability by CSLM demonstrated that DBD ACP effectively inactivated *P. aeruginosa* population in biofilms.

This study demonstrates the potential of a novel high voltage in-package ACP decontamination approach for inactivation of bacterial biofilms.

The research leading to these results has received funding from the European Community´s Seventh Framework Program (FP7/2207-2013) under grant agreement number 285820.

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# Inactivation process of *P. digitatum* spores evaluated by dose of ground-state atomic oxygen

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We have focused on the effects of neutral oxygen species on the inactivation of *Penicillium digitatum* spores. On the basis of measurement of densities of oxygen radicals such as ground-state atomic oxygen [$O(^3P_j)$] and singlet oxygen molecule [$O_2(^1\Delta_g)$], we showed that $O(^3P_j)$ is the dominant species responsible for the inactivation of the spores quantitatively[1][2]. Besides, with the oxygen radical treatment, the spores were inactivated without major morphological changes[3]. However, the inactivation process that oxygen radicals affect the spores has not been clear. In this study, we have investigated the process, including the inhibition of the function of cell membranes, the oxidation process of the spores, and nanostructural changes, using confocal laser fluorescent microscopy and transmission electron microscopy (TEM).

To eliminate the influence of atmospheric gases, the radical source and the sample were enclosed with a plastic cover. The spore suspension of 1 μl was spotted on a ϕ35 mm dish and dried. The samples were exposed to oxygen radicals 10 mm downstream from the radical head at a $O_2/(Ar+O_2)$ flow rate ratio of 0.6% with a total flow 5 slm from 1.5 to 7 min. Those treatment times correspond to $O(^3P_j)$ dose from $2.1\times10^{19}$ to $9.8\times10^{19}$ $cm^{-2}$ on the basis of measurement of radical density[2]. The ultrathin sections of the spores were prepared, stained by uranyl acetate and lead, and observed by TEM.

Figures 1(a), (b) and (c) show the TEM images of cross section of the control spore and those treated with $O(^3P_j)$ dose of $2.1\times10^{19}$, $9.8\times10^{19}$ $cm^{-2}$, respectively. As shown in Fig. 1(a), intracellular organelles, such as nucleus and mitochondria, in the control spore were observed. The structure in the spore was relatively kept at $O(^3P_j)$ dose of $2.1\times10^{19}$ $cm^{-2}$ as shown in Fig. 1(b). On the other hand, Fig. 1(c) shows that the structure was completely decomposed with that of $9.8\times10^{19}$ $cm^{-2}$. These results indicated that the intracellular structure was gradually decomposed according to the increase of $O(^3P_j)$ dose. We will discuss the relation of the decomposition with the oxidation of the spore on the inactivation process in detail.

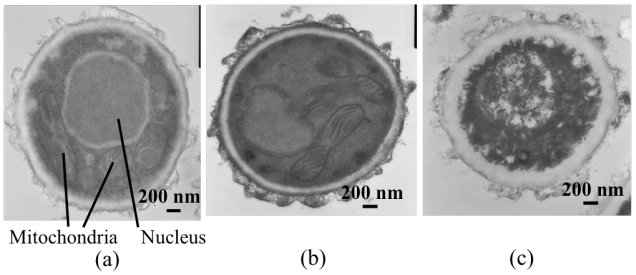


**Figure 1.** *TEM images of cross sections of P. digitatum spore: (a) control, and (b) $2.1\times10^{19}$ and (c) $9.8\times10^{19}$ $cm^{-2}$ in $O(^3P_j)$ dose.*

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# Effects of Low-Temperature Atmospheric-Pressure Plasma Irradiation on the Differentiation of Mouse Embryonic Stem Cells

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Recently various applications of low-temperature Atmospheric-Pressure Plasmas (APPs) irradiation to living cells and tissues were performed and therapeutic effects such as wound healing and tumor apoptosis were also demonstrated. However, the effect of plasma irradiating on cell differentiation is largely unknown. Therefore we focused on embryonic stem (ES) cell differentiation. The objective of this study is to examine the effect of plasma irradiation on the differentiation of mouse ES cells.

ES cells are derived from the inner cell mass of pre-implantation blastocysts [1] and have the characteristic features of self-renewal and pluripotency. They can differentiate into all cell types deriving from the different three germ layers: endoderm, mesoderm and ectoderm [2].

Embryoid bodies (EBs) are comprised of three germ layers. The differentiation process of embryo is mimicked by the formation of EBs, which can be induced from ES cells *in vitro*. We performed the irradiation of low-temperature APPs to mouse ES cells, and then analyzed the process of EB formation. The plasma irradiation affected the differentiation of mouse ES cells. This is the first finding of the effect of plasma irradiation on ES cell differentiation. The detailed results will be discussed.

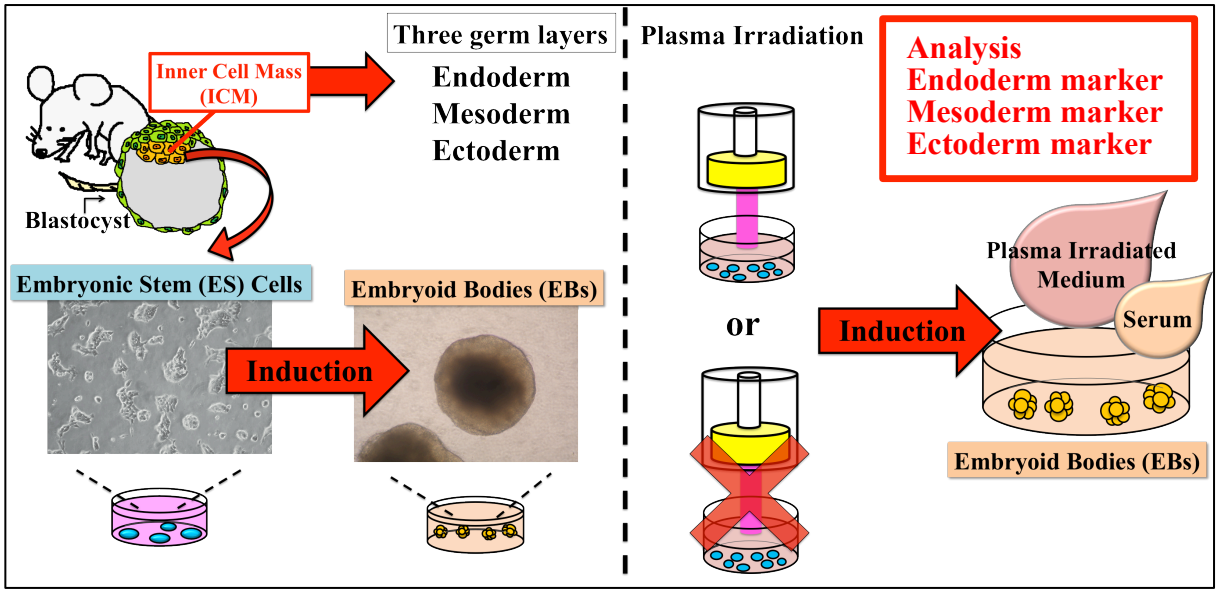


**Figure 1:** *The analysis of the effect of gas plasma irradiation on the differentiation of mouse ES cells*

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# COST Action MP1101: Biomedical Applications of Atmospheric Pressure Plasmas

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COST (European Cooperation in Science and Technology) is a framework aiming to support collaboration and integration across Europe in selected areas of science and technology. In particular, COST seeks to support emerging and interdisciplinary research areas with potential for socio-economic impact. Biomedical applications of atmospheric pressure plasmas is such an emerging area. The Action MP1101 [1] supports initiatives designed to bring coherence to a geographically dispersed research community with diverse aims, using instruments such as targeted workshops and exchanges of scientific personnel between participating laboratories, with the goal of developing a shared and coherent scientific approach. At the present time, 22 countries from the COST area are engaged in the Action. The Action has four working groups, one concerned with development and optimisation of plasma sources, and three addressing application areas, namely: materials processing, therapeutic applications of atmospheric pressure plasmas, and decontamination using atmospheric pressure plasmas. This paper will present an overview of the scientific programme of the Action, and will motivate initiatives described in greater detail in other presentations, such as the development of a reference plasma source and reference biological assay procedures. These activities address what we perceive to be a serious weakness in the collective programme of our community, namely a difficulty making valid comparisons between results obtained in different laboratories. This is a serious obstacle to achieving one of the key aims of the Action, a coherent research programe spanning the European research area. Although the focus of COST activities is necessarily in Europe, participation in Action events is open to interested parties from other countries, and can sometimes be supported by the Action.

[1] http://www.cost.eu/domains_actions/mpns/Actions/MP1101

# An atmospheric pressure plasma reference source and protocols for biomedical applications

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Studies of low-temperature atmospheric pressure plasmas and their interactions with biological samples have been difficult to compare when obtained from different plasma sources with varying biological protocols and across different research groups. Varying parameters including plasma geometries and materials, electrical power input, gas composition, ambient conditions, media composition, and plasma-biological interaction protocols make mechanistic understanding and comparing research studies challenging. While the effects of these variables are generally acknowledged there is still a lack of systematic studies. Within the frame of the EU COST Action *Biomedical Applications of Atmospheric Pressure Plasma Technology* a reference plasma source and associated biological interaction protocols, along with basic calibration procedures have been developed. Progress of this initiative will be presented.

It is acknowledged that there are various plasma sources needed to be developed within the community for specific needs and applications. An outline of one plasma reference source will be presented along with measurements comparing performance in different laboratories. The atmospheric pressure plasma source is chosen to allow: good diagnostic access to the plasma core and effluent regions, a simple geometry for modeling and simulations and a plasma effluent for biological interaction studies.

The plasma source consists of two plane parallel stainless steel electrodes; quartz windows enclose the discharge region. The core plasma channel has a cross-section of 1 mm x 1 mm over a length of 30 mm. Typical operational parameters are helium feed gas flow with molecular admixtures. One electrode is driven at 13.56 MHz, via an impedance matching network, while the other one is grounded. This produces a cold homogeneous glow discharge at ambient pressure. Species generated within the rf discharge volume are transported by the gas flow toward the effluent region, where they can be directed onto a sample. The charged particles and any electric fields are confined within the core plasma volume, thus leaving the plasma effluent charge free and consisting of reactive neutrals and UV radiation.

A number of identical plasma reference sources have been established in different labs and using simple optical emission spectroscopy and electrical characterisation the variability of the sources has been investigated. Advanced optical measurement techniques have been implemented for absolute measurements of reactive oxygen and nitrogen species. Results from a robust cell viability assay (Alamar Blue) have also been compared in different laboratories, with 3T3 mouse fibroblasts as a reference for eukaryotic cells, with the view to implementing the protocol as a biological reference for this plasma source.

**Acknowledgement:** The authors acknowledge EU COST Action MP 1101.

# Biological Standard Tests for an Evaluation of Different Plasma Sources and Treatment Regimes

K. Masur [1], N. S. Braithwaite [2], P. Bruggeman [3], T. Gans [4], A. Hirst [4], G. M. W. Kroesen [5], D.Marinov [2], K. Niemi [4], D. O'Connell [4], S. Reuter [1], D. Schröder [6], V. Schulz-von der Gathen [6], M. M. Turner [7], E. Wagenaars [4], K. Wende [1], A. West [4], and Th. von Woedtke [1]

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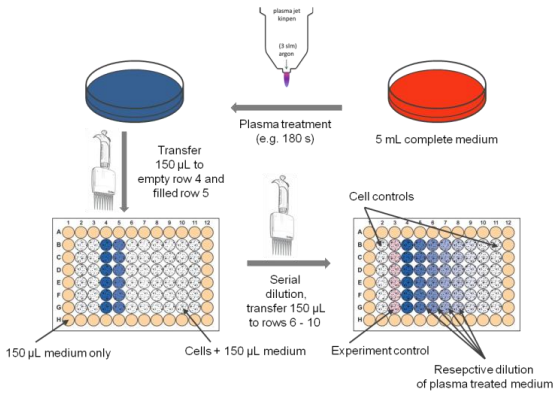
During the last decade the interests and efforts in Plasma Medicine increased with time. Since today there are several leading institutes developed their own plasma sources as well as treatment protocols for biological experiments. Due to the different sources including various feed gases there are dozens of physical parameters which differ with each set-up. In addition also the interest to introduce non-thermal plasma to micro- and cell -biology led to numerous experimental variations. Within the frame of the EU COST Action MP1101 "Biomedical Applications of Atmospheric Pressure Plasma Technology" a reference plasma source and associated protocols, along with basic calibration procedures have been developed. Progress of this initiative will be presented.
While engineers and physicists are working on the harmonization of physical parameters also biological benchmarks needs to be developed and adapted. Based on first discussions the least common denominator was the usage of 3T3 fibroblasts as a reference for eukaryotic cells; *Staphylococcus aureus* as a reference microorganisms, as well as spores from *Bacillus subtilis*. This also includes the usage of identical liquids and culture media, since those cellular environments heavily impact the results of the plasma treated organisms and cells. As a standard assay to assess the impact of a plasma source on mammalian cell systems the alamar blue assay was proposed. It allows the determination of the biological relevance of a plasma source or its parameter variation.




Here we present first data gained from a cell biological standard assay which tests the vitality of the reference 3T3 mouse fibroblasts with different eukaryotic cells - all treated with either the helium based atmospheric pressure plasma reference source or the kinpen, an argon plasma jet. These first results show that this test system is easy to handle and works stable independent of the laboratory. Furthermore, the experiments also revealed that both plasma sources display differences concerning their effects on the tested cells with respect to treatment times. But also due to different admixtures to the feed gas, we could proof that this test system is able to detect variations in the biological read out system.

Therefore, these biological assays are indeed helpful in order to define certain plasma parameters and to improve non-thermal plasma treatment of living cells. These first tests also showed that the need for biological as well as physical standardizations within the plasma medicine community can be solved with a reference plasma source and common basics in biology. This will help to compare the experimental findings of different groups with each other. The developed protocols and agreements will help to proceed with a more detailed research of each participating laboratory with their own sources and cells – while comparing to the references.

**The authors acknowledge EU COST Action MP 1101.**

# Introduction to the EU COST Action TD1208 - Electrical discharges with liquids for future applications

*Mirosław Dors, Bill Graham, Felipe Iza, Frantisek Krcma, Petr Lukes, Dragana Maric, Davide Mariotti, Stefan Matejcik, Cristina Paradisi, Stephan Reuter, Antoine Rousseau, Susana Sério Venceslau*

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This presentation will introduce the newly formed EU-COST action for building international networks on the topic of electrical discharges with liquids for future applications [1].
Plasmas generated in liquids or gas discharges interacting with liquids are now one of the recent frontier topics in plasma physics and plasma chemistry research with a broad field of potential applications. Plasma-liquid systems can create strongly non-equilibrium environments often with gas temperatures close to or at room temperature. They provide an ideal basis for developing novel chemistries and related technologies. The aim of this Action is to bring together a high level of experimental, simulation and theoretical expertise available around Europe in order to improve the knowledge of basic processes responsible for initiating and sustaining discharges in/on liquids and as well as to facilitate coordination and interdisciplinary exchange of knowledge and know-how between researchers from different scientific fields and countries in the field of electrical discharge plasmas in contact with liquids.
The COST Action chaired by F. Krcma (CZ) and B. Graham (UK) includes four work groups (for contact details see COST Action website www.cost-plasma-liquids.eu)

WG1 Plasmas generated directly in the liquid phase. (A. Rousseau, FR) (fig 1 a)
WG2 Atmospheric plasmas interacting with liquids. (P. Lukes, CZ) (fig 1 b)
WG3 Elementary physical and chemical processes initiated by discharges. (D. Maric, RS)
WG4 Interaction of plasma reactive species with materials and surfaces. (S. Reuter, GE)
with the sub WGs:

- WG4-1 Water treatment (M. Dors, PL)
- WG4-2 Bio applications (S. Reuter, GE) (fig 1 c)
- WG4-3 Nano and solid surface applications (D. Mariotti, UK) (fig 1 d)
- WG4-4 Applications in organic chemistry (F. Iza, UK)

Apart from the short-term scientific missions, which foster the exchange between the Actions members, two workshops and one training school have been held so far. Further Workshops are planned and a strong link to the world-wide plasma and liquid and their applications community is sought (see website for details).

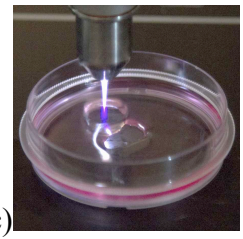
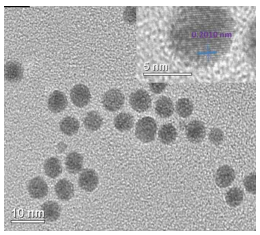
a)  b)  c)  d) 

**Figure 1:** *a) Plasma in liquid (École Polytechnique), b) above liquids (IPP AS CR, Prague), c) for biomedical applications (INP Greifswald) or d) for nanoparticle generations (Univ. of Ulster, UK)*

# Behaviors of Atmospheric-Pressure Discharge and its Interaction with Soft Materials as a Basis for Plasma Medicine

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Applications of atmospheric-pressure plasmas for medical treatments have attracted great attentions due to remarkable effects in medical treatment including cancer and thus have been extensively investigated worldwide as a new scientific field "plasma medicine" [1]. For development of innovative plasma technologies, it is of great significance to study basic characteristics of atmospheric-pressure discharge as well as fundamental processes involved in plasma interactions with biological molecules. In the plasma interactions with biological molecules, it is essential to be noted that the biological molecules are composed of organic materials (soft materials), of which the bond-dissociation energies are typically less than 10 eV.

Our research group has carried out a series of investigations on plasma interactions with soft materials on the basis of surface analyses using X-ray photoelectron spectroscopy (XPS) [2,3].

In this presentation, these studies are extended further to investigate the behaviors of atmospheric-pressure plasmas and thier interactions with soft materials in air and liquid for development of advanced plasma technologies, which are suitable for plasma medicine. Characteristics of atmospheric-pressure plasmas have been examined in terms of the discharge power source of high-voltage DC pulse to RF and UHF for investigations of frequency dependence on gas-breakdown properties. Dynamic behaviors of the plasma bullet from dielectric-barrier discharge plasma jet have been investigated using an intensified CCD (ICCD) camera, as shown in Fig. 1, in which O 777 nm spectrum line was selectively detected through optical band-pass filter.

Furthermore, the atmospheric-pressure plasma interactions with soft materials have been studied to understand the fundamental effects of plasma exposure on the modification of the molecular structure, which is considered to be essential in plasma medicine.

Acknowledgements
This work has been carried out partly under the auspices of The Grant-in-Aid for Scientific Research on Innovative Areas “Plasma Medical Innovation”(24108003) from The Ministry of Education, Culture, Sports, Science and Technology (MEXT).

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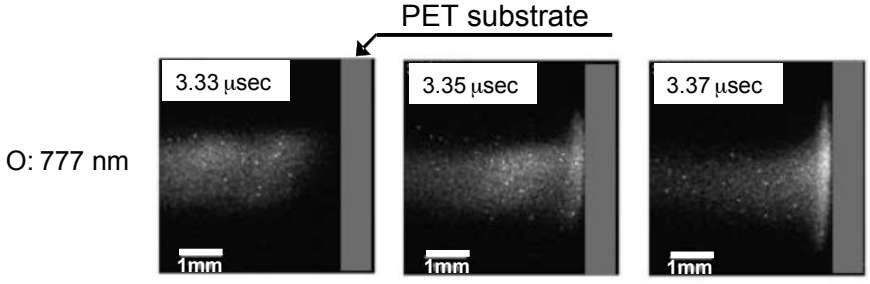


**Figure 1:** *ICCD images of plasma bullet impinging on PET substrate.*

# Non-thermal Atmospheric Plasmas in Dental Restoration: Improved Resin Adhesive Penetration

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Surface treatment by plasmas is a potential option that represents a process of changing surface energy of different materials and leads to an improvement of surface bonding characteristics. Recently studies [1-4] have demonstrated that non-thermal plasma treatment could improve the bonding strength of restorative composites to dentin. Nevertheless, more detailed mechanism of the bonding improvement, especially with regard to the influence of plasmas on the interfacial region, has not been understood yet. The purpose of this study is to investigate the influence of non-thermal plasma treatment on the penetration of a model dental adhesive into the demineralized dentin.

Prepared dentin surfaces were conditioned with etchant, randomly selected for treatment with an argon plasma brush [3-4] or gentle argon air blowing (as control). The plasma-treated specimens and control specimens were applied with a model adhesive containing 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy) phenyl]-propane (BisGMA) and 2-hydroxyethyl methacrylate (HEMA). Cross-sectional specimens were characterized using micro-Raman spectral mapping across the dentin, adhesive/dentin interface, and adhesive layer at 1~um spatial resolution. SEM was employed to examine the interfacial morphology.

The micro-Raman result disclosed that plasma treatment significantly improved the penetration of the adhesive, evidenced by the apparently higher content of the adhesive at the adhesive/dentin interface as compared to the control. Specifically, the improvement of the adhesive penetration using plasma technique was achieved by dramatically enhancing the penetration of hydrophilic monomer (HEMA), while maintaining the penetration of hydrophobic monomer (BisGMA). Morphological observation at the adhesive/dentin interface using SEM also confirmed the improved adhesive penetration. The results further suggested that plasma treatment could benefit polymerization of the adhesive, especially in the interface region.

The significant role of the non-thermal plasma brush in improving the adhesive penetration into demineralized dentin has been demonstrated. The results obtained may offer a better prospect of using plasma in dental restoration to optimize adhesion between tooth substrate and restorative materials.

This work was supported by US National Institute of Health (NIH) R01-DE021431.

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# A biological "tissue model" to study the plasma delivery of reactive oxygen species

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In this presentation I will discuss the utility of a biological "tissue model" to monitor the delivery of plasma jet-generated reactive oxygen species (ROS). Helium plasma jet interactions both across the surface and into the subsurface (defined as 150 μm to 1.5 mm) of the tissue model are investigated. The model comprises a gelatin gel encapsulating a homogeneously dispersed chemical or biological reporter molecule. Jet-surface interactions result in (i) star shaped patterns that resemble those previously reported for surface-plasma streamers on insulators (as imaged by Pockels sensing) and (ii) "filled" or hollow circular surface features, which resemble the "killing" patterns seen in plasma jet treatments of bacterial lawns.

The use of reporter molecules show that plasma can deliver ROS from 150 μm to 1.5 mm below the tissue surface. Subsurface delivery of ROS is consistent with the use of plasma to decontaminate wounds (covered by wound exudate and clotted blood), the deactivation of whole biofilms, plasma-enhanced drug delivery through skin and the destruction of solid tumours.

From the data presented, we argue that in these four cases (and others) ROS may be capable of directly accessing a tissue's subsurface, as opposed to other proposed mechanisms, which involve stimulating surface reactions that trigger a cascade of biomolecular signalling events (into the tissue).

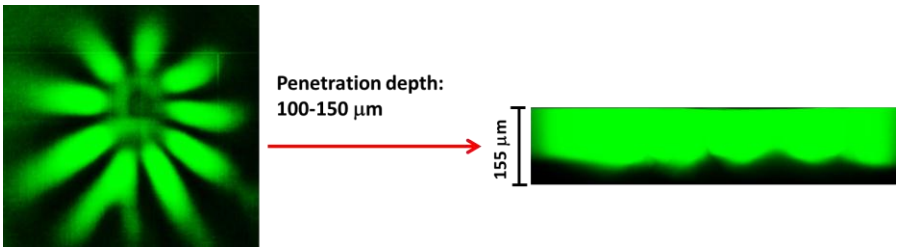


**Figure 1:** *Helium plasma jet delivery of ROS into the biological tissue model. ROS is detected in the material by the green fluorescence. The spatial distribution of ROS on the tissue surface resembles a star shaped pattern (left fluorescence micrograph) and ROS penetrates the material to a depth of 155 μm (right confocal micrograph).*

# Simulation Study of Virus Concentration Using Plasma-functionalized Graphite-encapsulated Magnetic Nanoparticles with Biotin-Avidin System

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In the present study, we prepared the graphite-encapsulated magnetic nanoparticles by using a DC arc discharge method and developed the plasma processing technology to modify the surface properties of nanoparticles for bio-medical applications. The primary amino groups grafted after Ar plasma pre-treatment followed by $NH_3$ plasma post-treatment appeared to play an important role to introduce the functional groups onto the surface of nanoparticles.[1-2] Then, we analyzed the population of amino groups introduce onto the nanoparticles using the conventional chemical approaches. From the analysis, we found that the population of amino groups was evaluated as roughly $5\sim7x10^4$ molecules per nanoparticle. The surface structure analysis by transmission electron microscopy indicated that no significant damages were found on the structural and morphological of the treated nanoparticles, denoting that the present technique is applicable for high-efficiency surface modification of the magnetic nanoparticles. For aiming at developing the feasibility of the selective detection of virus, we have carried out the immobilization of the antibody(C111) of influenza virus onto the surface of aminated magnetic nanoparticles, as shown in Fig. 1. We confirmed a significant enhancement of collection rate of H1N1 type influenza virus by magnetic collection of antibody-immobilized magnetic nanoparticles, as shown in Fig. 2. The present result suggests the possibility to use this method for selective influenza virus detection.

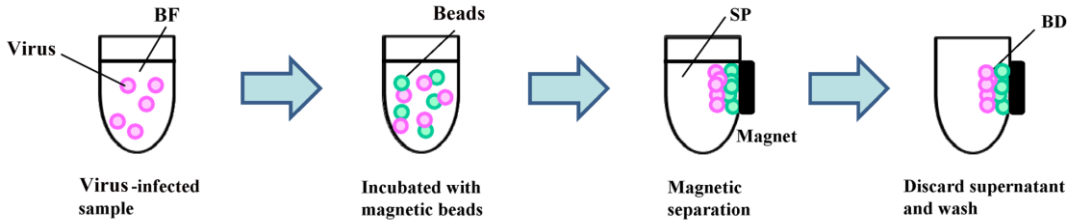


Fig. 1 Procedure of magnetic collection of influenza virus by antibody-immobilized magnetic nanoparticles(MNPs).

To simulate the virus concentration experiment, we employed the biotin-avidin system in place of antibody and antigen reaction. The detail of results will be presented at the conference.

This work was supported in part by a Grant-in-Aid for Scientific Research (Grant No. 2110010) from the JSPS.

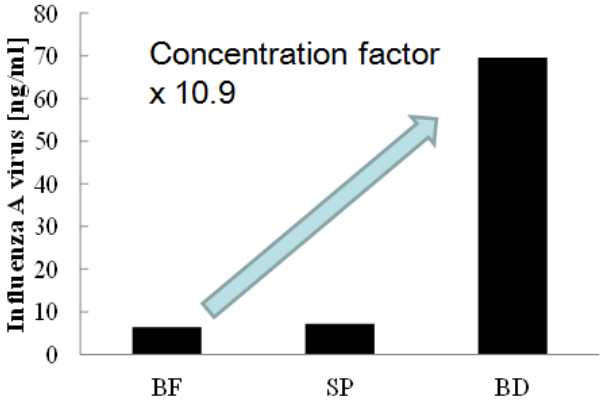


Fig. 2 Results of virus condensation for MNPs using C111 antibody.

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# Biodegradable copolymer coatings deposited by low pressure plasma polymerization for controlled drug delivery – first *in vivo* results

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In our recently published work [1],[2] we have demonstrated the possibilities to utilize catalyst and solvent free chemical synthesis for the preparation of multilayer, nano thick, biodegradable and biocompatible PCL-PEG copolymer coatings on different substrates by simultaneously introducing ε-caprolactone (ε-CL) and diethylene glycol dimethyl ether (DEGME) precursor vapours in an RF plasma reactor.

In this work, the same low pressure plasma process was used to deposit biocompatible and biodegradable copolymer coatings with tailored surface properties on biocompatible implants loaded with an anticancer drug; *cisplatin*. The functionalities of the plasma coating are i) to control the cell adhesion on the implant, ii) the control the drug release rate. The drug loaded coatings were implanted in 28 mice for duration of 3 months and the small animals were sacrificed (see fig 1.). No inflammation was observed on the tissues exposed to the plasma coatings. Moreover, whereas a conventional chemotherapy treatment with *cisplatin* leads to renal injuries in 25%-30% of the patients [3], no injury was observed in this study which indicates that the anticancer drug was released at a slow and controlled rate.

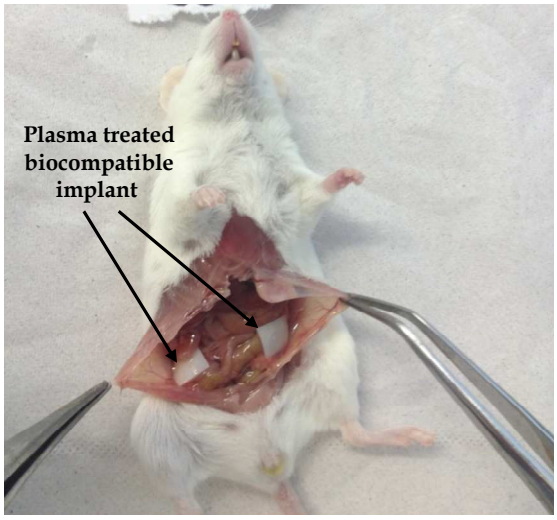


**Figure 1:** *Intraperitoneal biodegradable plasma treated implant in mice removed after 3 month. No inflammation was observed in the tissue exposed to the plasma coatings.*

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# Plasma-based stimulation of biotechnological processes in medicinal mushroom mycelia

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In biotechnological processes, high-yielding production strains are the condition for economic production of biomass and desired metabolites. High-yielding strains are generated by a combination of mutagenesis and selection using genetic engineering, X-ray, gamma-ray, UV ray or radical forming chemicals. The plasma glow contains components as UV radiation and highly reactive radicals which could possibly work synergistically in generation and improvement of production strains. There are few investigations demonstrating the efficiency of atmospheric-pressure plasma for breeding high-yielding bacterial strains. Till now such approaches have been limited on prokaryotic cells [1]. An interesting candidate for improving metabolite production is the medicinal mushroom *Ganoderma lucidum*, the famous Reishi mushroom. In Japan and other Asian countries *G. lucidum* is used for prevention and treatment of many different diseases. Meanwhile it has also got increasing importance in the Western hemisphere. Mycelia and fruit bodies contain about 150 structurally different triterpens, e.g. ganoderic acids and immunomodulating polysaccharids [2]. The aim of our investigation was to check for the first time if plasma influences growth and productivity of mycelial cultures of *G. lucidum* as an example for eukaryotic cells.

Mycelial cultures of the fungus were exposed to plasma [plasma jet, surface and volume DBD (S-DBD, V-DBD)]. Treatment times varied from 2 to 20 min. After cultivation mycelial biomass was separated from the culture medium, lyophilized and extracted by different solvents. Yield of biomass and of extracts was determined. To get an overview of chemical composition HPLC finger prints of organic extracts were taken. Dichloromethane extracts were further analyzed for content of whole triterpens by chemical methods as well as for quantity of ganoderic acid A and ergosterol by HPLC. The β-1-3 D-glucan content was determined in hot aqueous extracts by specific immunological and enzymatic methods.

Treatment of the mycelial cultures with plasma resulted in positive effects on extract yield (V-DBD), content and spectrum of some metabolites. V-DBD increased β-1-3 D-glucan while whole triterpen content was increased by the plasma jet. Both dielectric barrier discharge plasma sources, S-DBD and V-DBD, led to elevated ergosterol contents. All effects were dependent on the treatment time, plasma equipment, operating gas and treatment conditions.

These first results show that plasma could be principally useful for optimization of biotechnological processes not for only prokaryotic but also eukaryotic cells. Further investigations with other organisms and to get more insight into mechanisms are in progress.

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**Acknowledgement:**
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# Electrically-driven micro-bubbles assisted protein crystallization

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We have succeeded in protein crystallization by electrically-driven mono-dispersed micro-bubbles. The dispensed directional micro-bubbles whose air-liquid interface tends to combine the ambient molecules due to by their stiction force on the surface of bubbles. These characteristics enable to agglomerate the protein molecule which brings to successful protein crystallization in the low concentrate protein solution.

One of the conventional protein crystallization methods is hanging drop method and sitting drop method which use supersaturation of the protein solution and classified as vapor diffusion [1][2]. However the method tends to time consuming and it tend to produce good quality of the protein crystal. Figure 1(a) shows the proposed new method of protein crystallization. The electrically driven micro-bubbles were dispensed and coated with protein molecules by their stiction force on the surface of bubbles. Next, micro-bubble shrank and protein concentration on the bubble was increased because superficial area of the bubble was decreased. Finally, at the time of reaching protein concentration to supersaturation of the protein solution, protein crystal core was generated. This phenomenon can be occurred in low concentration of protein solution and this is important to produce good quality of the protein crystal. Figure 1(b)(c) shows experimental result of protein crystallization. A large number of protein crystals were produced in protein solution compared to the control condition within 1hour. We confirmed that electrically-driven bubble knife promoted protein crystallization.

The present novel method of protein crystallization induced by electrically driven bubble knife has many advantages over cost and time and efficiency. The expansion of the current research will contribute to the general organic crystallization in many fields of researches.

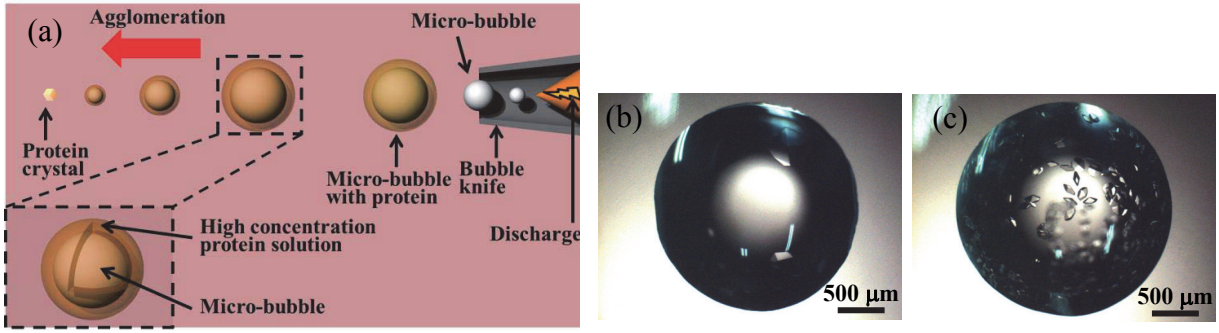


**Figure 1:** *(a) Concept of the protein crystallization assisted by electrically driven bubble knife, (b) protein crystallization by conventional hanging drop methods, (c) protein crystallization assisted with electrically driven micro-bubbles*

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# Measurement of reactive oxygen species in plasma-treated water

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Reactive oxygen species (ROSs) generated by plasma play an important role in inactivation of biomolecules. These radicals induce damage to DNA, lipid, and proteins. Therefore, analysis of damage on large DNA molecule in aqueous solution induced by non-thermal atmospheric pressure plasma jet (APPJ) has been studied in our previous research [1]. Here we measured the intensity of ROSs produced by APPJ using electron spin resonance (ESR) spectroscopy with spin trapping technique to assay the ROSs effects on the DNA strand breaks.

The APPJ generator consists with a glass tube, a stainless steel wire as a high voltage electrode, and a stainless steel mesh as a ground electrode. It was placed in an acrylic chamber. Dielectric barrier discharge was generated using a pulse power supply, and extended from the tip of the glass tube by flow of argon gas. DMPO and BMPO were used as a spin trapping agent, and DTPA was used as a chelating agent for metal ions. After exposing the APPJ or gas to the spin trap solution, it was immediately measured by ESR.

Hydroxyl (OH) radical spin adducts was observed in all samples. The highest signal intensity of both OH radicals and super oxide radicals were observed in the sample placed under the APPJ. OH radical spin adducts was also observed in other samples. They were located at places not to be affected by APPJ directly. This indicates that ozone or some other reactive radicals generated by the APPJ produce OH radicals in aqueous solution. However their signal intensity was significantly lower than the signal in the sample, which was placed under the APPJ. Therefore, exposing APPJ to liquid surface produce and confine OH radicals in the liquid efficiently.

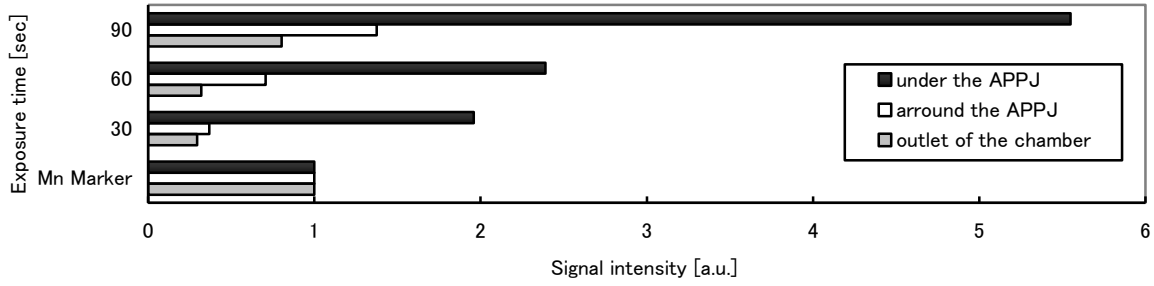


**Figure 1:** *BMPO-OH spin adduct signal intensity*

Acknowledgements
The work was partly supported by Grant-in-Aid for Scientific Research on Innovative Areas "Plasma Medical Innovation" form the Ministry of Education, culture, Sports, science and Technology (MEXT), Japan.

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# Tracking plasma generated $H_2O_2$ from gas into liquid phase and revealing its dominant effect on human skin cells

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It is long known that hydrogen peroxide ($H_2O_2$) is an important agent for influencing biological systems. Also in the field of plasma medicine its dominant role was emphazised by several groups [1-3]. In this work the complete pathway of $H_2O_2$ from the generation in the gas phase by an atmospheric pressure argon plasma jet, to its transition into the liquid phase and finally to its restraining effect on human skin cells is investigated for different feed gas humidity settings. Gas phase analytics like Fourier transformed infrared (FTIR) spectroscopy and laser induced fluorescence (LIF) spectroscopy on ˙OH are combined with liquid diagnostics such as chemical assays and electron paramagentic resonance (EPR) spectroscopy. Furthermore, the viability of human skin cells is measured by Alamar Blue assay.

By comparing the gas phase results with chemical simulations in the far field $H_2O_2$ generation and destruction processes are clearly identified. Amazingly, the production rate of $H_2O_2$ in the gas phase is almost identical to the $H_2O_2$ production rate in the liquid phase (figure 1). This indicates that dissolution of gas phase $H_2O_2$ is the major production mechanism in the liquid phase. Furthermore, it is shown that $H_2O_2$ concentration corralates remarkably well with the cell viability. Other species in the liquid like ˙OH or $O_2^{\cdot-}$ as well as the pH-value do not show this correlation.

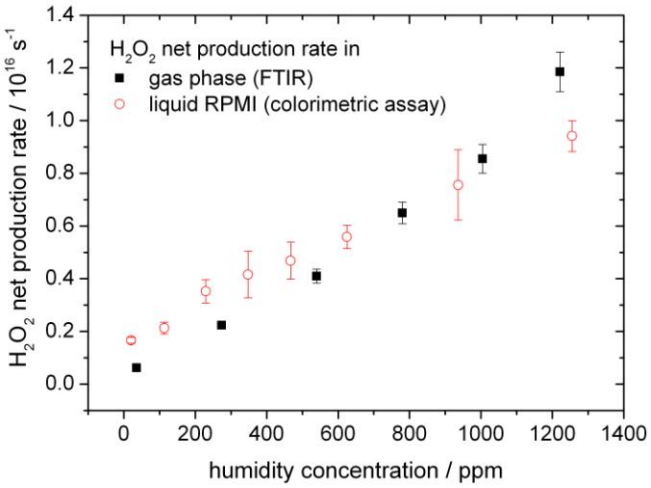


**Figure 1:** $H_2O_2$ production rate is identical in the gas and in the liquid phase

The Authors acknowledge the funding by the BMBF (03Z2DN12).

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# Degradation of DNA and Proteins Induced by Microplasma Jets

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In recent years, atmospheric pressure microplasma jets have attracted much interest because of the possibility of propagating non-thermal plasmas in open air. As so, reactive plasma species (e.g. radicals, positive or negative ions, electrons, UV radiation), and not only long-living afterglow species, can be delivered, at ambient pressure and temperature, to targets located some centimeters away from the main discharge zone. This property opens up a wide range of new and fascinating possibilities including, among others, decontamination and biomedical applications (odontology, dermatology, cancer research,…). To get a better insight into the biomedical effects of plasma jets, fundamental studies on the interaction of the plasmas with biological macromolecules are essential. In this context, we have exposed solutions of plasmid DNA and proteins to a microplasma jet. The studied plasma is created by a dielectric barrier discharge (DBD) with an axial symmetry [1]. Pure helium flows through the inner electrode at a flow rate in the range 50–1000 $cm^3$/min. Experiments have also been performed with low $O_2$ or $N_2$ admixture (<3%). High voltage pulses (3–10 kV) are applied between the electrodes at a repetition rate frequency of 20 kHz. The microplasma jet is set up vertically with the gas flowing downwards for interaction with solutions of plasmid DNA and proteins placed inside Eppendorf tubes. The plasma propagates through a capillary tube, and either the plasma or its gaseous effluent enters the biological solutions with no admixture of the surrounding air. The damages to the DNA (oxidized bases, strand breaks, abasic sites) have been analyzed through specific enzymes (Fpg, Nth and Ape1) by agarose gel electrophoresis, while the damages to proteins (in our study Bovine Serum Albumin or BSA) have been analyzed by SDS-polyacrylamide gel electrophoresis, UV absorbance and mass spectrometry. The degradation of plasmid DNA and BSA has been studied as a function of the applied voltage, gas mixture and flow, and exposure time. In parallel, the microplasma jet has been extensively characterized using a wide range of diagnostics, including laser and fast imaging techniques, and time and spatial resolved spectroscopy. In particular, absolute densities of singlet delta oxygen ($O_2(a^1\Delta_g)$) and ozone ($O_3$) molecules have been measured in the flowing effluent of the microplasma jet by infrared optical emission spectroscopy [2] and UV absorption spectroscopy [3], respectively. The influence of different parameters, such as gas flow and mixture, and power coupled to the plasmas, on the production of these reactive oxygen species by the microplasma jet has been investigated. The control of the operating conditions of the microplasma jet enables the tailoring of the reactive species composition of its effluent. This provides the scope to correlate the damages induced on DNA and proteins to the presence of specific reactive species produced by the microplasma jet. This will, eventually, allow to tune the microplasma jet for desired applications in biomedicine.

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# Minimally-Invasive Gene Transfection Using Atmospheric Pressure Plasma

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Gene transfection is expected to play an important role in molecular biology and the medical treatment such as gene therapy and creation of induced pluripotent stem (iPS) cells. However, the conventional gene transfection methods, such as a lipofection, an electroporation, and a viral vector method, have some problems that the survival fraction is low and the genes cannot be transferred into some specific lipid cells. On the other hand, recently, gene transfer using discharge plasma has attracted attention [1]. However, the mechanism of the gene transfection using the plasma is not clarified and there is no progress in the method in recent years. Therefore, we try to use controlled atmospheric pressure plasma and investigate the mechanism toward developing highly-efficient and minimally-invasive transfection [2].

Schematic of an experimental setup is shown in Fig. 1(a). We generate atmospheric pressure plasma using low frequency (LF) (frequency: 10 kHz, voltage: $V_{p\text{-}p}$ kV) with He gas flow, which is irradiated to the living cells covered with genes. In this experiment, preliminarily, we use fluorescent YOYO-1 ($c$ μM) instead of the genes and use LIVE/DEAD Stain for cell viability assay, and we simultaneously observed their fluorescence. By the fluorescence image, we defined the transfection efficiency η and viability as the ratio of the number of transferred and surviving cells to total cell count, respectively.

Figure 1 shows (b) the transfection efficiency **η** and (c) cell viability as a function of plasma irradiation time $t_d$, where the diffusion distance $d$ is 35 mm and 73 mm for $V_{p\text{-}p}$ = 7.8 kV, $c$ = 5μM. It is clarified that the transfection efficiency is strongly dependent on the plasma irradiation time and the diffusion distance with keeping high cell viability. This result indicates effects of charged particles are increased by using short diffusion distance.

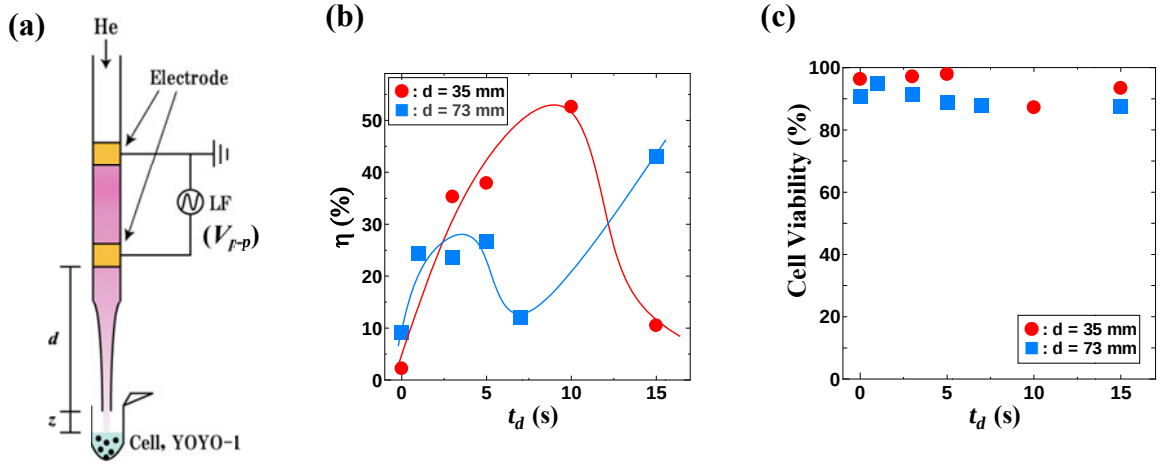


Fig. 1: (a) Schematic of an experimental setup for atmospheric pressure plasma irradiation, (b) the transfection efficiency **η** (%) and (c) cell viability (%) as a function of plasma irradiation time with the diffusion distance $d$ as a parameter for $V_{p\text{-}p}$ = 7.8 kV, $c$ = 5μM, $z$ = 5mm.

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# Differential protein expression and thiol oxidation pattern in human keratinocytes in response to non-thermal plasma to reveal activation route

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The functionality of non-thermal atmospheric pressure plasmas opens exiting potentials in the field of plasma medicine e.g. in wound management. Plasma generated species like reactive oxygen and nitrogen species (ROS/RNS) are thought to be major players by influencing cellular redox balance and cell physiology [1]. To identify the route of cell activation, human keratinocytes, lymphocytes and monocytes were treated using an argon plasma jet (kINPen). Protein expression and phosphorylation were determined using label free high resolution mass spectrometry, 2D gel electrophoresis or by western blotting technique.

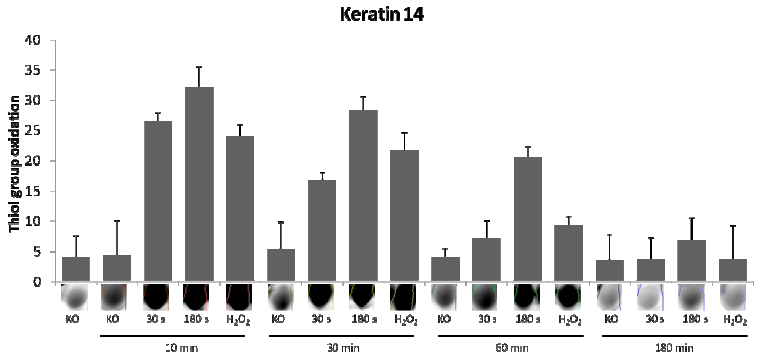


Figure 1: Thiol oxidation of intracellular keratin 14 after plasma returns to control within 3h

Protein expression was changed depending on cell type, treatment time and incubation after treatment. After classification of regulated proteins, prominent changes were found for proteins involved in metabolism, transport processes and membrane bound proteins. The oxidative stress response like nuclear factor erythroid 2-related factor 2 (Nrf2) related protein expression was activated in all cell types, indicating changes in cellular redox homeostasis. While a major chemical modification of proteins was not observed, redox-sensitive labeling of cellular proteins confirmed an immediate and reversible oxidation of thiol groups (Fig. 1), depending on treatment intensity. ROS/RNS signaling was also reflected by a time dependent activation of mitogen-activated protein kinases (p-38 MAPK, ERK) and cell fate regulatory proteins (p53, Chk1).
These results show that plasma treatment triggers a multiple responses in mammalian cells by the direct or indirect action of ROS/RNS including the activation of the natural antioxidant system.

The Authors acknowledge the funding by the BMBF (03Z2DN11).

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# A genome-wide profiling of response genes in eukaryotic cells to non-thermal atmospheric pressure plasma treatment

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Non-thermal atmospheric pressure plasma has been proved promising in many biomedical applications, such as decontamination, cancer inhibition and wound healing. However, the cell response mechanisms during these processes are far from clear. This is due to the complexity of both the plasma and biology system. Plasma has multi dimensional parameters including discharging modes, discharging gases, and treatment time. Cells on the other hand can response in multiple organelles such as cell membrane, mitochondrion, and endoplasmic reticulum, and with different pathways such as phosphorylation or ubiquitination. It is very important to look into them with a global view.

Here we report a genome-wide profiling of the cell responses to different parameters of plasma using a beneficial tool of the model organism budding yeast, the single deletion mutants collection [1]. The collection consists of 5153 different mutant strains, each strain having one ORF gene deleted. The 5153 strains were pool-cultured as one sample, and subjected to plasma treatment. The fitness (survival ratio) of each strain was then determined by high-throughput sequencing of its specific Barcodes (up and down tags implanted when the deletion strains were constructed) [2].

Plasmas of 12 different parameters were applied to the collection samples, and the gene response patterns were compared in these parameter groups. The DC jet we used could both do discharge in water (DIW) and produce plasma-activated water (PAW) for treatment to cells. The profiling result represented distinct response patterns between the DIW and PAW group. In each group, treatment time and discharging gases worked together to build a similar response. The major response genes were from mitochondrion genome maintenance, vacuolar acidification, and protein transport. We are also comparing the fitness data in our result with other groups' previous drug screening data to see whether we can find similar patterns between our plasma treatment and existing drugs.

This work can provide necessary information for a safer and better-understood biomedical application of non-thermal plasma.

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# Effects of microwave argon plasma on cell-wall-lacking *Mollicutes* bacteria

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Bacteria of the *Mollicutes* class are the smallest free-living organisms. Some *Mollicutes* species are human, animal and plant pathogens, which inhabit the surface of eukaryotic cells. The intrinsic feature of the *Mollicutes* is lack of the peptidoglycan-containing cell wall typical for other bacteria. Membranes of the *Mollicutes* have a composition similar to the composition of eukaryotic membranes [1].

To establish the effectiveness of the non-thermal plasma (NTP) against *Mollicutes*, we applied it to *Mycoplasma hominis* and *Acholeplasma laidlawii*, which are among the most widely spread *Mollicutes*. The previously described MicroPlaSter β source of the microwave argon plasma [2] was used to treat nutritive agar plated *M. hominis* and *A. laidlawii* for 30-300 s. Dependence of bacterial resistance on the medium composition was studied. Input of UV and ROS was evaluated.

A dose-dependent bactericidal effect on tested species was shown. Still, the effect was less pronounced than for other bacterial species tested under similar conditions. So, the maximal 10- and 100-fold drop was observed for *A. laidlawii* and *M. hominis*, respectively. Similarly treated *E. coli* and *S. aureus* demonstrated the $10^5$ and$10^3$ drop, respectively. Cholesterol presented in the cultivation medium affected resistance of *A. laidlawii*. Addition of 10 mM antioxidant butylated hydroxytoluene decreased mortality by a factor of 25-200. Exogenously added hydrogen peroxide $H_2O_2$ did not cause mortality. UV radiation alone caused 25-85 % mortality in comparison with the whole NTP. NTP treatment of *M. hominis* triggered growth of microcolonies, which were several tens fold smaller than a typical colony. A mean diameter of cells from microcolonies was 98 ± 6 nm while the diameter of control *M. hominis* cells ranged from 206 to 1320 nm (the mean was 689 ± 319 nm; $p<0,005$).

Obtained results indicated a high degree of heterogeneity and adaptation of *Mollicutes*. Despite a lack of the cell wall, A. *laidlawii* and *M. hominis* were more resistant to argon microwave NTP than other tested bacteria. The membrane composition seemed to be important for the increased resistance to NTP.

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# Non-Thermal Plasma Promotes Apoptosis and Cell-Cycle Arrest in a Lymphoma Cell Line

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Beneath the complexity of every cancer lie a limited number of critical events that propel tumor cell and its progeny into uncontrolled expansion and invasion. One of these is deregulated cell proliferation, which, together with the suppression of apoptosis, supports further neoplastic progression. Targeting of these critical events should have potent and specific therapeutic consequences.

Recent progress in plasma generation has led to the possibility of sustaining at atmospheric pressure plasmas with temperature as low as room temperature. Atmospheric pressure cold plasmas, providing a blend of chemical and physical components such as reactive species, charged particles, UV radiation and electric field, are a promising technology for a wide range of medical therapies. Several studies have reported on the selectivity of plasma treatments and some pioneering works show that plasma exerts anti-tumor effects on different in vitro and in vivo models.

The cytotoxic impact of dielectric barrier discharge plasma generated in ambient air was studied at cellular, molecular, and genetic levels in a mouse lymphoma cell line (L5178Y). Cells were treated with plasma (30-120 sec) and analyzed 6, 24 or 48 h following treatment. Apoptotic and/or necrotic events, cell-cycle progression, and reactive oxygen species (ROS) formation were evaluated by flow cytometry. A complementary experimental approach was used to detect the primary DNA damage (histone phosphorylation), as opposed to irreversible mutational effects (micronucleus) possibly resulting from DNA damage.

Treatment of cells with plasma caused cell-cycle arrest and apoptosis. The early effect appeared as an increase in the percentage of cells in S phase, accompanied by a compensatory decrease in G1 cells. After a longer post-treatment time, plasma led to a marked increase of cells in the G2/M phase. Cell death was observed starting from 6 h post-treatment, where a high % of necrotic cells was recorded. After 24 and 48 h post-treatment, a significant fraction of apoptotic cells appeared and became the dominant cell death type. The early appearance of cell-cycle arrest compared to cell death clearly indicates that cytotoxicity is a primary direct effect of plasma and not a consequence of cell-cycle block. Plasma induced DNA damage and irreversible mutational events. Micronucleus frequency was weak when compared with that recorded for the positive control in almost all the experimental conditions. Of note, it was higher than that of positive control only when cells were treated with plasma for 120 sec. This implies the possibility to select plasma treatment conditions with a limited genotoxicity. The genotoxic effect can be dependent on ROS generation, observed after plasma treatment.

The results of this study will contribute to open up new pharmacological prospects for cancer therapy. Future applications of such understanding will provide a clear rationale for designing *in vivo* experiments and pave the way for pilot clinical studies.

# RNase A is Permanently Inactivated by a Dielectric Barrier Discharge by Chemical Modifications

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**Abstract**
Proteins are the major players in most cellular processes. They differ in sequence, size, form, and function. Recent investigations show that different proteins are affected differently by plasma treatment [1,2]. We investigated the influence of a dielectric barrier discharge (DBD) [3] on the enzyme RNase A, which can be used as a model for highly stable proteins such as RNases or prions. RNases are problematic when working with RNA in the laboratory due to their high stability and ability to efficiently cleave any RNA samples. Standard RNase A inactivation is performed using concentrated $H_2O_2$, toxic RNase inhibitors, or repeated autoclaving. RNase A inactivation by plasma treatment would be a fast and safe way gentle to the equipment.

RNase A samples were treated with the DBD either dried or dissolved in *A. dest.* and enzyme activity was monitored. Enzyme inactivation was observed under both treatment conditions, though inactivation was much faster for samples treated in solution. RNase A activity was reduced by about 40% when dried enzyme was subjected to 300 s DBD treatment or total inactivation was achieved when the enzyme was in solution. To investigate the molecular mechanisms of inactivation, circular dichroism and Raman spectroscopy as well as mass spectrometry were employed. It was evident that RNase A was unfolded by DBD treatment and samples treated in solution were unfolded faster than dried ones. RNase A is capable of refolding after heat denaturing [4], but no regeneration of structure or enzyme activity was observed incubating plasma-treated samples for 18 h. The RNase structure is stabilized by four structural disulfide bonds [4]. Raman spectroscopy and mass spectrometry revealed cleavage of disulfide bonds and formation of sulfonic acid by DBD treatment at the cysteines most relevant for enzyme structure. Over-oxidation of cysteines to sulfonic acid prevents re-formation of disulfide bonds, thereby preventing regeneration of enzyme activity. Plasma-induced breaking and over-oxidation of disulfide bonds is promising for further applications to terminally deactivate proteins with critical disulfide bonds like RNases or prions [5].

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# Cryopreservation of plasma treated water (PTW) for disinfection

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For disinfecting human bodies in dental and surgical applications, the inactivation of bacteria in body fluid by low-temperature atmospheric-pressure plasmas is essential. The reduced pH method was developed that strong bactericidal activity can be achieved if the solution is sufficiently acidic [1], and supplied $O_2^-$• into solution was confirmed to key active species. Although such usual disinfection experiment is with direct plasma exposure to bacteria suspension, we found that the plasma treated water (PTW) has strong bactericidal activity with the reduced pH method [2]. Pure water exposed to the plasma was incubated for given time period and mixed to bacteria suspension. Half lifetimes of bactericidal activity depends on temperatures and lower temperature brings longer lifetime. Half lifetimes were in accordance with Arrhenius equation both in liquid and solid states (Fig. 1). These results show that this bactericidal effect was not brought by ozone ($O_3$), hydrogen peroxide ($H_2O_2$), and/or nitrogen oxide (NOx), because they are stable species. From the experimental results of ESR (Electron Spin Resonance) measurement of $O_2^-$• [3] against PTW with spin trapping method at each temperatures, the activation energy is equal to that of inactivation experiment.

Based on cryopreservation concept, sequential production apparatus of high concentration PTW was newly developed with 1 meter glass tube and cooling system. Its bactericidal activity is so high that 22 log reduction (i.e. $10^{-22}$) of spore cell (*B. subtilis*) was achieved with 100% PTW (Fig. 2). The disinfection by cryopreservation of PTW is a novel method of plasma disinfection. In contrast, the half lifetime at body temperature is estimated to a few second and it seems ill effect to human body seems low. This type of indirect plasma exposure would bring effective and safety plasma disinfection, because the selected supply of active species is possible.

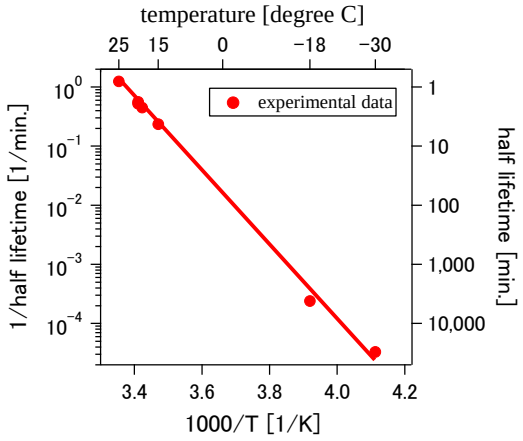


**Figure 1:** *Arrhenius plot for bactericid al activity of PTW.*

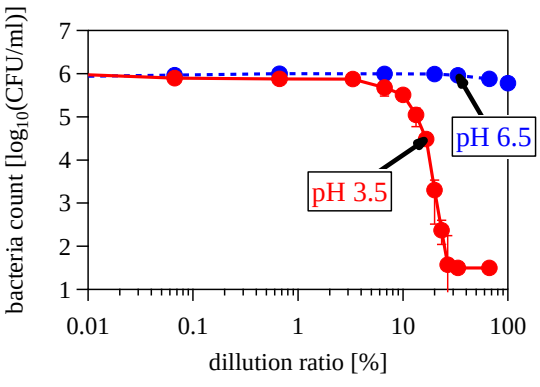


**Figure 2:** *Bactericidal activity (B. subt ilis) of diluted PTW with the reduced pH method.*

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# Modulation of Cell Activities by Changing the Plasma Composition

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Recent developments in plasma medicine indicated that it is possible to stimulate eukaryotic cells by applying non-thermal plasmas. However, there is the need to understand the processes of ROS /RNS effects inside the cells in order to find the balance between stimulating or killing the cells [1]. In the past much effort had been done by in order to control the plasma components and finally modulate biological activities [2]. Furthermore, it was shown before that a pure argon plasma treatment leads in a time dependent manner to an activation of the mitogen-activated protein kinases MEK, ERK and p-38 MAPK [3].
In this study we evaluated the influence of different feed gas admixtures as well as changes of ambient air surrounding the effluent of the plasma jet kinpen09 on the molecular processes of plasma treated human cells. Increasing feed gas humidity led to an elevation of $H_2O_2$ in the plasma treated liquids – and directly influenced cell viability [4]. However, here we show that $H_2O_2$ also stimulates the cells by accelerating the activation of signaling cascades such as the MAP kinase pathway. Therefore a controlled admixing of humidity to the feed gas represents one possibility to modulate cellular activities on a molecular level. But also by adjusting the oxygen to nitrogen gas composition surrounding the effluent results in a tunable modulation of cellular reactions. The resulting variation of ROS to RNS led to differentiated cellular reactions, such as a modulated secretion of growth factors like HB-EGF or cytokines like interleukin-6. These results show that changes in the plasma composition are modulating cellular responses with respect to signaling processes finally influencing cell survival, but also basic cell functions.

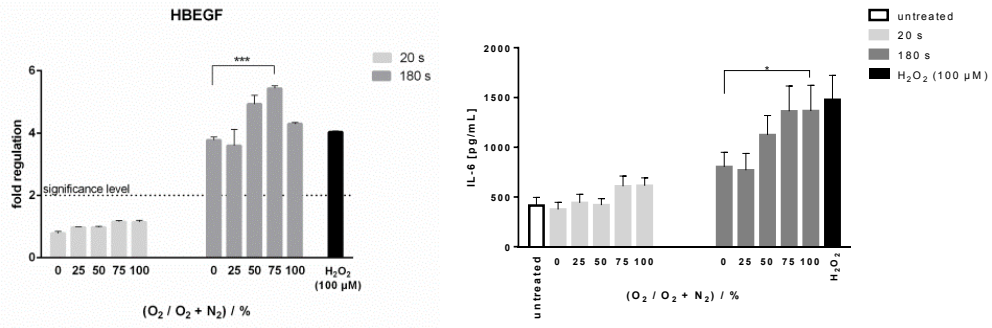


**Figure 1:** *Up-regulated gene expression of growth factor HBEGF (A), and increased IL-6 secretion (B) due to altered environmental conditions*

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# Selective Supply of Active Species using Plasma Treated Water (PTW) for Effective and Safety Disinfection

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Cosidering the medical applications of plasmas, which involve disinfection, wound healing, and so on, the supply of active species to solution is extremely important. For plasma disinfection, high bactericidal activity has been achieved in liquids via "the reduced pH method" where the solution is sufficiently acidic [1]. Superoxide anion radical ($O_2^-\cdot$) induced in acidic aqueous solutions (lower than critical pH 4.8) tends to capture a proton ($H^+$) to form hydroperoxy radical (HOO·), which shows considerably stronger bactericidal activity [2]. This result indicates that plasma-induced active species in liquid are crucial. Reactive oxygen species (ROS), produced by plasma jets from oxygen gas in ambient atmosphere, is divided into two groups: one is radical species like the above-mentioned $O_2^-\cdot$ and hydroxyl radical (OH·), and the other is non-radical species like singlet oxygen ($^1O_2$) and ozone ($O_3$). ROS is known to cause damages against nucleic acids, proteins, and lipids. For specific medical applications like plasma disinfection, it is necessary to supply desired active species and simultaneously avoid supplying unnecessary species to reduce unwanted affect to human body.

We investigated the formation of ROS including free radicals in water exposed to different types of contact or non-contact atmospheric-pressure helium plasma [6]. In contact plasma, all of those (relatively large amount of $^1O_2$) are induced in the liquid, whereas, in non-contact plasma, $O_2^-\cdot$ can be dominantly induced in the solution. Non-contact plasma is one of suitable methods supplying key species (i.e. selective supply) for the reduced pH method.

In addition, we investigated the formation of ROS including free radicals in plasma treated water (PTW), a pure water exposed to the plasma, because PTW also has strong bactericidal activity with the reduced pH method [7]. Its bactericidal activity is so high that 22 log reduction (i.e. $10^{-22}$) of spore cell (*B. subtilis*) is achieved. Half lifetime of bactericidal activity strongly depends on temperature (4 sec at 37ºC, several hours at 0ºC, more than several years at -85ºC) and lower temperature brings longer lifetime. These results show that this bactericidal effect is not caused by stable active species like $O_3$, hydrogen peroxide ($H_2O_2$), or nitrogen oxide ($NO_x$). ESR (electron spin resonance) measurements with spin trapping method and spin oxidation method show intense signal of $O_2^-\cdot$ and no signal of the others like $^1O_2$, $O_3$, or OH· in PTW. The activation energy of $O_2^-\cdot$ decay is concordant with that of bactericidal activity. Since high bactericidal activity via $O_2^-\cdot$ can be kept by cryopreservation and be deactivated soon at body temperature, PTW is another suitable method for plasma disinfection based on the reduced pH method.

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# Plasma therapy for large-scale wound treatments: development of a flexible plasma source

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Different types of cold atmospheric plasma sources are currently under investigation for biomedical applications. Some of them have already been testet in case studies or clinical trials, mostly being applied in dermatology. Meanwhile very few companies even succeded to gain medical device status for their plasma source. Clinical trials have shown very promising and impressive results concerning the ability of plasma therapies to stimulate wound healing [1]. However, due to their geomety most plasma sources only allow treatment of small wound areas at once. Large-scale wounds require to scan the area with the plasma source, which can become a time consuming and therefore costly process. This is an important issue in terms of plasma therapy acceptance by patients, doctors, clinic staff and of course reimbursement by insurance carriers.

Generally, dielectric barrier discharges are simply to use and easy to scale geometrically. However, due to usually firm and nonflexible carrier and isolator materials it's hard to cope with non-planar surfaces, e.g. large wounds on human extremities. This disadvantage can be avoided by using flexible materials for carrier, isolator and conductive structures [2]. Silicone-based polymers provide good dielectric properties and chemical stability as well as sufficient mechanical flexibility. For material selection, regulatory requirements in terms of biocompatibility and toxicology must be considered. It's even more important if a long-term application of the plasma source to a wound is being planned. Furthermore a risk analysis and assessment must be carried out.

In this contribution we present basic physical and risk assessment relevant properties of a flexible dielectric barrier discharge arrangement (Fig. 1). This includes the electrical characterisation (power consumption, patient leakage current), thermal properties, ultraviolet irradiance on the treated surface and concentration of reactive species. Additionally, selected results of material tests will be shown.

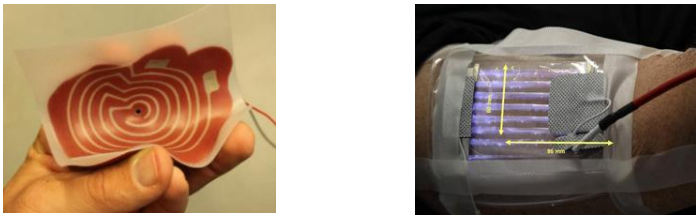

**Figure 1:** *Flexible dielectric barrier discharge arrangements*

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# Cold atmospheric plasma sources, plasma diagnostics and plasma factors at medical applications

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One of the directions of development in plasma medicine is a construction of various plasma sources and a search of their working regimes for decontamination of opportunistic pathogenic microflora living on tissue surfaces including mucous tunics and wounded surfaces of different etiologies as well as sterilization of abiotic surfaces. Various researches showed a high activity of cold atmospheric plasma (CAP) against wide range of causal organisms including pathogenic bacteria characterized by multiple antibiotic resistances. CAP is a flow of partially ionized gas under atmospheric pressure and environmental temperature. Biological action of CAP is determined by a cumulative effect of active components of plasma torch including photons, electrones, ions, exited moleculas and free radicals such as $NO_x$ and $O_x$. In our work we used several types of different plasma sources, including ferroelectric plasma reactor and also various sources of microwave plasma. Ferroelectric reactor is a device consisted of two grid electrodes, the area between of which is filled by dielectric grains with a high value of dielectric constant. After applying voltage to electrodes the grains get polarized. The external electric field generated by electric power supply is concentrated near connection points between the grains, where a great number of nanosecond pulsed discharges appear. The active radicals in this case are trapped by gas flow (argon or air) and are transported to a processed surface. For generating the plasma flow SHF power supplies was also utilized with a frequency 2.45 GHz and various shapes of electrodes and power from 50 to 200 W. As a plasma-supporting gas the argon of high purity (99,999%) was used as well as additional buffer gases such as $CO_2$, He and Air. The methods and results of CAP diagnostics were presented which allow analyzing the contribution of different plasma components. Comparison of the effects of different plasma factors for plasma sources in repetitively-pulsed and microwave discharges in regard to their influence on different causal eukaryotic and prokaryotic organisms is presented.

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# Single-cell-level Mobile Microplasma Jet For Cancer Cell Apoptosis

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The issue of single-cell-level control has recently attracted enormous interest which resulted in a very large number of top-level publications [1-3]. However, in spite of the presently achievable intracellular-level physiological probing through bio-photonics, nano-probe-based, and some other techniques, the issue of inducing selective, single-cell-precision apoptosis, without affecting neighbouring cells remains essentially open.

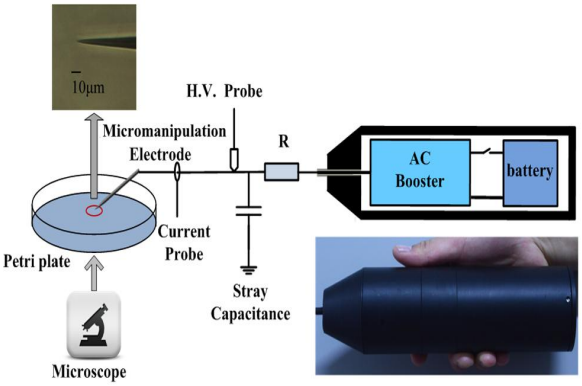


**Figure 1:** *Schematic of the microplasma jet setup and a sketch of the biomedical treatment*

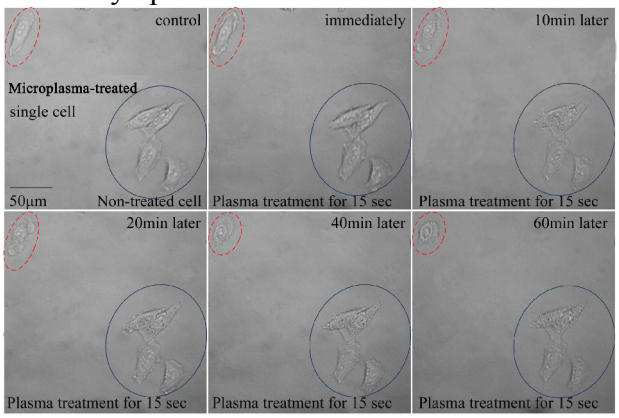


**Figure 2:** *Real-time monitoring of morphological changes at the single-cell level in HepG2 cell.*

Here we resolve this issue and report on the effective single-cell-precision cancer cell treatment using the reactive chemistry of the localized corona-type plasma discharge around a needle-like electrode with the spot size ~1 μm. When the electrode is positioned with the micrometer precision against a selected cell, a focused and highly-localized plasma discharge induces apoptosis in the selected HepG2 cancer cell only, without affecting any surrounding cells. This is confirmed by the real-time monitoring of the morphological and structural changes at the cellular and cell nucleus levels after the plasma exposure.The power delivered to the cell is very small (a few mW) yet sufficient to induce apoptosis selectively, without affecting neighboring cells. The plasma source is battery-operated and does not rely on any external power or gas supplies, which may be particularly useful in situations where external power supply is not available or device portability is an issue.

This advance may lead to next-generation single-cell-precision microsurgeries and may also lead to the step changes in the capability of addressable microarrays towards instantaneous inactivation of the as-detected malignant cells, where the needles in the arrays may be used as both the electrophysiological probes and the electrodes for the plasma generation.

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# Generation of micro plasma in water for biomedical applications

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Plasma in water has been studied in various fields such as water purification, sterilization, and material processing, since it is capable of generating reactive chemical species, shock waves, light emissions, electric fields and charged particles [1][2]. However, it is not suitable to apply to cell/tissues directly, because generation of plasma in water requires high voltage and it sometimes causes a spark which makes cells/tissue damage. Streamer in water is known as a much smaller discharge than a spark [3]. In this study, we focused on developing generation of a micro plasma in water such as a streamer.

Plasma was generated at a tip of needle electrode in a quartz cuvette filled with ultrapure water of 0.8 μS/cm. The polished tip curvature of the needle electrode was 40 μm and the voltage from 0 to 20 kV with 10 μs width was applied to the tip. A plate grounded electrode was set outside wall of the cuvette and the electrode gap was 10 mm. Plasma generation processes were visualized by shadowgraph at 100 Mfps with exposure time of 10 ns.

Figure 1 shows the typical pattern of a primary streamer during propagation. The primary streamer was developed spherically from the tip of the needle electrode and finally stopped propagation. The length of the developed streamer against the applied voltage is shown in Fig. 2. The applied voltage and the length of the streamer could be decreased to 7 kV and around 250 μm, respectively. In addition, a spark was not observed even at 25 kV. The micro plasma developed in this study can generate only a streamer which is safer and stable for biological applications.

This study was partly supported by a Grant-in-Aid for Scientific Research from JSPS. We acknowledge T. Nakajima and H. Fujita, IFS, Tohoku Univ. for discussion and support.

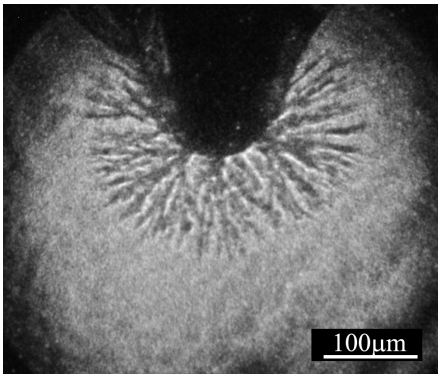


Fig. 1 Shadowgraph image of the primary streamer during propagation.

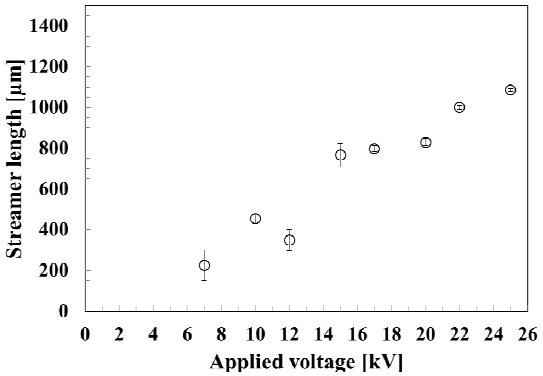


Fig. 2 Length of the developed primary streamer vs. applied voltage.

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# Propagation Difference of Atmospheric-pressure Helium Plasma jets Using Different Dielectric Materials

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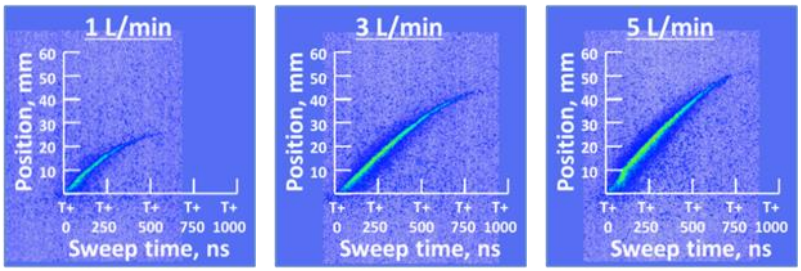
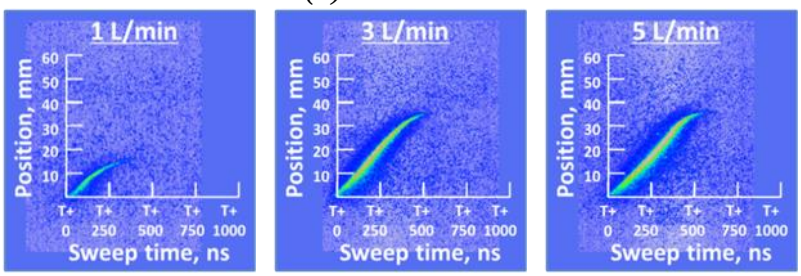
Atmospheric-pressure plasma jets (APPJs) have rapidly become widespread in the field of material modification and biomedical applications. These applications are based on various phenomena produced by the APPJs, such as UV light emission, electrons, ions, and radicals. In the past, it was reported that a plume-like emissive region in a centimeter order is elongated along a helium gas flow ejected from a glass tube into atmospheric air [1]. Furthermore, it was also reported that a bunched emission like a "plasma bullet" ejected from the dielectric tube exit is observed in the rise timing of applied voltage by an ICCD camera and it has a velocity of several tens of km/s, which is much faster than the gas flow speed [2]-[3]. However, the APPJs propagation comparison between different dielectric materials has not yet been adequately investigated and reported. The propagation difference of APPJs using glass and alumina tubes will be reported at the conference.




*(a) Glass Tube*




*(b) Alumina Tube*

**Figure 1:** *The positional dependence of the streak images of APPJs using (a) glass and (b) alumina tubes.*

**Acknowledgment:** This work was supported by JSPS KAKENHI Grant Number 25115.

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# Mass spectrometry of ions formed in atmospheric-pressure plasma jets

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A low-temperature atmospheric-pressure plasma (APP) has been studied widely in recent years. Due to its nature of low gas temperature, APPs are used for various medical applications such as sterilization and wound healing [1]. However, in many cases, the causes of the effects have remained unclear yet. For various technological applications of low-temperature APPs, a better understanding of plasma gas-phase reactions will facilitate the development of better controlled processes. In this study, to better understand the gas-phase reactions in ambient air by such plasmas, both negative and positive ions emitted from helium-based low-frequency (kHz) plasma jets were analyzed using mass spectrometry (MS).

The APP jet system consists of a glass tube, in which a He gas flows, and two brass electrodes wound around the glass tube [2]. The pulse frequency of power supply and the peak-to-peak voltage were in a range of 20–30 kHz and 3–9 kV, respectively. The He-gas flow rate was 1-3 l/min. MS of plasma jets was performed with a LC-mate double-focusing mass spectrometer (JEOL, Tokyo, Japan), which had three differential pumping stages [3]. The tip of the plasma jet was aligned with the center of the MS orifice, which was 130 μm in diameter. The distance between the plasma-jet tip and the orifice was 3 mm.

Figure 1 shows mass spectra of negative ions emitted from a He APP jet. It is seen that $COx^-$ ions (especially $CO_2^-$) are generated by the discharge. Negative ion water clusters $Y^-(H_2O)_n$ with core ions $Y^-$ being $OH^-$, $NO_3^-$, and $HO_2^-$ have been also observed. Mass spectra of positive ions will be discussed in detail.

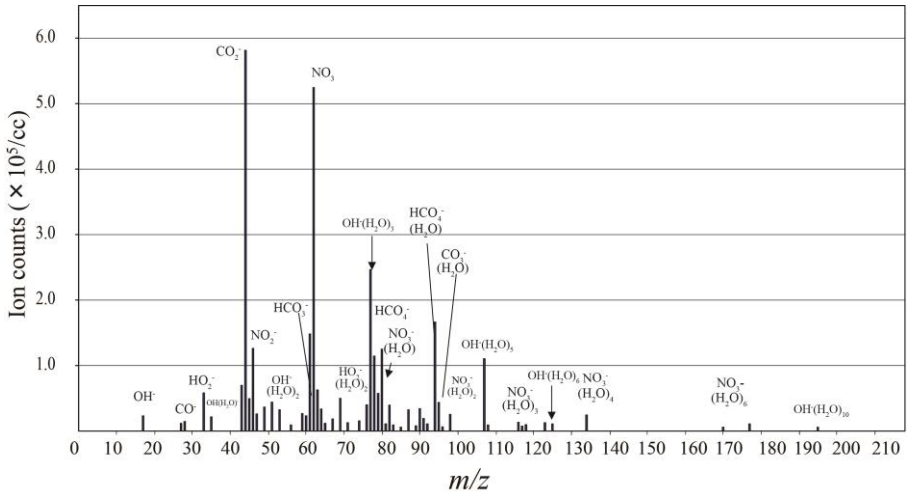


**Fig. 1.** *Mass spectrum of negative ions emitted from a He APP jet. The He gas flow rate was 3l/min and the peak-to-peak voltage Vpp was 9 kV.*

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# Atmospheric Pressure Dielectric Barrier Discharges in Air: Chemistry and Antimicrobial Effects

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Indirect atmospheric pressure air DBD plasmas have obvious advantages for biological applications, but device design and control of reactive species chemistry remain challenges. We report results on surface microdicharge (SMD) air plasmas interacting with either solid surfaces or water, in which the DBD operates with a powered disk electrode separated with a thin dielectric layer from a conducting mesh, where the discharge forms. This simple configuration has been used extensively for various biomedical and related applications. [1-3]

Results of SMD antimicrobial action on adjacent dry surfaces and in water are correlated with gas phase composition via FTIR and UV-VIS absorption. In the cases of plasma exposed to water, the water pH and composition are measured. Below ~0.1 W/cm$^2$, the air plasma generates mostly ozone ($O_3$). At higher power per unit area (> ~ 0.5 W/cm$^2$), the air discharges create mostly nitrogen oxides such as NO, $N_2O$ and $NO_2$; in the presence of water vapor, nitric acid ($HNO_3$) readily forms from reaction between $NO_2$ and $H_2O$. In addition, hydrogen peroxide ($H_2O_2$) and nitrite/nitrous acid ($NO_2^-$/$HNO_2$) are observed in water adjacent to the air plasma. If the water is unbuffered, the nitric acid (in the form of nitrate anion, $NO_3^-$) is primarily responsible for the observed acidic pH. Aqueous mixtures of $NO_2^-$ and $H_2O_2$ at low pH generate reactive peroxynitrite ($ONOO^-$). [4] This compound is also known to form naturally from the reaction between enzymatically generated superoxide anion ($O_2^-$) and nitric oxide (NO), both of which are formed by macrophages in the innate immune system. [5] It therefore seems likely that air plasma mimics, in part, the natural antimicrobial chemistry of the immune system.

We also report on antimicrobial synergy between light emitting diode (LED)-generated 369 nm ultraviolet photons (UVA) and species created by the plasma. Using mixtures of buffered water with deliberately added nitrite and hydrogen peroxide, we show that these species are responsible for the enhanced antimicrobial action of first plasma-exposed then UVA-exposed water. [6]

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# Biologically Relevant Species in Atmospheric Pressure Helium-Oxygen Plasmas Operated in Ambient Air

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Atmospheric-pressure plasma jets (APPJs) have been gaining attention because of their great potential in bio-plasma applications. In order to understand the underlying operating principles of such systems and to optimize their performance in applications, it is important to know the chemical kinetics of the reactive multi-species plasma.

Because of the presence of humid air, the plasma tends to produce significant amounts of reactive species and the plasma-induced chemical reactions are complex. We use a 0D global model [1,2] with the extended reaction scheme (over 1300 elementary reactions among 65 species) to describe the complex plasma-induced chemistry of both the neutral and ionic compositions in both the active plasma and afterglow regions of helium-oxygen APPJs.

Figure 1 shows the densities of 65 species in the core plasma and the afterglow as calculated by the global model for a radio-frequency-driven (13.56 MHz) He+0.5%$O_2$ APPJ with 250 ppm air fraction (relative humidity of 50%). The global model quantitatively reveals the behaviour of biologically relevant species, such as reactive oxygen species and reactive nitrogen species. The presented global model can provide valuable insights into the underlying chemical reaction kinetics of the afterglow and the interactions of plasma-induced species and biological targets.

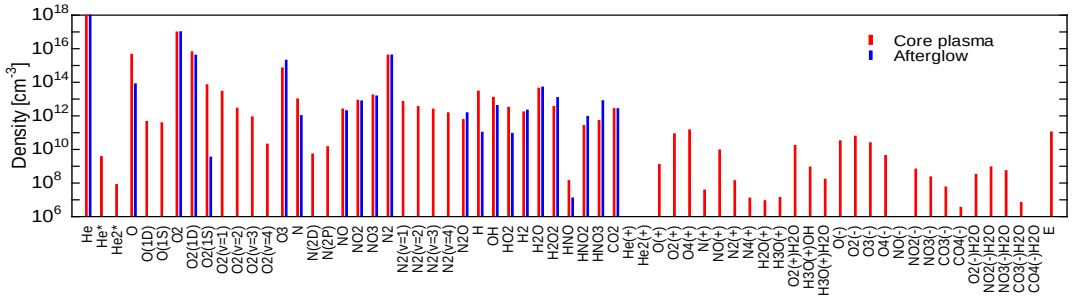


**Figure 1:** *The absolute densities of 65 species in He+0.5%$O_2$+0.025%humid air plasma*

This work was supported by KAKENHI (24110704 and 24561054), and the UK EPSRC (EP/D06337X/1, EP/H003797/1 and EP/K018388/1).

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# Plasma chemistry modelling in atmospheric pressure plasmas: Errors and uncertainty

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Modelling is an important tool for understanding the behaviour of atmospheric pressure plasmas, especially under circumstances where experimental data is sparse or even absent. However, the chemistry of these discharge is complex, and may encompass dozens of chemical species interacting through hundreds of reactions. Recent practice in assembling models for such chemistries may (with slight exaggeration) be characterized as making an eclectic selection of data from previous models. This procedure has several weaknesses. There is a tendency for errors and confusions to be inadvertently perpetuated, and the original reasons for choosing particular values for the rate constants are often obscured. In the best case, these values originate from experimental measurements with a defined uncertainty, but these uncertainties are not in recent practice systematically transmitted with the rate data. Under these conditions, quantitative statements about the accuracy to be expected from complex chemistry models are difficult, and suspicions that the combination of errors and uncertainties embodied in such models render them almost worthless may be entertained. The work reported in this paper seeks to address such concerns. Taking the moderately complex yet practically interesting chemistry of helium-oxygen mixtures as an example, we have systematically sought a primary source for every rate constant in the model. From these primary sources we have made a fresh determination of the values to be used for each rate constant, and with each rate constant we have associated an uncertainty. With these data, we have used Monte Carlo procedures to propagate uncertainty information from the rate constants into predicated species densities. These calculations not only quantity the uncertainty in prediction, they also associate the uncertainty in particular rate constants with the uncertainty in species densities, thus identifying sensitive parameters. We conclude that while the predictive uncertainty in chemistry modelling is significant (and, for example, should certainly be taken into account when comparing models with experiments) it is not such as to invalidate the modelling approach.

# Reactive Molecular Dynamics Simulations for the Interaction of Reactive Oxygen Species with Biomolecules

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The use of cold atmospheric pressure plasmas (CAPPS) in plasma medicine is envisaged for a wide range of applications including sterilisation, wound healing, etc. [1]. To date much experimental work has been done on the interaction between CAPPS and living cells and it is generally accepted that reactive oxygen and nitrogen species (RONS) play a crucial role in these interactions. However, little is known about the interaction chemistry on the molecular scale, which hinders the full understanding, development and optimisation of the intended applications. Complementary to experimental studies, computational techniques are ideally suited to tackle this problem. Using atomic scale simulations one might be able to predict the role of the different plasma species, in their interaction with biomolecules [1, 2].

In this work an overview is presented of our recent simulation results on the role of reactive oxygen species (O, HO, $HO_2$ and $H_2O_2$) on a range of biomolecules, employing reactive molecular dynamics (rMD) simulations.

First, results for Lipid A are presented. Lipid A is found in the protective outer membrane of gram-negative bacteria. Deviation from this structure (e. g., number of acyl chains) results in a diminished endotoxic activity. With rMD we investigated whether ROS are able to structurally change this biomolecule. Our simulation results go in line with experimental data obtained by Chung *et al.* [3].

Second, rMD simulations have been performed on the interaction of ROS with antioxidants, which form the natural defence system against oxidative stress [4]. Different antioxidants, both water and lipid soluble, are investigated, to aim for a better understanding of their role in plasma medicine.

Third, the effect of ROS on glucose molecules is investigated, as D-glucose forms the basis for many biochemical systems. Indeed, a clear view of the interaction chemistry with the basic molecular structures is essential.

Finally, the interaction of ROS with lipids, specifically α-linolenic acid, is illustrated, as a model system for the free fatty acids present in the lipid matrix of the skin [6]. It is found that the ROS give rise to H-abstraction of the free fatty acids, leading to the formation of e. g. alcohol or aldehyde functional groups. We expect that this will affect the hydrophilic character of the skin layer.

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# Numerical Simulation of Electric Double Layer in Contact with DBD - Effects of Mobility and Diffusion Coefficient of Liquid Ions -

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Plasmas in and in contact with liquid have attracted much attention because of their possible application fields such as nano materials synthesis [1], water treatment [2], and bio-medical applications [3]. In a plasma treatment of liquid, gas-phase active species in the plasma primarily encounter the liquid-phase species in an electric double layer (EDL). We previously performed numerical simulation of EDL formation coupled with the DBD [4]. We found that slower liquid ions are found to be left on liquid top surface, and that they are expected to preferentially interact with gas-phase species. In this work, we performed similar calculation for clarifying which transport parameter (mobility $\mu$ or diffusion coefficient $D$) is essential for realizing this tendency. Figure 1(a) is flux on the liquid and concentration of positive (X) and negative (Y) ions in liquid under standard conditions, where X and Y are assumed to be identical except for their polarity. Figure 1(b) shows the result calculated with assumption of $\mu_Y = 0.1\times\mu_X$, which do no marked difference from the standard result shown in Figure 1(a). On the other hand, Fig. 1(c), which is the results with assumption of $D_Y = 0.1\times D_X$, shows marked difference from Fig. 1(a) and 1(b). Slower negative ions tend to be left on the liquid to surface. These results mean that the preferential interaction between slower ions and gas-phase species is essentially governed by magnitude of diffusion coefficient of liquid ions.

This work has been partly supported by the Grant-in-Aid for Scientific Research on Innovative Areas "Frontier Science of Interactions between Plasmas and Nano-Interfaces" (No. 21110003) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and the Grant-in-Aid for Scientific Research (C) (No. 24540540) from Japan Society for the Promotion of Science.

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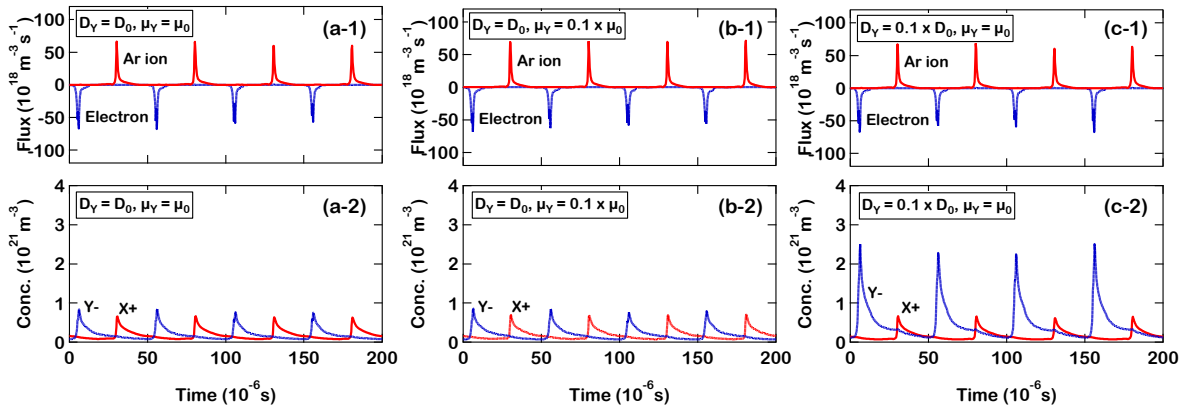


**Figure 1:** Effects of mobility and diffusion coefficient of liquid ions X+ and Y- on the concentration of these ions on the top of the liquid surface in contact with DBD.

# Electron Spin Resonance Study of Plasma-Biological Surface Interactions under Atmospheric Pressure Plasmas

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## 1. Introduction

Nonequilibrium atmospheric pressure plasmas (NEAPP) have feasibly applied blood coagulation as reported by Ikehara et al. [1] There are many models, however coagulation mechanisms have not clarified yet. Thus the plasma-biological surface interactions are required to study for understanding effects of the NEAPP plasma treatments. Here we focused on the blood and cells and on any mechanism on the basis of chemical changes through radical formations. For detection of radicals, we applied the real time in situ electron spin resonance (ESR) method, developed by our group.[2]

In this study, we have detected of free-radicals on bloods of edible meats under the NEAPP treatments. On the basis of experimental results, we will discuss about the plasma-biological surface interactions in particular of blood coagulation.

## 2. Experimental

Samples were blood component extracted from homogenized edible meats of bloods. 3 μl of the blood sample was dropped on a quartz plate. Immediately after the drop, the NEAPP generated with Ar gas flowing with a flow rate of 5 slm by application of high voltages of 60 Hz. The samples were set in the ESR cavity and measured by the X-band ESR spectrometer.

## 3. Results and discussion

After the plasma treatment of bloods, ESR signals with g-value of approximately 2.004 were observed clearly as shown in Fig. 1. Compared with signals from hemoglobin and albumin, similar ESR signal was detected in the case of hemoglobin. The results summarized that the signal has not identified completely yet but a candidate for heme b in bloods. As the NEAPP treatment time increased, intensities of ESR signal were increased and saturated as the time exceeds 5 min.

In summary, we speculate that gaseous active species such as O atom and oxidative radicals may cause to the chemical changes on peptides and proteins of cells and tissues. Free radical generation plays an key role for chemical changes on the biological surface under plasma treatments.

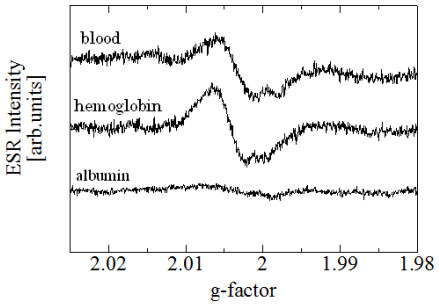


**Fig.1** *ESR spectra for blood, hemoglobin, and albumin on the quartz plates measured after plasma treatments for 10 min.*

**Acknowledgements**

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# Atmospheric Plasma Processing to Form Organic Coating on Ceramic Nanoparticles for Biomedical Imaging

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Over 1000 nm (OTN) near infrared (NIR) wavelength region has been known to be more transparent one for applying fluorescence biomedical imaging [1] due to much less scattering loss in the currently used wavelength region below 1000 nm. One of the candidate fluorescent agents for the fluorescence in the OTN-NIR region is rare-earth doped ceramics nanoparticles (RED-CNPs). Among the RED-CNPs, yttrium oxide ($Y_2O_3$) nanoparticles (NPs) doped with rare earth ions such as Er or Ho ions have been studied as one of the most useful candidates for the OTN-NIR fluorescence because of their fluorescence efficiency and controllability of the particle size. To apply the $Y_2O_3$-NPs for the biomedical fluorescence imaging, one of the most important issues is the surface coating of the NPs by organic polymers for applying chemical durability and biological functions on them. The authors have proposed various kinds of wet processes for the coating such as the use of ionomer-polyethylene glycol block copolymer [1]. On the other hand, the existence of a non-fluorescent layer on the surface of the $Y_2O_3$-NPs consisting of hydroxyl carbonate has come to be known, which may be formed due to the water and carbon dioxide in the atmosphere. The use of wet solution processing for the polymer coating may enhance the formation of the hydroxyl or hydroxyl carbonate layer even if the $Y_2O_3$-NPs are stored in a dry atmosphere. Therefore, the development of dry processing for forming an organic layer on the $Y_2O_3$-NPs is one of the important studies for achieving the OTN-NIR biomedical imaging.

Plasma processing has been known as a method to form organic coating on substances by introducing carbon source gas to the plasma atmosphere. Normally, low pressure plasma is used for the layer formation on the NPs. However, for three dimensionally homogeneous formation of the coating on the NPs, a floating mechanism of the NPs in the plasma is required. Fluidization of the particles can be one of the methods to float the NPs in plasma. Atmospheric plasma is more efficient to fluidize the NPs compared to the low pressure one. For achieving the dry process to form the organic coating on the $Y_2O_3$-NPs, the authors have developed a new equipment of the atmospheric plasma coating on the fluidized NPs.

In a plasma chamber made of silica tube, $CH_3$ gas diluted with He was introduced to fluidize the $Y_2O_3$-NPs and for the organic coating layer on the NPs by plasma formation by applying 20 kHz nano-pulse with 5 nsec duration. The FT-IR measurement and the thermogravimetric analysis on the processed $Y_2O_3$-NPs revealed that organic coating was formed on the NPs, which thickness increased by increasing the processing time. The acid durability test revealed the improvement of acid durability of the samples by the plasma processing. Therefore, a dry processing for forming organic layers on the $Y_2O_3$-NPs for giving acid durability was successfully developed.

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# Diagnostics of intracellular signaling systems of glioblastoma brain tumor cells treated with plasma-activated medium

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Diagnostics and modeling are essential methods to understand molecular mechanisms in Plasma Medicine. Plasma-tissues/cells interactions contain multiple step reactions from plasma inputs to physiological outputs. Electrons, radicals, lights, and other components in plasma interact with surrounding environments in gas phase and liquid phase, and those components interact with biological systems such as cellular membrane and intracellular signaling networks.

We have recently reported that plasma activated medium (PAM) selectively induced apoptosis (a programmed cell death) on glioblastoma brain tumor cells [1] and drug-resistant ovarian cancer cells [2]. Diagnostics and modeling in biological systems are needed to understand intracellular molecular mechanisms of apoptosis in PAM-treated cancer cells.

We investigated whether survival and proliferation signaling networks were affected by PAM on glioblastoma brain tumor cells because the signaling networks are abnormally activated in cancer cells [3]. Western blotting analysis showed that the activities of hub molecules in survival and proliferation signaling networks such as AKT, ERK, and mTOR were downregulated by PAM. These results suggest that PAM induced apoptosis by inhibiting the survival and proliferation signaling networks.

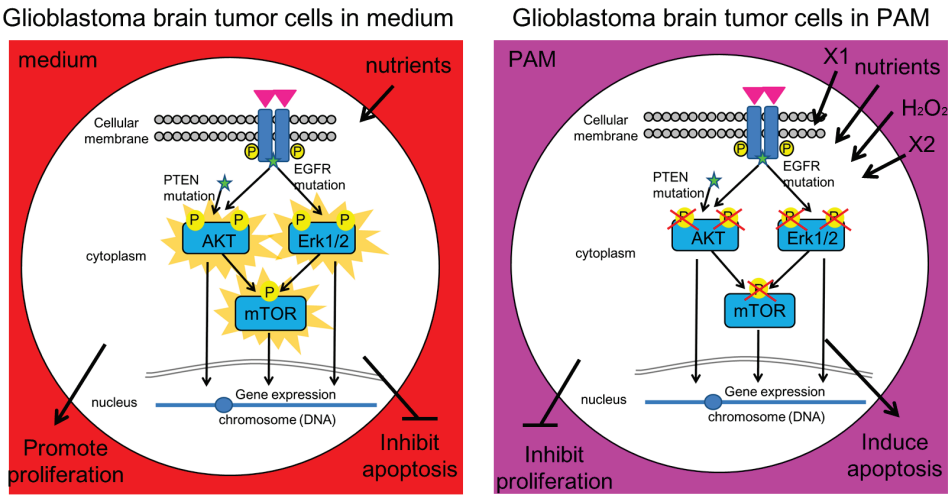


Figure1: Intracellular molecular mechanisms of apoptosis of PAM-treated glioblastoma brain tumor cells

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# Plasma Stimulates Angiogenesis

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Angiogenesis is the growth of new blood vessels and normally occurs in the process of healing wounds and restoring blood flow after injury or insult. The role of fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) in angiogenesis is well defined[1]. Our laboratory has recently demonstrated the stimulation of fibroblast growth factor (FGF) production by porcine endothelial cells following plasma treatment[2]. Angiogenesis occurs when endothelial cells sprout from pre-existing vessels to form new structures, and we hypothesized that plasma treatment could induce stimulation of angiogenesis following treatment of excised murine aortic rings, a well-defined *ex vivo* model of neovascularization [3]. For these experiments, mouse thoracic aorta was sectioned, subject to plasma treatment using a microsecond DBD discharge. The tissue was exposed to plasma for 10 seconds at different doses (22kV, 20 microsecond pulse and frequencies of 50 Hz, 500 Hz, 800 Hz and 1.66 kHz), placed onto growth factor reduced matrigel, and incubated in standard growth media. Some rings contained VEGF as a positive control. Sprouting from rings was analyzed daily, and on the eighth day photographed and outgrowth area quantitated by image analysis. Preliminary results suggest plasma treatment could induce sprouting from aortic rings in a dose-dependent manner with 500 Hz being the most effective, compared with un-treated controls. Future experiments will determine angiogenic gene expression induced by plasma treatment. These data indicate that plasma treatment can induce microvessel sprouting from aortic rings in a 3-dimensional tissue model, and suggests the potential that plasma treatment could be considered a therapeutic modality.

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# Two-Dimensional Numerical Simulation of Mass Transfer of Reactive Species through Plasma–Liquid Interface

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Two-dimensional numerical simulation of mass transfer of reactive species through a plasma–liquid interface was conducted. Pulsed argon plasma was formed between a needle electrode and water surface when a pulsed voltage with duration of 200 ns and a frequency of 1 kHz was applied. Behavior of reactive species generated by the plasma was investigated by considering electron-impact reactions, gas- and liquid-phase reactions, and mass transfer assuming gas–liquid equilibrium on the interface. Commercially available software COMSOL Multiphysics® was used for the simulation.

First, the distributions of electron density and electron temperature in the plasma were obtained. Then, the gas-phase reactions and mass transfer of the generated species—OH radical (•OH), hydrogen peroxide ($H_2O_2$), and hydroperoxyl radical ($HO_2$•)—were calculated. Figure 1 shows the distribution of •OH in gas phase after 100 ns from the voltage rise. The dominant reaction of the •OH generation was dissociation of water molecule by argon metastable atom. The •OH density was high near the water surface where the electron density and electron temperature were high. Because most the OH radicals generated in gas phase were consumed by the self-quenching reaction, which generated $H_2O_2$, very few OH radicals dissolved into water. In water, the OH radicals existed within a very small region, less than 10 μm from the water surface, for about 10 μs because of the reactions with •OH, $H_2O_2$, $HO_2$•, and organic compounds included in water. However, it was experimentally confirmed that there is a liquid-phase flow induced by the plasma as reported in [1]. Thus, not only diffusion but also convection of the liquid-phase species has to be considered.

This work was supported by JSPS KAKENHI (25790071).

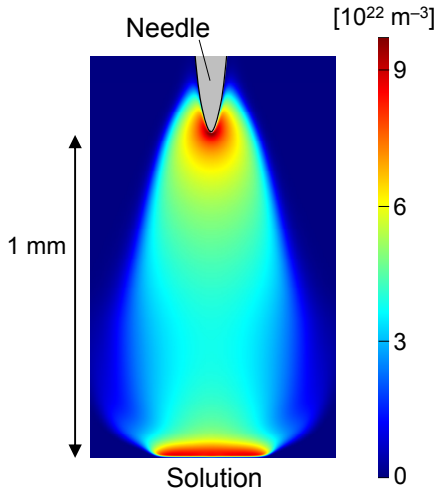


**Figure 1:** *Distribution of OH radical in gas phase after 100 ns*

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# Tailored Reactive Oxygen Species and their generation mechanisms from the plasma, the gas and the liquid phase to human cells

S. Reuter[1], H. Tresp[1], J. Winter[1], M. Hammer[1], A. Schmidt-Bleker[1], S. Iseni[1], A. Barton[1], M. Dünnbier[1], K. Masur[1], R. Bussiahn[2], K. Wende[1], Th. von Woedtke[2], K.-D. Weltmann[2]
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Research in Plasma Medicine requires a detailed insight into reactive oxygen and nitrogen species (ROS/RNS) pathways from their point of generation to their cellular effect [1]. Tailoring plasma sources to generate a specific ROS/RNS composition helps to identify these pathways. In this work, precise optical and spectroscopic diagnostics follows the generation processes from the discharge region to the gas phase and the transition into the liquid phase. Correlating the reactive species dynamics with biological effects reveals the role of different reactive species groups in cell response[2, 3].

The work unravels by space and time resolved diagnostics interaction mechanisms of plasma jet guided streamers with ambient air species and how turbulent species transport (see figure 1) influences plasma dynamics. A diagnostic of long living species combined with a simple reaction kinetics model shows the generation mechanisms and the most relevant chemical reactions occurring within the ambient surrounding [4].

Liquid diagnostics correlated with the plasma diagnostics reveals the origin of reactive species within the liquids. Here, generation and transport are discussed and it is shown that VUV radiation has a non negligible influence on ROS generation in biologically relevant liquids. The identification of these mechanisms forms the link between plasma reactive species generation and cellular effect.

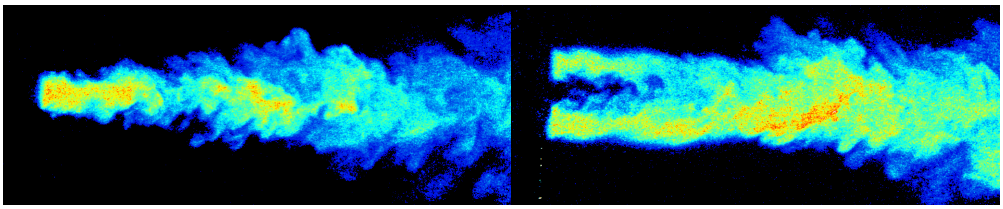

**Figure 1:** *Gas flux image of a plasma jet nozzle 3 slm (left) and shielding gas 5 slm (right) revealing the turbulent mixture of ambient species with feed gas for the plasma off case. [5]*

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# Key reactive species in cold atmospheric pressure plasmas: absolute measurements

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Quantitative measurements of short- and long-lived reactive species in cold atmospheric pressure plasmas are essential for understanding fundamental processes of the plasma dynamics and associated chemical kinetics, benchmarking models and simulations, controlling delivery of energy carrying species to biological samples, and developing safe and reliable future technologies in plasma medicine.

We have developed and employed a broad variety of advanced diagnostic techniques. We use phase resolved optical emission spectroscopy (PROES) for direct measurements of the nanosecond electron dynamics [1], molecular optical emission spectroscopy for gas temperature measurements [2], laser diode absorption spectroscopy for metastable density measurements [3], UV-LED absorption for ozone density measurements and infrared emission spectroscopy for singlet oxygen density measurements [4].

Key to understanding the chemical kinetics is the measurement of absolute atomic oxygen and nitrogen ground state densities. However, these measurements are particularly challenging in the collision-dominated environment of atmospheric pressure plasmas, requiring extremely high temporal (picosecond to nanosecond) and spatial (microns) resolution. The most versatile approach is a combination of diagnostic based modelling (DBM) [5], two-photon absorption laser induced fluorescence (TALIF) [6] and high-resolution synchrotron VUV absorption spectroscopy [7].

Detailed investigations of radio-frequency driven atmospheric pressure plasma jets will be presented for operation with different power coupling mechanisms, i.e. continuous radio-frequency operation and kHz pulsed operation. The influence of molecular gas admixture variations is studied for oxygen, nitrogen, air (dry & humid) and water. The obtained results show very good agreement with numerical simulations of the electron dynamics and chemical kinetics. This allows us to tailor plasma properties toward desirable conditions for technologies in plasma medicine.

**Acknowledgment**
Support by the UK EPSRC (EP/K018388/1 and EP/H003797/1) is greatly acknowledged.

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# Plasma Jet (V)UV-Radiation Impact on Biorelevant Liquids and Cell Suspension

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In the present work the generation of radicals in plasma treated liquids and eukaryotic cell suspensions were studied. In order to understand the contribution of plasma (vacuum) ultraviolet (V)UV and ultraviolet (UV) radiation on the species investigated, different cases are studied: UV radiation of the plasma jet only, UV and VUV radiation of the plasma jet combined, and (V)UV radiation with the plasma effluent, which include all reactive components. The plasma emitted VUV radiation was examined by optical emission spectroscopy (OES) and its effect on radical concentrations in cell suspension was analyzed. Radical formation was investigated in ultrapure water, as well as in more complex and biologically relevant solutions like Dulbecco's phosphate buffered saline (DPBS) solution, Rosewell Park Memorial Institute (RPMI) cell culture media, and Dulbecco´s modified Eagle´s medium (DMEM).
It could be shown that due to their various compositions different reactive species were formed by plasma treatment [1,2].For example, superoxide anion ($O_2^{\bullet-}$) and hydroxyl ($^{\bullet}OH$) radicals were detected by the use of electron paramagnetic resonance (EPR) spectroscopy in DPBS. Additionally, glutathione thiyl radicals ($GS^{\bullet}$) were found in cell suspension (figure 1).
An important result for plasma medical research is that the amount of generated radicals due to the VUV radiation of the plasma jet is one third of the formed concentration by the treatment using all reactive components of the argon plasma jet together.

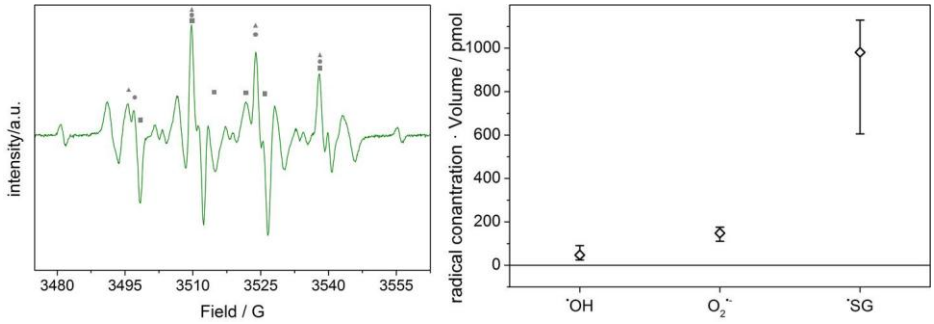


**Figure 1:** *EPR spectrum after 180 s treatment with VUV radiation of an argon atmospheric pressure plasma jet and hydroxyl (marked in the spectrum with (▲), superoxide anion (●) and glutathione thiyl (■) radical concentrations.*

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# Evidence about Formation of Peroxynitrite in Air Plasma-Treated Water through a Second-Order Post-Discharge Reaction of $H_2O_2$ and $HNO_2$

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Post-discharge reactions in plasma-treated water may contribute significantly to the biological effects induced by gas phase discharge plasmas produced at the gas-liquid environment. For solutions that were treated by air-liquid-phase plasmas, the antimicrobial properties of plasma-treated water were tentatively attributed to the synergetic effect of acidic pH and to the $H_2O_2$ and nitrite/nitrate remaining in noticeable concentrations in the solution. However, the exact mechanism and contribution of these species in biological effect of these plasmas are not fully understood yet. The great interest is in transient species produced by plasma at the gas-liquid interfaces such as hydroxyl OH• and nitrogen radicals NO• and $NO_2$•, peroxynitrite and the role of these species in plasma-induced biocidal effects in plasma-treated water. However, these species are difficult to measure due to their short lifetimes and fast disproportionation in the plasma/liquid systems.

In this work formation of peroxynitrite in water being treated by air discharge plasma was studied using two different approaches. First, phenol was used as the chemical probe to characterize some reaction pathways of ROS and RNS produced by gas phase plasmas in contact with water in dependence on the composition of the gas atmosphere (20% oxygen mixtures with nitrogen or with argon) and the pH value of plasma-treated water controlled by buffers (pH 3.3, 6.9 and 10.1). Second, the formation of peroxynitrite was determined through the kinetic study of the post-discharge reaction between hydrogen peroxide and nitrite ions occurring in water after being treated by air discharge plasma. Evidence of formation of peroxynitrite was proved by detection of specific products of phenol in plasma-treated water. Nitrated products of phenol (4-nitrophenol, 2-nitrophenol, 4-nitrocatechol, and 4-nitrosophenol) were detected in addition to hydroxylated products (catechol, hydroquinone, 1,4-benzoquinone, and hydroxy-1,4-benzoquinone). These products gave clear evidence about formation of $NO_2$•, NO• and OH• radicals and $NO^+$ ions in plasma-treated liquid. A close, 1:1 concentration ratio between hydroxylated and nitrated products was determined in plasma-treated water and in model water mimicking content of plasma-treated water, which has been shown to be a result of the post-discharge processes in plasma-treated water mediated by peroxynitrite. Formation of peroxynitrite was further demonstrated through kinetic study of the post-discharge reaction between $H_2O_2$ and nitrite ions in plasma-treated water. Excellent fit was determined between experimental data from post-discharge evolution of $H_2O_2$ and $NO_2^-$ measured in plasma-treated water and the pseudo-second-order reaction between $H_2O_2$ and $NO_2^-$. The third-order rate constant $k = 1.1 \times 10^3$ $M^{-2}$ $s^{-1}$ for the reaction $NO_2^- + H_2O_2 + H^+ \rightarrow ONOOH + H_2O$ was experimentally determined in plasma-treated water at pH 3.3 with the rate of ONOOH formation in the range $10^{-8}$ to $10^{-9}$ M $s^{-1}$. The yield of formation of OH• and $NO_2$• from ONOOH was estimated to be 25-35% of the total amount of peroxynitrite theoretically formed in plasma-treated water through reaction of $H_2O_2$ and $NO_2^-$.

This work was supported by the Academy of Sciences of the Czech Republic (project No. M100431203).

# PLASMA TREATMENT OF HUMAN SKIN TISSUE

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Plasma Medicine is a challenging field of research requiring interdisciplinary collaborations between physicists, cell biologists and physicians. While physicists gain deep insights into the diagnostics of the plasma itself, cell biologists try to understand the transfer of reactive species and energy to living cells and tissues. To date most knowledge of plasma interaction with biological systems was gained by working with either cell suspensions or monolayers in vitro [1]. This led to a detailed comprehension of the balance between cell stimulation and the induction of cell death based on the findings on the transcriptomic as well as proteomic level [2, 3].

Recently a plasma jet has been developed for medical applications and was certified as a medical device class IIb in 2013[4]. Although plasma analytics are well advanced little is known about the plasma tissue interaction and the possible risks. In order to fill this gap ex vivo skin explants were exposed to plasma for different time lengths and cultured for 24 hours. Thereafter, skin samples were analyzed for distinctive markers for proliferation, apoptosis, DNA-damage and differentiation (keratin 1 and 14) by employing immunofluorescence. Moreover, secreted cytokines and growth factors were measured in the culture supernatant. Markers were chosen based on the findings in molecular biology of cell culture experiments. Here we present data of plasma treated skin biopsies which clearly show stimulating effects indicated by the activation of proliferation markers like Ki67 consolidated by a secretion of cytokines (such as IL-6). We also proved that the effects were mediated into deeper layers of the epidermis, without harming the cells on top of the treated skin samples. Interestingly the results revealed an increase of proliferating keratinocytes for 3 minutes treatment while induction of apoptosis strongly increased after 5 minutes. However, DNA-damage using H2A.X immunoreactivity was relatively constant between all samples. The expression of keratin 14 and 1 within the human epidermis remains grossly unaltered by plasma application.

Our results strengthen a safe application of this plasma device (kinpenMED) in human tissue, confirming previous in vitro results in situ for the first time. Keeping in mind that this study was performed on healthy skin, for an introduction of plasma medicine into the clinics similar studies on diseased skin are under way in order to proceed towards a safe treatment of real patients.

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# Poster Presentations

## Monday, May 19

# PLASMA – TISSUE INTERACTIONS

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Plasma is being recognized as a "wonder drug" with applications ranging from anti-microbial [1] (prokaryotic cell) treatment to killing of cancer cells [2] (eukaryotic cells) and everything in between. It is also now fairly well established that the different effects such as wound healing or stem cell-differentiation depend on the dose applied. At higher doses damage to cellular macromolecules, membrane lipids and DNA from oxidative stress has been documented by many labs [3, 4]. As we move toward use of plasma as a routine therapeutic regime, calibration of plasma delivery is a key unresolved parameter.

The non-penetrating nature of plasma is an additional obstacle in its transition to direct clinical applicability. In addition, we will have to consider the interaction of the multi-organ system homeostatic effects in an intact animal. Examination in 3-D models *in vitro* would be a first step in evaluating the efficacy of these modalities as well as a step toward identifying any cell signaling in complex tissues. While several studies have provided *in vivo* evidence [5] that plasma may be used to achieve specific biological outcomes, these laboratory models do not address the potential complexities that may be involved in treating patients.

We are examining alternative approaches to use plasma for different cellular effects. These include treatment of leukemic cells to push them to differentiate into functional immune cells instead of inducing immediate apoptosis. We are also treating aortic rings *in vitro* as a 3-D tissue model to induce angiogenesis. We are additionally investigating the possibility of immune stimulation that may provide not only a therapeutic option but also result in immune surveillance and control of metastatic cells. The paper will present results from our ongoing experiments.

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# Mapping the Effects of Low Temperature Plasma Treatment of Prostate Cancer Cell Lines and Primary Cells: Along the Path to Cell Death

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In recent years, investigations into the application of low temperature atmospheric pressure plasmas (LTPs) for biomedical purposes have demonstrated great potential in areas such as surface sterilisation, wound healing, biofilm inactivation [1], and now cancer therapy. In this study, we assess the efficacy of LTP on prostate cancer cell lines.

Prostate cancer is the most common form of the disease in men, and accounts for around 13% of all cancer cases [2]. There are over 40,000 new diagnoses and 10,000 deaths every year in the UK alone. Despite new therapy options and improvements to existing ones, the prognosis for sufferers can remain bleak, with unpleasant side effects. In this study, we have assessed the *mechanisms* of cell death after LTP treatment of *both* benign and cancerous prostate epithelial cells, mapping the immediate cellular responses which ultimately result in cell death.

The device used for the investigations was a dielectric barrier discharge (DBD) jet configuration, with helium as a carrier gas, and 0.3% $O_2$ admixture [3]. Reactive oxygen and nitrogen species (RONS) produced by the plasma are believed to be the main mediators of the plasma-cell interaction and response [4, 5]. We found that the concentration of reactive oxygen species (ROS) induced inside the cells increased with plasma exposure. Exposure to the plasma for >3 minutes showed high levels of DNA damage compared to untreated and hydrogen peroxide controls. At time periods up to 96 hours post-treatment, cell viability was significantly reduced. Cell recovery was found to be greatly inhibited following treatment times of up to 10 minutes, with results suggesting the cells die through necrotic mechanisms. All the findings were common to both cell lines, suggesting the potential of LTP therapy for both benign and malignant disease.

The next stage of research will include a thorough review of the plasma-biological interaction, in order to determine the RONS produced by the plasma, and how these relate to biological response. It will be important to determine optimum parameters for maximum biological efficacy, such as: exposure time, gas composition, and driving frequency and waveform.

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# Healing Burns Using Atmospheric Pressure Plasma Irradiation

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Atmospheric-pressure plasmas are indispensable for sterilizing, disinfecting, decomposing hazardous materials, and modifying material surfaces. Clarifying the mechanisms of plasma technologies that are used in practical applications is of critical importance. To achieve this, it is important to understand plasma technology. Against this background, we are trying to clarify the mechanism by which the atmospheric-pressure plasma (APP) reactor promotes regeneration of living tissues [1].

A schematic diagram of the experimental setup is shown in Figure 1. The APP reactor with a coaxial structure is composed of a 1-mm-diameter tungsten wire inside a glass capillary (plasma generation area, 8 mm ID; tip area, 1 mm ID) that is surrounded by a grounded tubular electrode. The AC/DC amplifier and Multifunction Synthesizer controlled by a PC provides a high voltage for plasma generation.

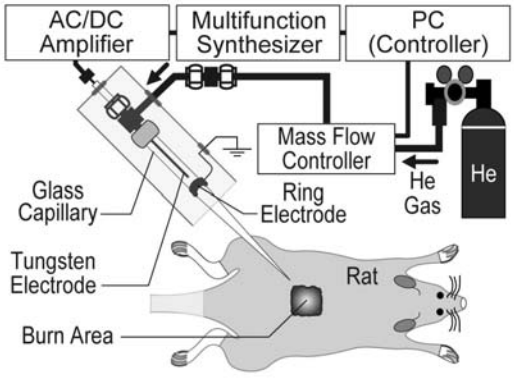


**Figure 1:** *Experimental setup*

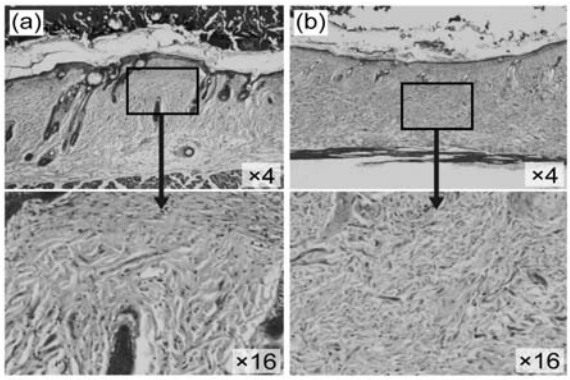


**Figure 2:** *Histological findings from rat skins*

A separate group of six new male Wistar rats (SPF, 8 weeks old, weight: 200–220 g) was used to observe histological findings from rat skins. A full thickness burn (5 $mm^2$) was made a rat's back, using an electric scalpel. One was then irradiated with the plasma once a day; the other one was not irradiated. Seven days after injury, fluorescein isothiocyanate (FITC)-labeled tomato-lectin was injected into the rats for visualization of neo-vascular vessels in the burn tissues. The burn tissues were subsequently extracted, and the tomato-lectin labeled neo-vascular vessels in the tissue specimens were observed using a confocal laser microscope. The histopathological findings from the burn area are shown in Figure 2. In the control example shown in Figure 2(a), there is marked proliferation of blue-stained connective tissue between hair follicles (tissue surrounding the coat is below the pores). The arrangement of the hair follicles has become irregular. On the other hand, in the plasma irradiation example shown in Figure 2(b), the arrangement of the hair follicles is regular. These results suggest that plasma irradiation promotes the healing sequence of hair follicles, as denoted by their regular arrangement in the irradiated example. Plasma irradiation may facilitate re-formation of normal tissue building in the process of post-burn skin regeneration.

This study was supported by a Grant-in-Aid for Scientific Research (B) (No. 21340173) and a Grant-in-Aid for Scientific Research on Innovative Areas (No. 24108010) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

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# Relationship of Nitric Oxide Concentration in the Blood Pressure Lowering in Rats Following Atmospheric Pressure Plasma Inhalation

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Recent investigations have demonstrated the use of atmospheric pressure plasma to promote wound healing. Plasma irradiation was shown by G. Fridman *et al.* to promote the recovery of a diabetic foot ulcer [1]. However, the recovery mechanism has not been elucidated. Furthermore, the safety of plasma irradiation of living tissue is unconfirmed. The purpose of this study was to investigate the biological safety of plasma inhalation on a living body by measuring vital signs. We have selected the electrocardiogram and blood pressure as measures of circulatory system function from the point of view of life support. The reason for using inhalation rather than irradiation of the plasma was because plasma is a gaseous substance and is thought to be absorbed by the mucous membranes and lungs, so a noticeable and rapid effect would be measured. In this report, we measured electrocardiogram, blood pressure, and nitric oxide concentration in the blood following plasma inhalation.

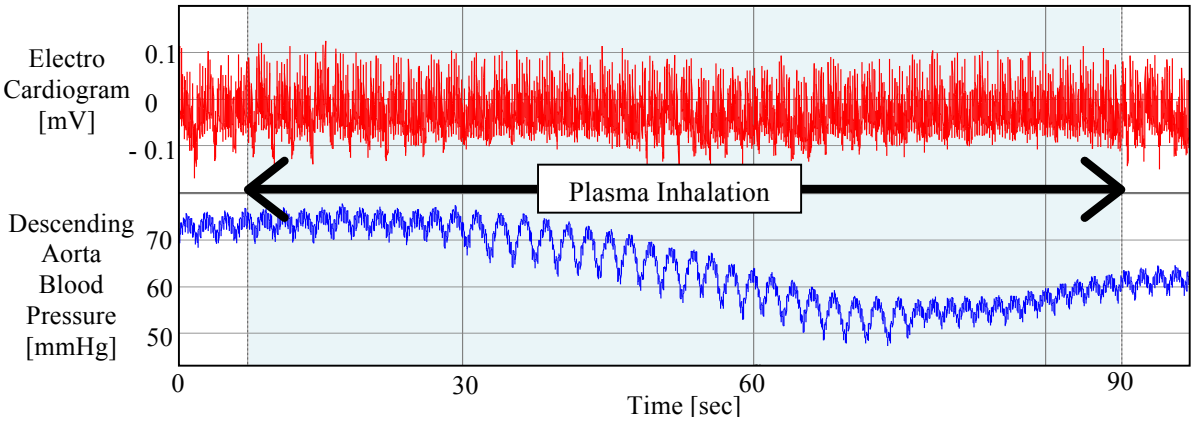


**Figure 1:** *The results following inhalation of atmospheric pressure plasma in rats.*

In the beginning, we were inhaled plasma on rat for 90 seconds under inhalation anesthesia. Figure 1 shows the results following inhalation of atmospheric pressure plasma in rats. A blood pressure- lowering effect was observed following plasma inhalation. There were no changes in heart rate and amplitude of electrocardiogram during plasma inhalation. This mechanism responsible for the phenomenon is too complicated to be examined in detail now. However, it is quite likely that pressure-lowering was caused by relaxation of peripheral blood artery.

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# OH radical as a major factor for cell adhesion by cold atmospheric plasma

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Cold atmospheric pressure plasma (CAP) is a new technique for biological application especially for treatment of tumor cells. Several ROS (OH, O, and $H_2O_2$) and RNS (NO) are considered to be the important radicals in biological effects. Here we used a 6 mm diameter plasma jet device to produce various radicals. At the conditions of 3LPM of He gas, 10 kHz, 11kv powered, 25 mm distance with 100ul PBS in each 24 wells, plasma could induce RPMI8226 myeloma cell death. Interestingly, when we stained the cells with trypan blue, we found 3 clear circles after plasma treatment. The center circle is dead cells, and then is the void area that cells were detached from the cultured plates, lastly is the live cells. The void area is positively correlated with the plasma treatment time and the power voltage, indicating some radicals in the plasma is involved in cell adhesion. So we analyzed the spacial distribution of several radicals by emission spectrometer. Although all of the spectrum lines (309nm, 337nm, 707nm, and 777nm et.al) are intensive at the center and weaken at the border, we calculated a relative intensity clear shows that intensity of OH radical is lower at the center while stronger near the edge. The relative OH intensity is perfectly correlate with the void area of the cells (Fig1), indicating OH radical may be the major factor for cell adhesion. We will further analyze the distribution of radicals by mass spectrometer and using OH scavenger to confirm this phenomenon and figure out the molecular mechanism of the interaction between OH radical and cell adhesion molecular.

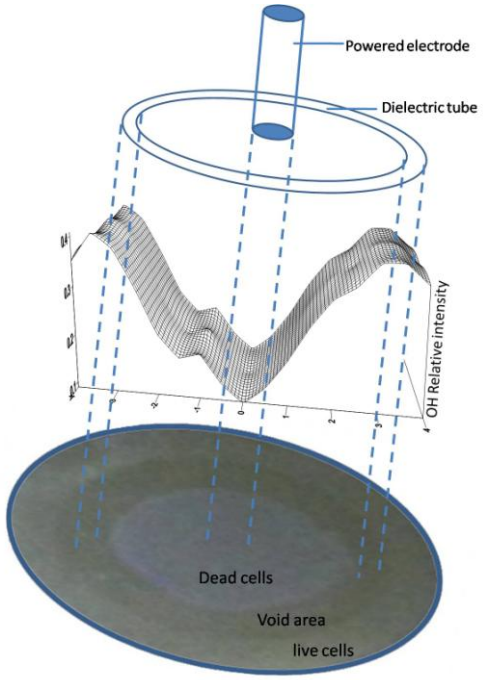


**Figure 1:** *Space corresponding of plasma jet, emission spectrum and cell culture distribution*

# Possible Clinical Application of Electron Discharge at Extremly Low Energy Level for Suppression of Oxidative Stress

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**[Background and Aim]**
Nonalcoholic steatohepatitis (NASH) has become a significant problem with increase of obesity and type II diabetes mellitus. Although oxidative stress has been implicated in the pathogenesis of NASH, anti-oxidative treatments have thus far exhibited only limited success. We previously showed that electron discharge at the extremely low energy level exhibited anti-oxidative efficacies against $H^+$, glutathione disulphide and $Fe^{3+}$ in vitro [1]. Here, we examined the reductive potency of the electron discharge at the low energy levels on the rat model of type II diabetes-induced NASH.

**[Methods]**
We designed a non-invasive device using dielectric barrier discharge system, which is capable to supply the plenary discharge electron onto the skin surface at nano ampere level/$cm^2$ [2].
We have prepared the type II diabetic NASH model rats induced by administration of streptozotocin and a high fat diet. The efficacy of the discharged electron on the NASH rats was examined with an electron treatment at 5μA for four weeks.

**[Results]**
The rats at 8 weeks old exhibited elevation of blood glucose, serum alanine aminotransferase (ALT), and hepatic peroxide product, malondialdehyde (MDA). When the rats were treated with 5μA, values of ALT and MDA were significantly decreased ($p<0.05$). In addition, gradual but steady decreasing tendency of glucose levels was observed comparing to the untreated group, although the values did not reach statistical significant ($p=0.074$). The serum values of MDA, ALT, and glucose were correlated significantly. Progression of fibrosis as measured by serum hyaluronic acid and histological examination was not affected by the treatment in this model. The present studies suggest that possible application of the discharged electron treatment at a micro ampere level should be examined by performing clinical studies on the diseases in which oxidative stress is implied as a pathogenesis factor.

**[Conclusions]**
Anti-oxidative electron treatment attenuated the pathogenically elevated liver inflammation and oxidative stress, together with presumably impaired glucose metabolism in NASH rat model.

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# Compact microwave atmospheric plasma devices for biomedical applications

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Interests in the biomedical applications of atmospheric pressure plasmas are increasing rapidly [1]. Various devices have been used for wound healing for hospitals. Considering market size, safety, and convenience, portable microwave plasma devices for home use and esthetics are highly required. There have been (bulky) microwave Ar or He plasma devices for hospital or laboratory uses and hand-held compact low-frequency (that produces prohibitive amount of ozone) air plasma devices but there has been no compact home-use microwave air plasma devices.

We present a portable ozone-free microwave atmospheric pressure air plasma jet (MPen). New device is developed based on previous coaxial transmission line resonator (CTLR) [2]. Modified structure at the open end enables to generate 10 mm air plasma jet with less than 10W of microwave power. No significant ozone is generated from the air plasma jet because of its high temperature at the discharge region (more than 200 °C). Gas temperature cooled to 36 ~ 40 °C by a cooling gas at the treatment region which is low-temperature. MPen can be applied for home care such as acne and oral care including teeth whitening.

We present compact microwave Ar plasma devices for skin care (SC2-Ar). Four compact size CTLR are assembled for a SC2-Ar header. The header of SC2-Ar is designed to treat circular area with 4 cm diameter, because relatively large area is required for esthetics. Stable Ar plasma jets are produced in microwave frequency. The gas temperature can be controlled in the range of 30 ~ 42°C. SC2-Ar is expected to be applied for effective skin care including wrinkle care, whitening and acne care.

Blood coagulation experiment is presented using MPen and SC2-Ar. The microwave air plasma in MPen shows fastest blood coagulation in three mins, the Ar plasma in SC2-Ar takes ~ three mins for blood coagulation, in comparison to natural coagulation which takes more than 20 mins.

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# A novel plasma based teeth whitening process

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Recently, dental bleaching has become one of the most requested procedures in cosmetic dentistry. To meet costumers demands, tooth bleaching gels based on hydrogen peroxide ($H_2O_2$) were developed, which effectively enable the removal of chromogens deposited on teeth enamel and dentin; however, the high concentrations of $H_2O_2$ required may cause several problems such as tooth sensitivity, alteration of enamel, pulp damage and gingival inflammation.
Various studies have reported promising results regarding the enhancement of bleaching effect adopting non-thermal atmospheric plasma instead of conventional light sources for whitening gels activation, increasing the rate of decomposition of $H_2O_2$ into free radicals (especially OH). While almost all these studies relied on the use of $H_2O_2$ gels as an intermediate agent between teeth and plasma, *Pan et al*. reported on the effects of direct plasma treatment of teeth, applying on their surface a saline solution instead of using $H_2O_2$ based gels [1].
In this work we present a cold atmospheric pressure plasma process for teeth whitening which does not require the use of either $H_2O_2$ or saline solution and which is capable of treating the entire dental arch simultaneously, in a fast and continuous way. The plasma source was applied for 15 min on a reconstructed dental arch made from extracted teeth previously pigmented with coffee. The effectiveness of the treatment was evaluated analyzing teeth color change by means of a spectrophotometer specifically designed for tooth's color measurement (SpectroShadeMicro, MHT) and using the International Commission on Illumination color value system (CIE-Lab), as usually done for these analyses. The obtained results show a clear and significant whitening effect after the plasma treatment, with a brightness improvement comparable to that obtained using standard $H_2O_2$-based techniques applied in similar conditions.

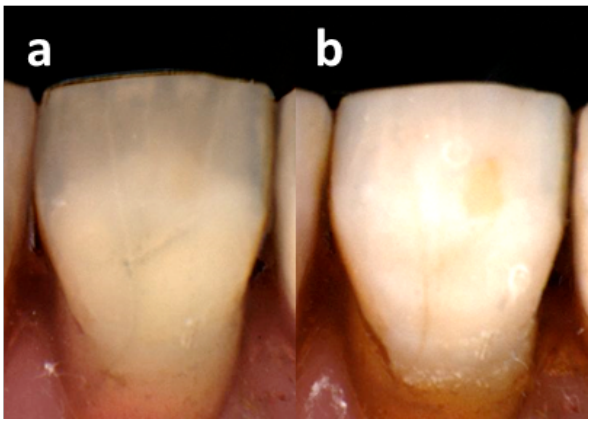


**Fig 1:** *(a) Untreated tooth (b) plasma treated tooth*

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# Diagnostic Imaging of Plasma-Treated Rat Hypoxic Ischemic Encephalopathy Model Using X-ray CT

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Hypoxic ischemic encephalopathy (HIE) bring on the various symptoms caused can be fatal condition by shortage of oxygen supply to the brain. Brain cells start to die within as little as five minutes without oxygen. There are various causes for HIE, and many of disorders and heath conditions can create shortage of oxygen in the brain. However, no cure for HIE has yet to be found. On the other hands, the atmospheric pressure plasma is indispensable not only for sterilization, disinfection, decomposition of hazardous materials, and surface modification, but also regenerative medicine and skin healing. In recent years, with angiogenesis effects of plasma irradiations indicated, we can expect treatment effects on HIE by improved blood flow in brain. However, these mechanisms have not been clarified. Against this background, we aim clarification of the healing mechanism and improvement of the brain function using plasma-treated rat HIE model and X-ray CT scanner. We prepared HIE model rats by drug administrations.

Drug administration: kainic acid has been widely used in experimental research of neurological disorders. We intraperitoneally administered kainic acid to two rats

According to the experimental results, no abnormality in the visual observation. Each rat was perfused fixation after neuropathologic alterations assessed. Because it is required to examine by observers to the histopathologically.

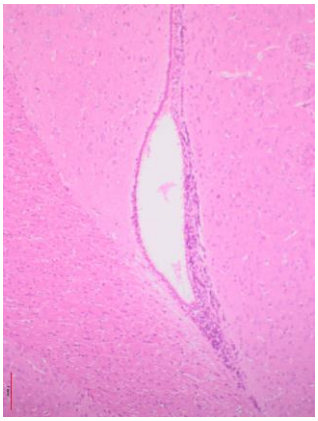

**Figure 1:** *Lateral ventricle of the rat*

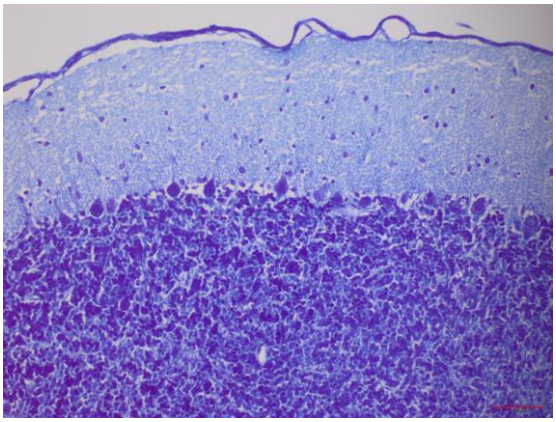

**Figure 2:** *Cerebellar Purkinje cell*

As indicated in Figure 1, we observed an abnormity of thickening chorioid plexus in the lateral ventricles of the kainin-administered rats. And also, as shown in Figure 2, we can see dropouts of the cerebellar Purkinje cells. These results suggested that the brains of the kainic-acid administered rats, at a cellular level, developed abnormities similar to HIE. On the day of presentation, we intend to report the results of the plasma inhalation to these model rats, and the examinations by the X-ray CT scanner.

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# Analyses of Reactive Oxygen and Nitrogen Species induced by atmospheric pressure guided streamers in a physiological liquid medium

Dezest[1], Gazeli[1-2], Quinton[3], Arbault[3], Chavatte[1], Girard[4], Cambus[5], Valentin[4], Reybier[4], Nepveu[4], Svarnas[2], Bulteau[1*], Clément[1*]

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Cold Atmospheric Plasmas (CAPs) are now well-known for their potential applications in biology and medicine. Energy levels implied in such partially ionized gases are numerous (excited states, charges, electric field, temperature, …) and synergetic effects may have a crucial role in the interaction between CAPs and the living cells, depending especially on the environment (air and liquid around). In this study, one type of CAPs has been used to follow the production of Reactive Oxygen and Nitrogen Species in a physiological liquid medium (Phosphate Buffered Saline; PBS).

The process consists in the propagation of ionization waves guided by a dielectric ceramic tube and has been named Atmospheric Pressure Guided Streamers (APGS, so called plasma jets in the literature). The electrodes configuration and the pulsed electrical parameters are fixed. Helium has been used as plasma carrier gas but small percentages of nitrogen or oxygen have been added in order to modify the chemical reactivity.

Optical emission spectroscopy has been used to characterize APGSs produced, allowing the identification of some specific excited states (NO, OH, $N_2$(SPS), $N_2$(FPS), $N_2^+$(FNS), O) as well as rotational and vibrationnal temperature of molecular probes.
Electrochemical analyses and ESR (Electron Spin Resonance) analyses have been realized in order to identify and to follow the production of long and short-lived reactive species as a function of exposure time. Durable chemical activity induced in PBS is analysed, leading to a better understanding of plasma/liquid interactions.

# Molecular Mechanism of Plasma-Induced Chemical Reaction on Protein and Amino Acid in Aqueous solution

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Plasma medicine is an attractive new research area, but fundamental understanding remains elusive. It is noteworthy to consider how the plasma generated active species react with biomacromolecules in aqueous solution. From the viewpoint of biochemistry, we investigated the chemical effects of low-temperature atmospheric pressure on biomolecules in solution [1, 2]. Low-frequency (LF) plasma jet was irradiated to the surface of protein solution, and then the enzymatic, structural, and chemical changes of the protein were analyzed using lysozyme as a model protein. The enzymatic activity, which correlates with a state of protein, decreased as the plasma exposure time (Fig.1). As protein conformation influences enzyme activity, the treated lysozyme was analyzed by circular dichroism (CD) spectroscopy. The CD spectra showed that the secondary structure slightly changed to the random coil structure. While the enzymatic activity of lysozyme decreased by 67% after the plasma treatment for 30 min, MALDI-TOF MS spectrometry revealed that the molecular weight (14,306) increased by only about 90, implying chemical modifications of lysozyme. These results indicate that the inactivation of lysozyme was related to the denaturation of native structure due to the slight chemical modifications on the protein. Considering that protein consists of amino acids, the chemical modifications of lysozyme resulted from chemical modifications of amino acids. For further fundamental understanding of molecular mechanism, competitive reaction experiment using 20 kinds of amino acids mixture solution was done. It showed that sulfur-containing amino acids (Met and Cys) and aromatic amino acids (Trp, Phe, and Tyr) were preferentially degraded by the plasma treatment (Fig.2), suggesting that the oxidation of these amino acids triggers inactivation of the protein. This is the fundamental step for elucidating chemical reactions by plasma, but it is essential for understanding the effect of plasma to human body.

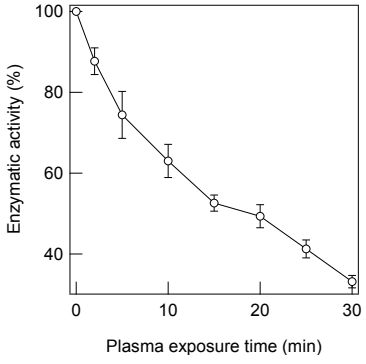


**Figure 1:** *Inactivation of enzymatic activity (lysozyme) by plasma treatment.*

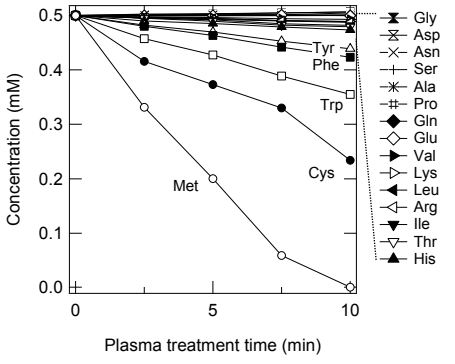


**Figure 2:** *Concentration reductions of 20 kinds of amino acids by plasma treatment.*

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# Interaction of Ambient Air Corona Discharges with Aqueous Solutions and Simple Biomolecules

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Non-thermal atmospheric pressure plasma devices are gaining increasing attention for medical applications wherein the delivery of reactive nitrogen and oxygen species (RONS) is desired [1], [2]. Numerous studies have shown the importance of RONS in biological systems, from the inactivation of bacteria [3] to the intricacies of cell signaling pathways [4].

While RONS are known to be important in biological systems, less is known about the conditions under which specific reactive species are transported to or formed in the liquid phase. Further study of the fundamental interactions between gas phase plasma (and the species generated) and liquids (typically aqueous solutions) is needed. Understanding the mechanisms by which reactive species are formed in the liquid phase will aid in the rational design of plasma devices for treating aqueous systems or tissues, and may enable the delivery of tailored cocktails of reactive species.

In this study, we focus on the interaction between atmospheric pressure air corona discharge of both polarities and an aqueous electrode (either distilled water or phosphate buffer solution with a submerged wire to ground). The geometry of the corona discharge allows direct contact between the air plasma and aqueous solution (above a submerged electrode), and also produces a slight convective air flow (ionic wind) which aids in the transport of gaseous species into the solution. The bactericidal effects of a similar device have been previously demonstrated [5]. The reactive species present in the treated aqueous solution are identified (ozone, nitrites, nitrates, hydrogen peroxide) and their mode of generation discussed (formed directly in the liquid or partitioning across the gas/liquid interface).

We also examine the effect of the reactive species generated on aqueous solutions of simple biomolecules (amino and nucleic acids). The reactive species present in solution and their effects on small biomolecules were probed using techniques including UV-vis absorption spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and electrospray ionization mass spectrometry (ESI-MS). These results will serve as a platform to investigate the effects of direct discharges on peptides and other biomolecules in aqueous systems.

The authors would like to acknowledge Dr. Anthony Iavarone[1] for his assistance in obtaining high resolution mass spectra of the amino and nucleic acids treated in this study. This work was supported by the Department of Energy, Office of Fusion Science Plasma Science Center.

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# A comparative *in vitro* study of different non-thermal atmospheric pressure plasma-jets concerning cell adhesion capacity on rough titanium alloys

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In implantology the use of titanium (Ti) implants with rough surfaces are common, but implant-related infections caused by bacterial biofilms are a growing problem. In addition to surface cleaning, a re-establishment of surface characteristics is essential to create the preconditions for bone regeneration [1]. Previous research showed the creation of a hydrophilic and thus cell-adhesive surface after treatment with atmospheric pressure plasma operated with argon and an admixture of 1 % of oxygen [2]. The objective of this experimental study was the comparison of five different non-thermal atmospheric pressure plasma-jet sources concerning their influence on cell growth.
For the experiments, Ti alloy samples (Ti6Al4V) with corundum blasted surfaces [3] (diameter of 11 mm, DOT GmbH, Germany) were used. Cellular experiments were performed after treating the Ti samples with plasma (duration 5 min), using different non-thermal atmospheric pressure plasma jets working with argon and 1 % of oxygen admixture (kHz-Jet, kINPen08, kINPen09, kINPenMed) (INP Greifswald, Germany) For technical reasons the kHz-Jet were arranged centrally atop the Ti samples. Whereas the other devices were set into a three dimensional table, moving the plasma-jets in straight lines with a distance between the lines of 0.5 cm. Subsequently human osteoblastic cells MG-63 (ATCC) were cultivated on the plasma-treated Ti samples (30.000 cells per sample) for 60 min and 24 h in complete Dulbecco´s Modified Eagle Medium with 10 % FCS at 37 °C, 5 % $CO_2$. Cell morphology was investigated by scanning electron microscopy. The cell area was then analyzed using ImageJ.

The cell areas indicated that a treatment of the Ti samples with kINPen08 results in significantly larger cells after 60 min of cultivation (kINPen08: 658 ± 450 µm²; control: 427 ± 166 µm²; $p = 0.006$). It seems that many of cells are able to spread faster, which is indicated by the relatively high standard deviation. With exception of the kHz-Jet (615 ± 271 µm²), cell areas using kINPen09 and kINPenMed were similar to the control (427 ± 166 µm²).

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# Estimation of Radical Intensity and Apoptosis Induction Activity of Aqueous Media using Single-Molecule DNA Measurement

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Recently, non-thermal atmospheric pressure plasma has been studied in biological and medical applications. Especially an atmospheric pressure plasma jet (APPJ) is widely used because it can treat subjects without thermal loading, and length of the plasma jet can be adjusted by flow condition of noble gas. Among them, reactive oxygen species (ROS) in water injected by the plasma exposure play an important role. To elucidate the cellular responses induced by exposure to NTP, we focused on (1) identification and quantification of reactive chemical species in aqueous media using electron spin resonance (ESR) spectroscopy with spin trap agents such as DMPO, (2) strand breaks on large DNA molecules suspended in aqueous media estimated by a single-molecule-based method [1], and (3) cellular response of mammalian cells (viability and apoptosis induction). In ESR experiment, we observed DMPO-OH spin adduct in the plasma-treated liquid. Then the correlation between DMPO-OH signals intensity and the number of strand breaks obtained from single-molecule DNA measurement was examined. The signal intensity was highly correlated with the number of strand breaks. Furthermore, apoptosis induction in human lung cancer cells was observed after the APPJ exposure under the same condition of the above two experiments. Our single-DNA-based analysis could be used in estimation of ROS intensity and apoptosis induction in cancer cells.

This work was partly supported by Grant-in-Aid for Scientific Research on Innovative Areas "Plasma Medical Innovation" (24108005) from MEXT, Japan.

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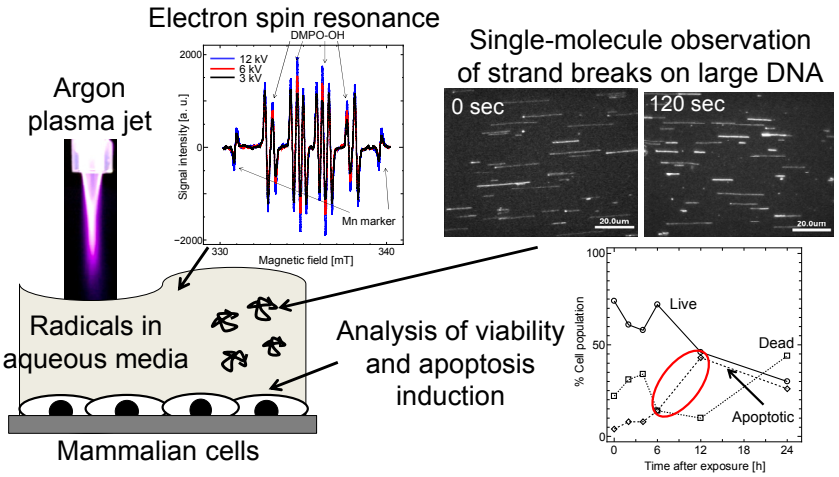


**Figure 1:** *A schematic overview of this study and the typical results of three experiments*

# UV absorption of water induced by APPJ irradiation

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Atmospheric-pressure plasma jets (APPJ) is recently well studied and proved its potential to use in many applications at plasma-bioscience interface since non-thermal characteristic. Typically, gas temperature $T_g$ of APPJs is room temperature around 300 K [1]. Here we modified distilled water by an APPJ of helium and investigated transmittance of the liquid by a UV-VIS spectrometer (U-3900, Hitachi).

Configuration of the atmospheric-pressure plasma source is similar to some previous works [2][3]. The plasma consisted of a glass capillary (2.4 mm inner diameter) and a copper ring electrode, and it working with a bipolar high voltage pulse of 7 $kV_{p-p}$ at a fixed low frequency of 10 kHz. Helium gas flow rate of 5 L $min^{-1}$ was fixed, thus gas speed of around 20 m/s was calculated. For safety use of a high voltage driven APPJ, an electrode was set in a Teflon unit as shown in figure 1. Transmittance of water was investigated in a wide range between 190 nm and 900 nm with a resolution of 0.2 nm.

As results, we observed no difference the transmittance in near infrared and visible ranges. However, transmittance of UV region was significantly decreased as shown in figure 2. The results show the measured transmittances in both cases 3-mL-water: one was exposed by the APPJ for 30 min and the other was unexposed. We here assume the reflectance is no change, then the absorbance of the plasma treated water was calculated as use of a transmittance of the distilled water as background. UV absorption increased as increase of exposure time up to 30 min, as shown in figure 3. The possible reasons are now investigating, it may be strongly correlated by reactive oxygen species (ROS) created by an interaction between an APPJ and ambient air species [3], and then ROS were diffused into the water.

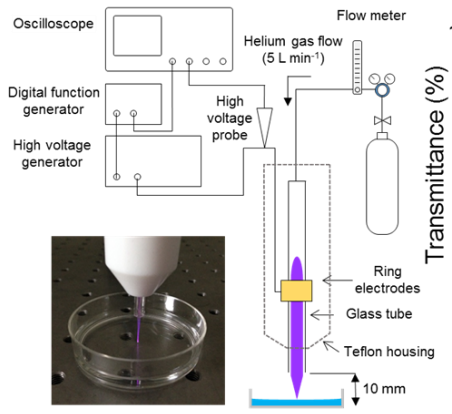


Figure 1

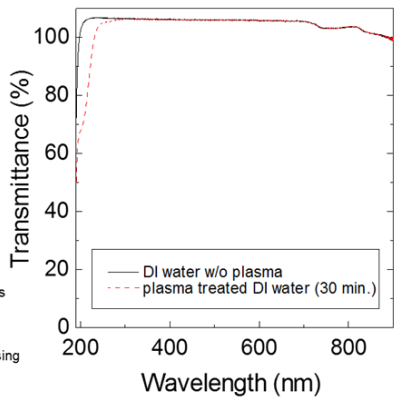


Figure 2

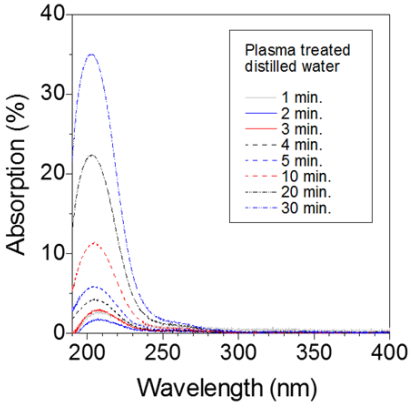


Figure 3

**Fig. 1** schematic of an experimental setup for plasma treated water. **Fig. 2** UV-VIS transmittance of distilled water and plasma treated water (30 min.) of 3 mL. **Fig. 3** UV absorption increased as increase of exposure time.

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# Determining the effect of plasma on bacterial DNA at the single cell level

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Plasma is an effective delivery system of reactive nitrogen and oxygen species (RNOS) that can be deleterious for bacteria as they damage cell membranes, proteins and nucleic acids [1]. Previously, oxidative damage was shown in DNA isolated from liquid cultures of the bacterium *Escherchia coli* that was exposed to plasma [2]. However, this reflected the average of the DNA in the population, and spatio-temporal variations in occurred damage may take place.

**AIM:** To demonstrate for the first time the effect of plasma on bacterial DNA integrity at the single cell level, using a low-temperature atmospheric pressure dielectric barrier discharge jet configuration on *Salmonella enterica*.

**RESULTS:** Adapting the DNA damage diffusion assay for plasma experiments, DNA damage was observed in *S. enterica* located up to 40% of the radius of the kill zone. This was plasma treatment-dependent ($P<0.001$) (Fig. 1) with gas-treated and DNAse I recombinant-treated controls showing no spatial variation. Such effect on DNA is not likely to be due to UV radiation alone, as UV radiation above 121 nm alone did not induce DNA damage or affect bacterial growth.

**CONCLUSIONS:** Our study at the single cell level shows that plasma can induce DNA damage in a 2D spatial-dependent fashion. UV radiation generated by the plasma participates in the chemical reactions for the generation of RNOS but would not be solely responsible of the DNA damage and inactivation that was observed here for *S. enterica*. Our results provide insights on the mechanisms of action of plasma in biological samples and highlight the importance of spatial distribution of RNOS in bacterial elimination.

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# Degradation of fatty acids by nitrogen flowing afterglows at reduced pressure

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Flowing afterglows at reduced pressure (1-20 Torr) are able to produce large amounts of reactive (atoms, metastable states) and radiative species at room temperature, which confer them interesting properties for the decontamination and the sterilization of the reusable medical instrumentation. During the last decade, mixtures of nitrogen and oxygen were extensively studied to this end and have demonstrated their anti-bacterial capabilities. More recently, pure nitrogen afterglows were investigated with the advantage to reduce the oxidation of the exposed materials.

In the present study, considered as a first step to get a better comprehension of the interaction between the N-atoms and biological organisms, different saturated (palmitic 16:0 and stearic 18:0) and unsaturated (oleic 18:1 and linoleic 18:2) fatty acids, considered here as model bio-molecules, have been exposed to the nitrogen afterglow flow.

We report that 15' of nitrogen afterglow exposure of increasing linoleic (18:2) and oleic (18:1) fatty acid concentrations (200 to 800 nmoles), allowed to completely remove 100% of these fatty acids, in contrast to the saturated palmitic (16:0) and stearic (18:0), which were not or only slightly altered (minus 10 and 2%, respectively). Gas chromatography and mass spectrometry analysis revealed the absence of degradation products with lower molecular weight. Likewise, we did not detect any lipid oxidation product formation, nor cytotoxicity for vascular cells, in the remaining fatty acid mixture after nitrogen afterglow treatment.

We checked whether the treatment alters the metabolism of remaining fatty acid. For this purpose, 800 nmoles of the fluorescent pyrenedodecanoic fatty acid (P12, an analog of oleic acid), were exposed to the nitrogen afterglow flow. A 30 min. treatment generated more than 75 % of P12 loss, comparable to the data observed in a mixture oleic acid/stearic acid (75:25). The remaining P12 metabolism in vascular endothelial cells, was significantly decreased, accordingly, by comparison to untreated P12.

These data suggest that interactions formed between N-atoms and fatty acid double bounds, induce a degradation into shorter undetectable degradation products, whereas saturated fatty acids are not or poorly altered. These experiments allow to decipher in part, the physical mechanisms of degradation evoked by nitrogen afterglow treatment on biological agents constitutive of eukaryotic cells.

**Aknowledgments**

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# Bactericidal and wound healing properties of the air plasma generated by the ferroelectric generator

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The work put its aim to study bactericidal and wound-healing effects of the afterglow produced by the ferroelectric reactor. The ferroelectric reactor is a container made of inert fluorocarbon polymer with two wire mesh electrodes and filled with spherical granules of alloyed $TiBaO_3$ that has a dielectric constant ranged 1000-10000 in dependence on the type of alloying. The construction of the reactor prevents UV irradiation of a treated surface. Pathogenic bacteria *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria monocytogenes* and the non-pathogenic *Escherichia coli* strain with the mutation in the reparation system were used.

The composition of the afterglow formed by microdischarges between granules was dependent on a feeding gas. There was no any oxygen or nitrogen active species when argon was used as feeding gas that determined absence of biological effects. Afterglow of air plasma was dependent on gas velocity. Nitrogen monoxide NO concentrations measured at 1 cm distance from the source decreased from 350 to 0 ppm upon velocity increased from 0 to 10 l/min. In contrast, the ozone $O_3$ concentration increased with increasing of air velocity. The maximal bactericidal effect was achieved at stationary conditions with 0 l/min air velocity after 5 minutes of treatment. It ranged from 24-fold (for the *S. aureus* Sa 78) to 813-fold (for *E. coli* JM 109) reduction in colony counting. Prolongation of treatment up to 30 minutes did not cause further drop in amount of survivors that is in line with known bactericidal properties of NO. Increasing of air velocity decreased a bactericidal effect.

A murine model of infected superficial slash wounds was used to assay wound healing activity of the ferroelectric generator. The air plasma afterglow formed under stationary conditions was applied to wounds infected with *S. aureus*. The reduction of bacterial loads after single 10 min treatment ranged from 6,9 to 124,5 folds (the mean 44, $p<0,05$). The statistically relevant increase in wound construction was observed starting from the 2$^{nd}$ of daily treatments.

Obtained data suggested that the afterglow produced by the ferroelectric generator possesses bactericidal and wound healing properties. Absence of UV irradiation makes it safer in comparison with other NO generator based on plasma technologies.

# Influence of plasma-treated liquids on structure and function of lipid membranes

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The interface of every cell to the environment is the membrane. Due to this, it is the first interaction site of any externally applied substances including reactive species in plasma-treated liquids. Proteins and lipids are the constituents of cell membrane, the later responsible not only for the structural properties e.g. barrier function but also involved in signaling. Lipid peroxidation is a well-known process of oxidative substances such as reactive nitrogen and oxygen species.

Here we describe experiments on biological relevant liquids treated with the cold atmospheric plasma source kinpen equipped with a device that isolates the jets effluent from surrounding atmosphere. By this, the gas composition interaction with effluent can be chosen and therefore the chemical production pathways controlled. As a result, the reactive species concentration and composition that is generated in liquids can be designed, a necessary step for controlled cellular reactions due to plasma-treatment.

Furthermore, we show experiments on the effect of plasma-treated liquids on lipids and lipidic model membranes like liposomes and solid-supported bilayer. Used methods include Raman microscopy, chromophore based light spectroscopy, dynamic light scattering, and small angle x-ray scattering (SAXS). The influence of plasma-treated liquids on lipid model membranes was an increased of bilayer thickness, biphasic change of fluidity, stable liposomes due to zeta-potential, and lesion formation.

A detailed molecular mechanism for the observed formation of transient lesions is proposed based on our experimental findings and published literature. These lesions allow a “self-mediated in- and efflux” of plasma-born reactive species and cell’s signaling molecules. Biological consequences are discussed.

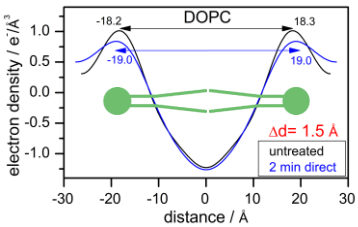


**Figure 1:** *Increase of bilayer thickness after plasma-treatment as measured by SAXS.*

The Authors acknowledge the funding by the BMBF (03Z2DN12) within the ZIK plasmatis at the INP Greifswald.

# Stimulation of Intracellular Reactive Oxygen Species in Uniform and Non-Uniform Regimes of Nanosecond Pulsed Dielectric Barrier Discharge Treatment

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**Research Aims:** Non-thermal plasma treatment of cells have been efficacious in areas of bacterial sterilization, cancer treatment, and even wound healing. While many of these studies have utilized plasma jets, direct plasma treatment with dielectric barrier discharges (DBD) have also been proven to be useful and sometimes even more effective [1]. However, DBD plasma treatment studies have been done with microsecond pulsed power supplies which generate filamentary structures in the discharge; the resulting plasma is in the non-uniform regime. Nanosecond pulsed plasma has been shown to generate diffuse discharge, without filaments[2]. Not only do these filaments in the non-uniform regime lead to uneven treatment of cells, but they also generate high electric fields which result in higher electron energies compared to those in the uniform regime. The electron energies influence the plasma generated species, and so changes in the plasma regime can also influence plasma chemistry. Therefore, it is crucial to understand how the different regimes of plasma influence cellular response. The goal of this study was to compare the effects of uniform and non-uniform nanosecond pulsed plasma treatment of C3H-10T1/2 mesenchymal cells [3]. Cell viability and intracellular reactive oxygen species were measured to assess safety of treatment and cell stimulation. **Methodology:** The C3H-10T1/2 mouse mesenchymal cell line was chosen for these experiments. The cells were treated in 6-well plates and stained with two fluorescent markers: MitoSOX Red, which detects $O_2^-$ in the mitochondria and propidium iodide, which is a live/dead stain. The energy per pulse was measured at 29.4kV for both uniform and non-uniform discharges and frequency adjusted so the total energy delivered to the cells (dose) could be compared between regimes. Data collection and analysis were performed using image cytometry and flow cytometry software (FCS), respectively. **Results:** Initial studies showed that uniform treatment increased intracellular $O_2^-$ while cell death remained the same for treatment in both regimes. **Conclusion/On-Going Work:** The uniform regime of plasma may be more efficacious in stimulating intracellular ROS with lower doses. On-going work include studying the roles of different plasma generated species for stimulation of ROS in cells. **Acknowledgments:** We acknowledge Dr. Theresa Freeman for her suggestions. This project was in part funded by NIH Grants 1 R01 EB 013011-01 (Freeman).

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# Degeneration of amyloid-ß fibrils in aqueous solution by low-temperature atmospheric-pressure plasma

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Alzheimer's disease (AD) is characterized pathologically by proteinaceous deposits mainly consisting of fibrils of amyloid-ß (Aß) peptides in the brain. Aβ fibrils are toxic fibrillar β-sheet-rich aggregates of protein. Aβ fibrils are highly stable, and undegradable by protease as biological defense mechanisms. We have reported the inactivation of protein in aqueous solution due to the chemical modification by low-temperature atmospheric pressure plasma (LTAPP) [1]. Here we demonstrated that LTAPP treatment changed the protease resistance, ß-sheet structure, and surface properties of Aß fibrils in solution using an *in vitro* system [2]. Our results indicate that Aß fibrils were not directly degraded by plasma exposure but modified to be easily degraded by biological systems.

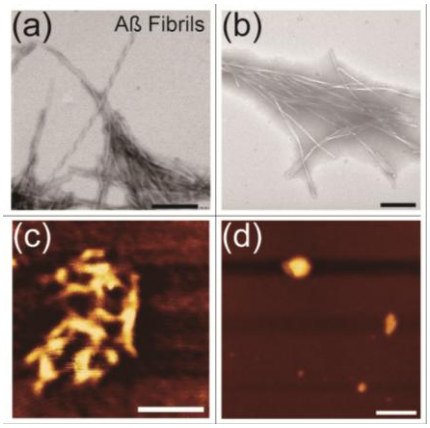


**Figure 1:** ***(a, b)*** *The effect of plasma treatment for morphology. TEM images of Aβ fibrils (a) before and (b) after the plasma treatment for 30 min.* ***(c, d)*** *The effect of plasma treatment for protease resistance. AFM images of the Aβ fibrils that were treated to the plasma for (c) 0 min and (d) 30 min and then incubated with a protease (trypsin) for 24 h. The scale bars represent 200 nm.*

A low-frequency plasma jet was used for the LTAPP processing in a similar system as described previously [1,3,4]. Considering the wet condition of body fluid, the Aß fibrils should be treated as solution by LTAPP, which generates active species in solution. To investigate the effects of plasma exposure for the protease resistance, we added a protease (trypsin) to Aβ fibrils that were treated to the plasma for 0 min and 30 min, respectively. TEM and AFM images showed that the protease resistance of the Aß fibrils was degraded by plasma exposure, although the morphology of the Aß fibrils was unchanged by plasma exposure of several minutes (Fig.1). The ellipticity at 218 nm in the far-UV circular dichroism (CD) spectra of the Aß fibrils decreased with increasing plasma-exposure time, i.e. the regular cross-ß structure of the Aß fibrils was destroyed. Fluorescence assay using a hydrophobic probe, 8-anilino-1-naphthalenesulfonic acid (ANS), showed that surface hydrophobicity of Aß fibrils was decreased by the plasma exposure. These results indicate that the degeneration of Aß fibrils induced by plasma expose would cause the loss of their protease-resistant property. In conclusion, the degeneration of Aß fibrils in solution can be achieved by plasma treatment and the fact suggests prospects for utilizing LTAPP for the elimination of neuritic plaque associated with AD by accelerating the proteolysis of Aß fibrils.

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# Evaluation of oxidative stress inside cell membrane by the penetration of HOO radical with the reduced pH method for plasma disinfection

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Plasma disinfection is required the inactivation bacteria in liquid. We have developed an effective method, reduced pH method [1], which can inactivate bacteria 100 times faster in lower pH condition than in neutral pH of body fluid. Super oxide anion radical ($O_2^-$•) was thought to key species from experiments with radical scavenger SOD (superoxide dismutase). The low pH increases the penetration rate of radicals across the cell membrane. In this paper, we discuss the inactivation rate of bacteria and the oxidative stress by an artificial model cell constructed by micelle, based on the concentrations of radicals at various pH values.

Three kinds of bacteria were inactivated by plasma treatment under various pH conditions. Bacterial inactivation rates were proportional to concentrations of hydroperoxy radicals (HOO•) calculated by the theoretical model of chemical reactions (Fig. 1). Although the supply rate of $O_2^-$• is constant, the concentration of HOO• is theoretically calculated to higher in lower pH. To evaluate the penetration of radicals into the cell membrane, a bacterial model using dye-included micelles was used. Decoloration rates of the model were also in proportion with the calculated HOO• concentrations (Fig. 2). The molecular mechanism is as follows. $O_2^-$• in liquid supplied by plasmas is changed into HOO• with acid dissociation equilibrium (p*K*a 4.8). The electrically neutral molecule of HOO• is more permeable across the cell membrane than $O_2^-$•. Thus, we concluded that the high permeation of HOO• into the cell membrane plays a key role for efficient bactericidal inactivation using the reduced pH method [2]. For further understanding of plasma medicine, discussions must be based on not the parameter of plasma generation but that of active species.

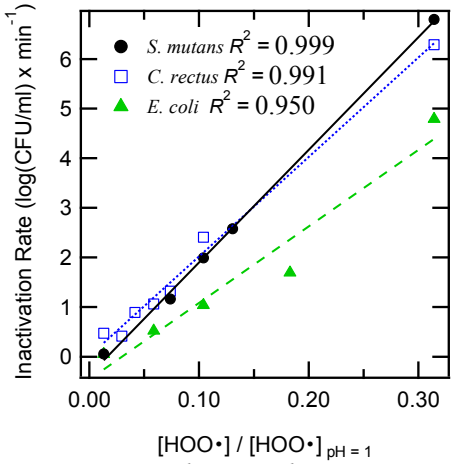


**Figure 1:** *Correlations between inactivation ratios of bacteria and [HOO•].*

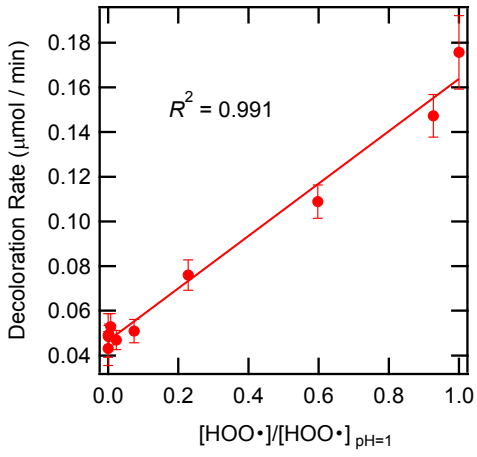


**Figure 2:** *Correlation between decoloration ratios of micelle and [HOO•].*

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# Analysis of intraperitoneal application of TTP on murine small bowel

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**Introduction:** Microorganisms have developed multiple resistances against many antibiotics threatening the survival of patients suffering from severe infections. Therefore, the development of novel anti-infective substances is a central topic in medicine. The application of tissue tolerable plasma (TTP) could prove useful as a novel technique in decontaminating infected tissues [1]. However, when using TTP it has to be born in mind that TTP may also cause a dose-dependent damage of tissues and that different tissues may show different susceptibilities to TTP treatment.

**Aim:** To investigate the tolerance of murine bowel tissue to TTP: TTP has potential intra abdominal value in decontaminating the abdominal cavity after gastrointestinal perforation or following post-surgical leakages after surgery of the gastrointestinal tract.

**Methods**: Female 10 – 12 weeks old C57Bl/6 underwent laparotomy. The bowel was carefully moved out of the abdominal cavity and placed onto a special plotting device. The plasma source kINPen Med was employed in pulsed mode technique using argon as the carrier gas. TTP administration followed a standardized protocol using different application time intervals and flow rates. Control groups were treated with laparotomy and gas flow only or laparotomy only. Macroscopical as well microscopical analyses were carried out at time points 1h, 24h, and 7 days following initial experiments.

**Results:** The application of TTP up to 48s on murine bowel led to hyperplasia and thickening of the muscle layer. Also, an inflammatory reaction of the peritoneal layer was observed. This included the presence of a leucocytic infiltrate. Further changes could not be detected. No signs of necrosis or any other damage was seen. However, a similar hyperplasia and thickening of the muscle layer of the bowel was found following treatment using argon gas flow only as well as following sham operations. In fact, no significant differences of wall thickness could be measured. In addition, the above inflammatory changes of the bowel could be observed in both groups, too.

**Conclusion:** TTP is an exciting new treatment option not affecting the usually vulnerable bowel. It may prove useful in the treatment of different forms of peritonitis using TTP as a possible adjuvant in bacterial decontamination.

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# Efficacy and Safety Considerations for the Use of Atmospheric Cold Plasma in Wound Treatment

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Atmospheric Cold Plasma (ACP) produced by a dielectric barrier discharge (DBD) effectively generates reactive species capable of inducing a range of bio-medically relevant interactions [1]. These include triggering natural mechanisms of blood coagulation [2], stimulating cell proliferation [3] and broad-spectrum antimicrobial effects against free and biofilm associated microorganisms [4]. Thus this technology has promise for wound healing applications. While the ability of cold plasma to coagulate blood despite the presence of anti-coagulants has been demonstrated [2], the underlying biochemical mechanisms plasma-assisted blood coagulation are largely unknown. There is little evidence quantifying blood clot formation and or the impact of system parameters on the intensity of the blood clot. In this study parameters of distance from plasma source, treatment time and plasma gas composition were investigated and the resulting clots were quantified using hemoglobin absorbance measurements and compared for a plasma jet and a dielectric barrier discharge (DBD) system. Optical emission spectroscopy was employed to correlate species generated in the plasma effluent to the impact on coagulation cascade. A maximum treatment time of 4 continuous minutes is required for blood clot formation. Irrespective of other process parameters, increasing the distance from the plasma source has a negative impact on blood clot formation.

Mammalian cells also show high sensitivity to oxidative stress from plasma induced reactive species. Safe operating windows for plasma devices in wound treatment not only depend on parameters guiding blood coagulation or microbial inactivation efficacies but also on the absence of cytotoxic effects in surrounding tissues. The cytotoxicity of DBD-ACP was assessed as a function of treatment times using HeLa cells as a model cell system. Cytotoxicity was strongly dependent on the treatment milieu. In the absence of serum, exposure to ACP for longer than 30 seconds at 70kV strongly inhibited the HeLa cells' ability to re-adhere to surfaces and abrogated cell growth. This suggests that serum proteins possess strong scavenging potential for reactive species and that cytotoxic activity is retained if the culture medium is not replaced post-treatment. Results from these studies will guide development of novel plasma devices for wound treatment applications.

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# Proliferation mechanism of budding yeast cells with oxygen radical treatment

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Various stimuli or stresses cause different responses of microorganisms, such as activation, functional depression, and cell death, depending on the dose or flux. On the basis of measurement of the densities of neutral oxygen radicals, such as ground-state atomic oxygen [$O(^3P_j)$] and singlet oxygen molecule [$O_2(^1\Delta_g)$], we showed that the $O(^3P_j)$ is the dominant species responsible for the inactivation of *P. digitatum* spores quantitatively[1][2]. In addition, we investigated the effects of oxygen radicals on the activation of the budding yeast cells, which is one of famous model organisms. We showed that oxygen radicals different effects, such as promotion, depression of cell growth and cell inactivation, according to the increase of dose of oxygen radicals[3]. In this study, we have investigated the mechanism of the promotion of the yeast cell growth treated with oxygen radicals in detail.

To eliminate the influence of atmospheric gases, the radical source and the sample were enclosed with a plastic cover. The budding yeast cells (*Saccharomyces cerevisiae* W303a) were suspended with phosphate buffered saline. The suspensions were treated with oxygen radicals 10, 15 and 20 mm downstream from the radical head for 30 s. The $O_2/(Ar+O_2)$ flow rate ratio and total flow rate were 0.6% and 5 slm, respectively. Recovered cells were arranged to be $1.0\times10^3$ cells/ml, and cultured with yeast extract peptone dextrose (YPD) medium at 30°C. We counted the number of cells every 24h.

Figure 1 shows the growth of yeast cells with oxygen radical treatment for 30 s as a function of exposure distance. According to the increase of the exposure distance, $O(^3P_j)$ density drastically decreased while $O_2(^1\Delta_g)$ density was constant[2]. Control cells were increased to be $2.8\times10^7$ cell/ml after 72 h. At the distance of 10 mm, the proliferation of the cells was promoted to be approximate 1.2 times. On the other hand, the promoted effects decreased with an increase in the exposure distance. These results suggest that the effects of $O(^3P_j)$ on the liquid phase lead to the promotion of the cell growth. We will discuss the mechanism of the yeast cell growth treated with oxygen radicals in detail.

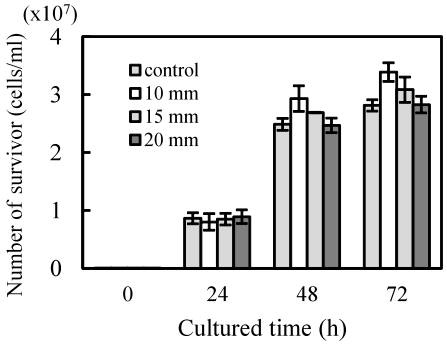


**Figure 1:** *The number of yeast cells treated with oxygen radicals at different exposure distances.*

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# Oxydative stress responses induced by atmospheric pressure guided streamers on bacteria *Escherichia coli*

Dezest[1], Gazeli[1-2], Chavatte[1], Salvayre[3], Cambus[3], Penny[4], Svarnas[2], Clément[1*], Bulteau[1*]

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Atmospheric Pressure Guided Streamers (APGS, so called plasma jets in the literature) are known to have bactericidal effects but the mechanisms of interaction are not well understood.

In this study the bacteria *Escherichia coli* is used as a model and exposed to APGSs, produced in helium and in electrical pulsed conditions. The cold atmospheric plasmas formed are optically studied by varying the carrier gas composition with adjunctions of small percentages of nitrogen or oxygen, in order to identify the main gaseous reactive species.

This way the different compositions of reactive gases are applied on suspensions of bacteria in order to follow their survival. First macroscopic observations indicate that APGSs induce bacteria death from decontamination up to sterilization levels. This result is emphasized by a remarkable delay effect: the 6log reduction in the bacteria concentration is obtained several hours after the APGS exposure. This delay effect is depending on the initial gas compositions and on the exposure time, suggesting different death processes, which are confirmed by MEBE analyses of the cellular structures.

Biochemical analyses of the bacteria have been thus realized in order to follow the oxidative stress induced by APGSs on the cell components. We show in this work chemical modifications on specific proteins, lipids but also on DNA and try to establish a correlation between these oxidation processes and the chemical reactivity of the gases in APGSs.

# Frugal Air Spark-like Plasma for Antimicrobial $NO_x$ Generation

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Air discharge plasma operated at atmospheric pressure can be utilized for a wide variety of potential applications at low cost using robust, well-established technology. We term these applications '*frugal plasma,*' thereby suggesting this is an example of *frugal innovation* [1-2]. Air plasmas can be operated to generate either primarily ozone ($O_3$) or primarily nitrogen oxides ($NO_x$), among other chemical species.

We demonstrate and analyze the generation of nitrogen oxides and their antimicrobial efficacy using atmospheric air spark-like plasmas in simple, inexpensive devices. Spark-like discharges in air in a 1 L confined volume are shown to generate $NO_x$ at an initial rate of over 2.2 x $10^{16}$ $NO_x$ molecules/J dissipated in the plasma. An inexpensive power supply dissipating about 12 W in this confined volume generates ~3000 ppm $NO_x$ in ten minutes. Over 90% of the $NO_x$ is in the form of $NO_2$ after several minutes of operation in the confined volume, suggesting that $NO_2$ is the dominant antimicrobial component. The strong antimicrobial action of the $NO_x$ mixture after several minutes of plasma operation is demonstrated by measuring rates of *E. coli* disinfection on surfaces and in water exposed to the $NO_x$ mixture. The spark-like discharge systems generating these species can operate with inexpensive power supplies, simple automotive spark plugs, and relatively small sources of electricity that could be provided by compact solar panel systems to recharge modest-sized batteries. [3]

Some possible applications of frugal plasma generation of $NO_x$ (perhaps followed by dissolution in water) include disinfection of surfaces, skin or wound antisepsis, and sterilization of medical instruments at or near room temperature. This is especially promising for circumstances in which conventional sterilization, disinfectant, or antiseptic supplies are not available, such as in emergency conditions, refugee camps, or isolated, low-resource settings in general.

**Figure 1:** *Photo of the 5-mm spark-like discharge in an automotive spark plug.*

This work was supported in part by the Department of Energy, Office of Fusion Science Plasma Science Center. ZM was supported by the Slovak-American Foundation.

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# Investigation of biological effect and toxin degradation using temperature controllable multi-gas plasma jet

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In recent years, atmospheric non-thermal plasma has attracted attention in the medical field because of effective and fast sterilization, blood coagulation and wound treatment. It is thought that the active species, which are generated by the plasma, significantly contribute to these results. However, the generated active species depend on the plasma gas species, with conventional plasma sources placing limits in the generation of active gas species, and the effect of gas composition on the plasma is not well studied. To generate various gas plasmas, the multi-gas plasma jet source was developed in our laboratory[1]. It can generate stable plasma with helium, argon, oxygen, nitrogen, carbon dioxide, air and their mixture gases under atmospheric pressure. In addition, it can generate stable plasma with desirable gas temperature from below freezing point to over 100ºC with independent of the discharge power using lab made temperature controllable system[2].

In this study, effects of plasma treatment on bacteria, living cells and toxin were investigated using the temperature controllable multi-gas plasma jet. To investigate sterilization effect in liquid, *Candida albicans* was treated with temperature controlled 20ºC various gas plasmas. As a result, nitrogen plasma exhibited the optimal capability for bacterial inactivation, and *C. albicans* with a population of $10^6$ was sterilized within 60 s. As plasma treatment to living body, fibroblast cells of mouse were treated with nitrogen gas plasma. The survival rate was almost no change with each 20ºC plasmas, by contrast, survival rate was decreased with each 60ºC plasmas as shown in Fig. 1.

From the aspect of detoxication, we proposed the use of multi-gas plasma for the decomposition of Tetrodotoxin (TTX), which is known as puffer fish toxin and resistant to decomposition by either heat or chemical reaction. TTX solutions were treated by various gas plasmas and analyzed by LC-ESI-TOF-MS. The TTX mass spectrum signal was reduced by plasma irradiations like fig. 2. Nitrogen plasma demonstrate 80% decomposition of TTX in 5min. Therefore, the plasma jet is expected to use for sterilization and toxin removal of heat-sensitive targets such as foods and living body. The details of the results of these experiments will be presented.

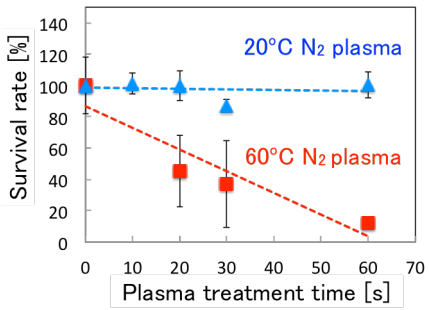


Fig. 1. Survival rate of fibroblasts by high and low gas temperature of $N_2$ plasma treatment

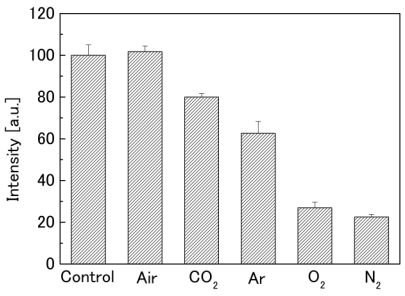


Fig. 2. Decomposition of TTX by 5min various gas plasma treatment

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# Atmospheric Pressure, Non-Thermal Plasma for Control of *P. aeruginosa* Biofilms: Effect of Biofilm Components on Phenotypic Resistance

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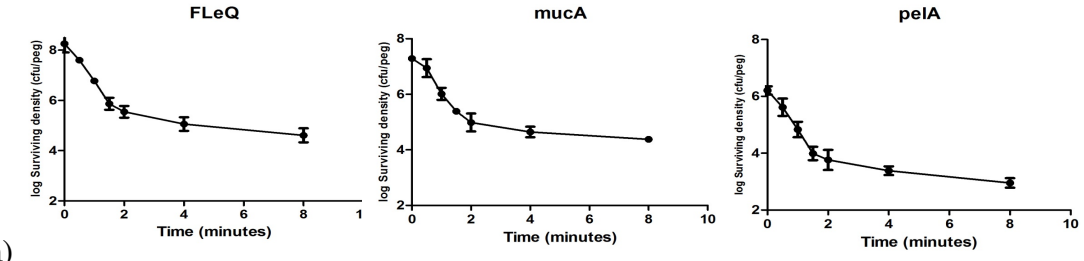
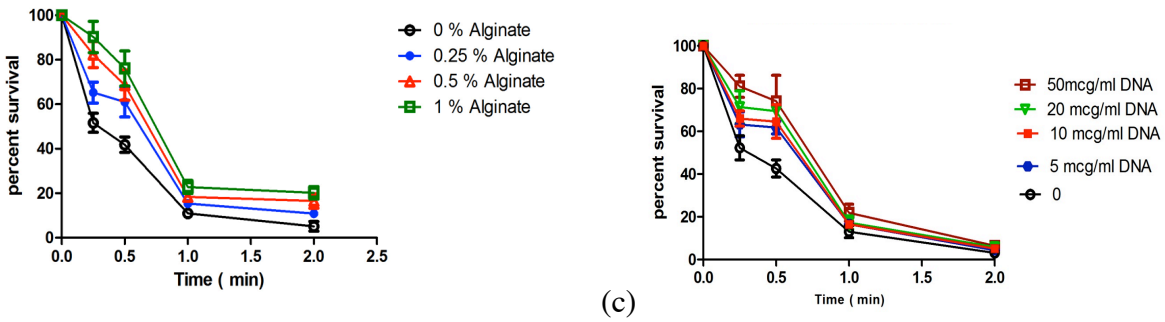
The utilization of atmospheric pressure nonthermal atmospheric plasma (APNTP) represents a novel method for sterilization of surfaces contaminated with high resistant microorganisms [1]. In this study we have investigated the phenotypic and genotypic factors mediating elevated tolerance to APNTP observed in *P. aeruginosa* biofilms. In addition, the effect of gene knockouts of specific adhesion/biofilm components (*fleQ*, *pelA* and *mucA*) on biofilm tolerance to plasma was evaluated.

Mucoid strains of P. aeruginosa exhibited increased phenotypic resistance than non-mucoid strains. This may be related to the protective role of overproduced alginate rich matrix in mucoid strains. The *mucA* mutant strain, which overproduces polysaccharide matrix, showed higher tolerance to plasma treatment than *pelA* and *fleQ* mutants. Addition of alginate and DNA to planktonic cells decreased the sensitivity to APNTP. This elevated tolerance confirms the role of extracellular biofilm components, such as alginate and eDNA in mediating the tolerance in sequestration of antibacterial species produced by the plasma.




( a)




(b) (c)

**Figure 1:** *(a)* Survival curve of mutant strains. (b) Effect of Alginate (c) Effect of DNA.

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# Comparative Study on the Use of Different Metal Electrodes in Low Pressure Glow Discharge Plasma Sterilization

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The sterilizing efficacy of low pressure direct current glow discharge hydrogen peroxide (H2O2) plasma generated by a planar parallel plate plasma source using the Plasma Enhanced Chemical Vapor Deposition (PECVD) facility was tested. This study compares the effect of using different metals (copper, stainless steel and aluminum) as electrodes in plasma sterilization of stainless steel dishes. Test samples were exposed to $H_2O_2$ plasma under different set of discharge currents and exposure times. *Bacillus subtilis* was used as the test organism and microbial analysis was made by means of the standard plate count method of serial dilution and pour plating. Evaluation of microbial death was done using survival curves, percent reduction and decimal reduction value. Results showed that sterilization using copper electrodes exhibited the highest decimal reduction value (D-value) and percent reduction among the three electrodes.

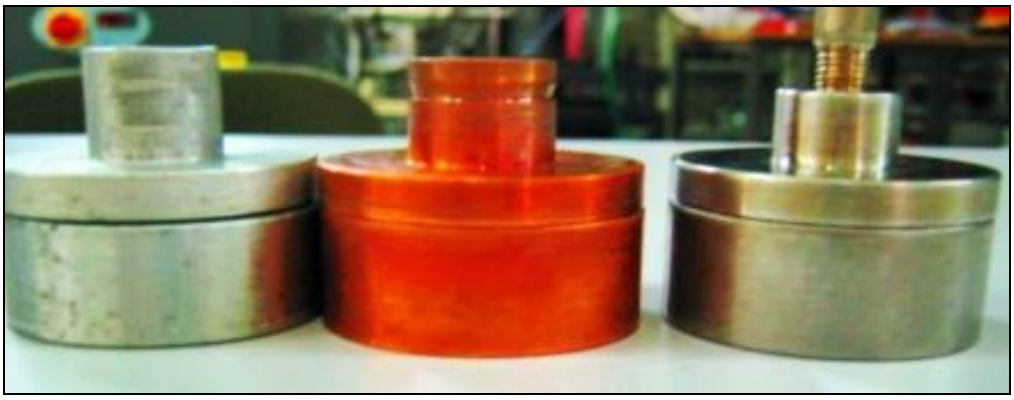

**Figure 1:** *Different types of metal electrodes in plasma sterilization: stainless steel, copper and aluminum*

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# Decontamination of the inner walls of a narrow tube at atmospheric pressure using long distance propagation discharge in argon

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Non-thermal plasma at atmospheric pressure is a useful tool for bio-decontamination because it produces reactive species (atoms, radicals, ions and stable species such as $O_3$ and $H_2O_2$) and UV emission, while temperature remains close to ambient. Therefore, non-thermal plasma technologies have been investigated for surface decontamination of thermally sensitive materials, in particular of small diameter tubes[1,2] that is of interest for medical applications. In this work, a pulsed corona discharge was propagated over 49 cm on the inner walls of a quartz tube (8 mm inner diameter) in which argon (Ar) was flowing at atmospheric pressure. A tungsten needle placed at the tube inlet was connected to a HV power supply that produced positive voltage pulses of up to 35 kV peak and 200 ns-5 µs duration at a rate of 500 pulses/s.


**Figure 1:** *Photograph of the argon pulsed discharge propagating over 49 cm tube*

*Escherichia coli* DH1 was used to evaluate the bactericidal effect; two droplets ($10^8$ bac./mL) were deposited on the tube inner surface, 2 and 44 cm from the HV electrode. In the case of pure Ar at 44 cm, 3 log reduction from the initial bacterial load was measured in 20 min; addition of 760 ppm of water in Ar enhanced the bactericidal effect to 4 log. We obtained full reduction (6 log) within 30 min of exposure in both cases. The effect of near UV emission (308 nm) alone was quantified by placing the bacteria on the outside surface of the quartz tube, and accounted for 7-48% of the plasma treatment bactericidal efficiency, depending on the sample condition (liquid/dry) and Ar water content. VUV (126 nm) is also expected to have an impact; however this was not quantified here. The effect of $H_2O_2$ was investigated; its aqueous concentration was 222 and 53 mg/L $H_2O_2$ at 2 and 44 cm, respectively, after 5 min exposure of water droplets to the humid Ar discharge. Incubation of bacterial cells in solutions with the same $H_2O_2$ concentrations for 5 min resulted in a 0.1 log reduction only (compared to 1.3 log with 5 min plasma exposure). The samples treated in Ar flow only controlled for the effect of anoxic conditions and desiccation accounted for less than 0.25 log reduction in 20 min. The temperature of the gas and quartz tube surface remained below 29°C. These results suggest that the major contributors to the measured bactericidal effect are OH radicals with synergistic effects with the secondary factors investigated in this study. Pulsed discharge plasma in argon is promising for bio-decontamination of inner walls of long tubes because it operates at atmospheric pressure without overheating the treated surfaces and does not produce toxic by-products such as ozone and nitrogen oxides.

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# Influence Investigation of Gas Temperature on Inactivation of Oral Bacteria using Temperature-controllable Plasma Jet

Takaya Oshita[1], Akitsugu Kawate[1], Toshihiro Takamatsu[1], Junki Yamada[2], Hidekazu Miyahara[1], Takashi Takiguchi[2], Matsuo Yamamoto[2] and Akitoshi Okino[1]

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There has been increasing use of atmospheric plasma for medical and dental application. Especially, treatment of living body such as sterilization is under active investigation because of the advent of non-thermal plasmas. To treat living body, the plasma with the following two characteristics is required. First, for killing bacteria efficiently, it would appear that high power plasma is required to produce more reactive species. Secondly, for safety for living cell, low gas temperature and accurate temperature control are required. However, the gas temperature of conventional non-thermal plasma is higher than the room temperature and not controlled accurately. So sterilizing effect depending on plasma gas temperature is not investigated. In addition, to suppress the temperature, methods such as limiting the discharge power or increasing the gas flow rate have been used, so the energy per unit volume provided to the plasma is reduced by these methods.

Thus, we developed a temperature-controllable plasma source, which can control the plasma gas temperature independent of the input power (patent number in Japan: 4611409). In this plasma source, the gas temperature is controlled before plasma generation as shown in fig.1. With the developed device, control of the helium plasma gas temperature over a range from -54°C to 160°C with a standard deviation of 1°C was achieved.

In this study, to investigate the sterilizing effect depending on the plasma gas temperature, survival number of *S. mutans* was measured after plasma treatment. *S. mutans* is bacterial species causing dental caries. It was cultured on agar plates (90 mm in diameter). The outlet of the plasma was placed just above the canter of agar plate with a distance of 3 mm. Carbon dioxide ($CO_2$) plasma was generated by plasma source (PCT-DFMJ02, PCT). The flow rate of $CO_2$ was 5 slm. After plasma treatment, bacterial number of center area 10 mm diameters was measured. As shown in fig.2, regardless of the plasma gas temperature, the number of *S. mutans* was decreased 6-digit in 300 s. With 20 and 40°C plasma treatments, the time for 6-digit decreasing was decreased to 120 s. This result shows sterilization efficiency changes depending on plasma gas temperature. Sterilization effects on microbial biofilms and other bacterial species were also investigated. These results will be presented.

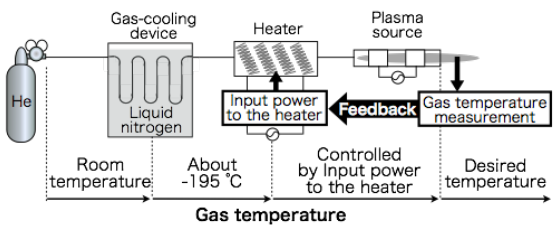


**Figure 1:** *Temperature controllable plasma jet*

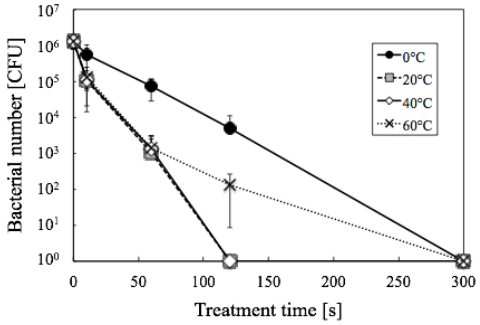


**Figure 2:** *Bacterial number of S. mutans*

# Water Sterilization by a Nano-second-pulsed Plasma Discharge in Gas Bubbles

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Nano-second pulsed plasmas in gas bubbles were applied for sterilization in water and generation of active species, e.g., ozone ($O_3$), hydroxyl radical (•OH), and oxygen radicals (•O), are investigated in a discharge reactor shown in Fig.1 (a). Gas bubbles were introduced from a bottom separator into the water and a pulsed high voltage was supplied between the wire electrode in the gas phase and the grounded plate in the liquid phase, with 40 ns in pulse duration using semiconductor opening switch diodes [1].

Bacillus subtilis was used as test object in the sterilization experiment. When air was used as working gas, the sterilization efficacy was better than those with the other gases (He, Ar, $O_2$, $N_2$). Further the sterilization efficacy was significantly improved by decreasing the pH value of the water as reported previously [2]. When increasing the gas flow rate and/or decreasing the conductivity of the water, the energy consumed in the discharge was decreased, which gives a better effect on the sterilization efficacy. These results propose that the efficient discharge technique is required for the further efficient sterilization. As shown in Fig.1 (b) ozone generated by plasma discharge in $O_2$ bubbles and hydrogen peroxide ($H_2O_2$) from adjunction which belongs to advanced oxidation processes (AOPs) were used to sterilize bacillus subtilis and the sterilization efficacy increased significantly as the $H_2O_2$ concentration increased. It was revealed that the increase of •OH had a dominant role in this process.

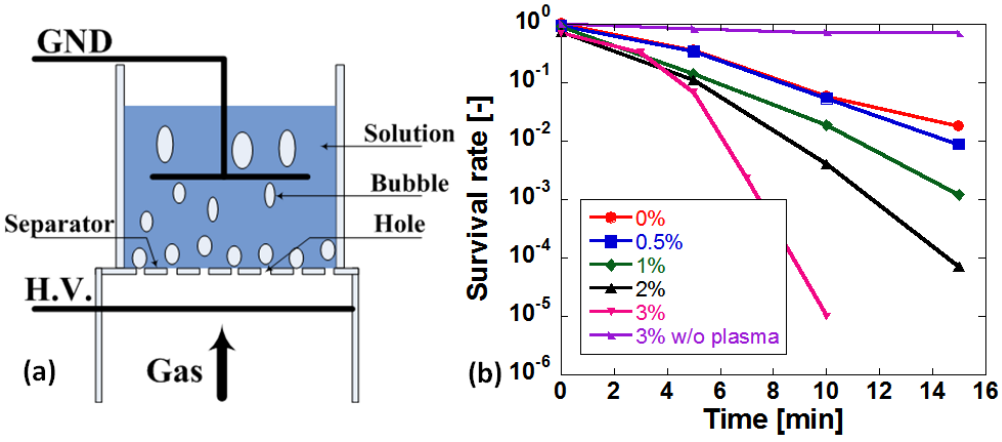


**Figure 1:** *(a) Schematic of the bubbles discharge reactor*
*(b) Change of bacillus subtilis survival rate using AOPs*

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# ffect of gas species on plasma- u ling sterilization

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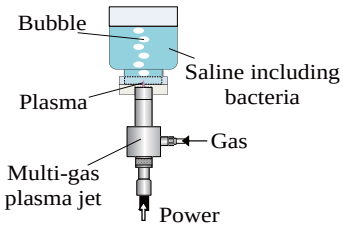
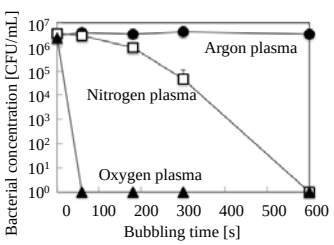
Recent years, research of sterilization using atmospheric non-thermal plasma has attracted much attention in various fields because it has high processing ability at low temperature. Sterilization effect of plasma is considered to be contributed by chemical reactive species such as hydroxyl radical (HO・) and singlet oxygen ($^1O_2$). Under dry condition, these reactive species affect bacteria directly. On the other hand, under wet condition, reactive species reach bacteria through interaction with liquid. Generally, for sterilization in water, plasma is irradiated to bacterial suspension from above. However, in this method, amount and kinds of reactive species introduced into water is limited because they depend on atmosphere.

In this study, to sterilize bacteria in water efficiently, we used a method to process liquid by plasma bubble. As shown Fig. 1, the outlet (1 mm in diameter) of the plasma souce is connected to the bottom of a water-filled container. Then the water was bubbled by the plasma. In this method, the plasma does not contact with atmosphere and so reactive species are transported to water directly. Additionally, since water is stirred by irradiating plasma from underneath, whole of water is processed evenly. As a plasma source, multi-gas plasma jet (PCT-DMFJ02, PCT) was used. This plasma source can generate stable plasma with various gases. Therefore, various reactive species could be introduced to the water.

To verify sterilization effect of plasma-bubbling, the effect of bubbled water on *E. coli* and *B. safensis* was investigated. *B. safensis* is spore forming bacterium, and it has very high viability. *E. coli* or *B. safensis* in 200 mL normal saline was bubbled by oxygen, nitrogen or argon plasma at 3 L/min gas flow rate. As shown in Fig. 2, with oxygen and nitrogen plasma processing, *E. coli* was decreased 6-digit within 60 and 600 sec, respectively. On the other hand, with the argon plasma, the number of the *E. coli* was not changed. These results show that sterilization effect is different by plasma gas species. In the case of *B. safensis*, it was decreased 5-digit within 600 sec with the oxygen plasma, and also decreased 1-digit for 600 sec with the nitrogen plasma. By taking time more than the case of *E. coli*, sterilization effect on *B. safensis* was obtained. To investigate the factor of sterilization, HO・ and $^1O_2$ in bubbled water were measured. 100 µL normal saline bubbled by plasma for 180 sec was added to 100 µL capture reagent of reactive species. The mixed solution sample was measured by electron spin resonance (ESR). In the 200 mL normal saline bubbled by nitrogen plasma and oxygen plasma, concentrations of HO・ were 0.057 µM and 0.037 µM, respectively. On the other hand, in the normal saline bubbled by oxygen plasma, 2.3 µM of $^1O_2$ was detected. This result shows that HO・ and $^1O_2$ have possibilities of contributing to sterilization effect of plasma-bubbling.




**igure** *Device configuration*




**igure** *Survival curve of E. coli*

# Roles of Oxygen and Nitrogen Atoms in $N_2/O_2$ Plasmas on Inactivation of Spore-forming Microorganisms

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To figure out the plasma inactivation mechanism of spore-forming microorganisms is important to improve the plasma sterilization property. In our previous work with the $N_2/O_2$ gas mixture surface-wave plasmas for the inactivation of spores, the effect of VUV/UV was studied by putting a small metal chamber covered with various optical filters to block the radicals inside the plasma chamber[1]. It is shown in Fig. 1(a) that the UV radiation is the most important lethal factor in $N_2/O_2$ plasma. In addition to the UV effect, the neutral species, such as excited O and N atoms, also play a role in the inactivation of spores. Recently, we have successfully carried out the measurement of the atomic O concentration in $N_2/O_2$ plasma using the VUVAS method, and found a strong correlation between the atomic O density and spore size changing, as shown in Fig. 1(b).

In this study, our purpose is to figure out the effect of N and O atoms on inactivation of the *Geobacillus stearothermophilus* spores by the $N_2/O_2$ plasma irradiation, by measuring the absolute N and O atom densities with the VUVAS method. The surface-wave plasma device consists of a stainless steel cylindrical vacuum chamber with a 2.45 GHz microwave generator and a microwave launcher. The compact plasma light source was installed on one side of the chamber and the VUV monochromator was fixed at the opposite port. We used the three emission lines at around 120 nm for N atom density measurements, and emission lines at 130 nm for O atom density measurement. The theoretical absorption property as a function of atom density was calculated based on the theoretical expression given in Ref. [2]. We are now investigating the operating condition of the light source to get the optimal emitting intensities and minimize the self-absorption effect in the light source. At the conference, we will present the results of N and O atom densities under different plasma discharge conditions and discuss their effects on inactivation of spore-forming microorganisms.

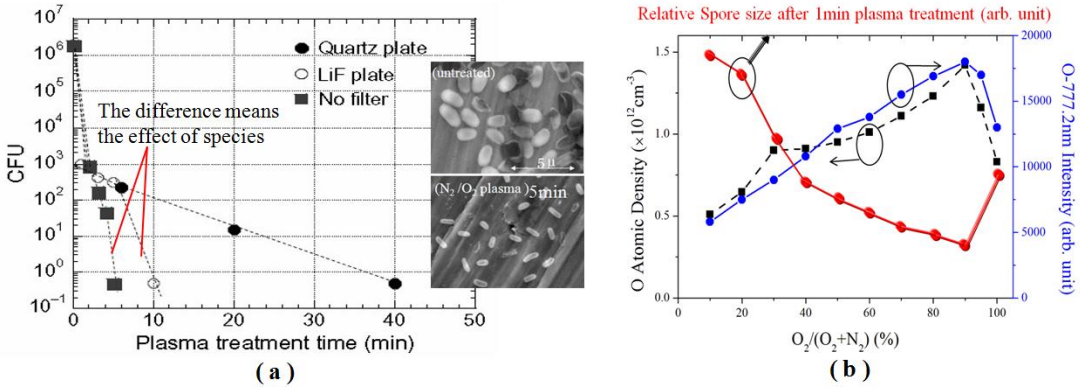


**Figure 1:** *(a) Survival curves and spore size changing of spores treated with* $N_2/O_2$ *plasma (b) The effect of O atom density on spores.*

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# Detection of membrane damages in *Escherichia coli* after plasma treatment

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Non-thermal atmospheric plasma has been investigated as the efficient treatment method for bio-decontamination of liquids. Plasma decontamination depends on various operating conditions and produces chemical and physical processes leading to death of bacteria. Processes potentially involved in plasma bio-decontamination are a heat, UV radiation and generation of reactive species and charged particles. Nevertheless, the detailed mechanism of plasma-induced microbial inactivation in water is still not well understood.

In this work we investigated plasma-induced pathways of *Escherichia coli* inactivation caused by atmospheric pressure plasma jet (APPJ) with emphasis how plasma affects cell membrane. We assayed bacterial counts with conventional cultivation on agar plates and fluorescent method LIVE/DEAD® BacLight™ Bacterial Viability kit. We determined a decreasing number of bacteria with increasing APPJ treatment time by both methods (Fig. 1). However, after a longer treatment time, we did not detect visibly cultivated colonies whereas the LIVE/DEAD kit still estimated a high percentage of living cells. Interaction of plasma with bacteria induces stress which does not lead to the death of bacteria, but to the loss of their ability to be cultivated – so called viable but non-culturable state (VBNC).

Consequently, the LIVE/DEAD kit, based on propidium iodide (PI) penetration, detected membrane damages in treated cells. Fig. 2 shows increasing formation of malondialdehyde (MDA) which was released from *E. coli* with increasing treatment by plasma. MDA is final product of lipid peroxidation and can be used as indicator of oxidative damage of membrane lipids caused by reactive oxygen species produced by plasma – mainly of OH radicals. In addition, due to clefts in a cell envelope nucleic acids leaked from bacteria to plasma treated solution. These results indicate contribution of lipid peroxidation and DNA damage in bacterial inactivation caused by APPJ treatment.

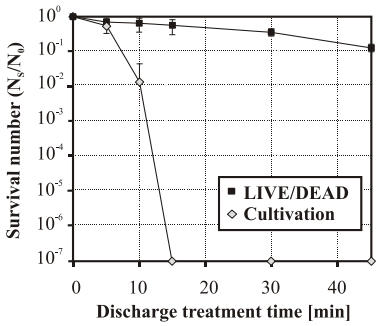


**Figure 1:** *Inactivation effect of the atmospheric pressure plasma jet assayed by conventional counting colony forming units and staining method LIVE/DEAD® BacLight™ Bacterial Viability kit.*

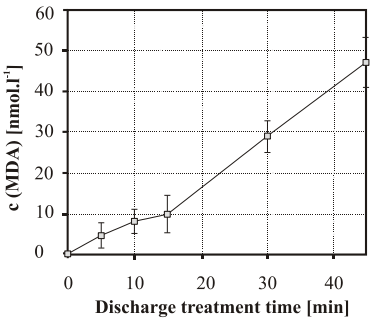


**Figure 2:** *Kinetics of increasing concentration of MDA in E. coli solution.*

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# Inactivation of bacteria and cells by DC transient spark discharge

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Non-equilibrium plasma produced by positive DC-driven transient spark (TS) discharge in atmospheric pressure air [1] and its use for the inactivation of *Escherichia coli* in water solutions with various initial pH, electrical conductivities, and buffering activities; and the treatment of normal (VERO line) and tumor (HeLa line) human cells in culture media were investigated. The bactericidal effect of the discharge on *E. coli* was evaluated by plate count method and linked with chemical species and effects induced by the discharge in the treated water solutions. Changes of pH and conductivity of the solution and concentrations of hydrogen peroxide $H_2O_2$, nitrites $NO_2^-$ and nitrates $NO_3^-$, dissolved ozone $O_3$, and peroxynitrites $ONOO^-$ generated by the discharge were analyzed by absorption and fluorescence spectroscopy. The cells in culture media exposed to the discharge were analyzed for viability, apoptosis and cell cycle. Direct exposure of cells to the discharge was compared with a remote exposure, where culture media exposed only to the discharge activated air flow. The results on the treatment of cells by DC transient spark in air were compared with those obtained with pulsed plasma jet in helium performed in parallel experiment. Two system setups of TS discharge were used. The first setup ('water electrode system') consisted of the high voltage needle electrode placed above the inclined plane with a narrow channel with grounded electrode. The flow rate of water in the channel was controlled by a peristaltic pump (<30 mL/min), and the discharge power (<2 W), its frequency (<4 kHz) and the treatment time (<20 min) were varied. The second setup ('water electrospray system') utilized the delivery of the water solution into the discharge zone via hypodermic stressed needle with a constant water flow rate (0.5 mL/min) and was described in detail in [2]. Finally, the plasma jet system was generated by dielectric barrier discharge at a constant gas flow rate (3 L/min) and frequency (2 kHz) and was described in detail in [3]. The results for non-buffered solutions showed that their acidity, conductivity and temperature monotonously increased with the discharge treatment time and their relative changes in both TS systems were found quite similar. Concentration of hydrogen peroxide (<1 mM), nitrites and nitrates (<1 mM), peroxynitrites (<100 μM), and dissolved ozone (~ 1 mg/L) were measured and the reduction of *E. coli* population (up to ~ 6-log) was evaluated as functions of initial pH, conductivity, buffer presence, and treatment time. Direct treatment of cells with TS systems led to the decrease in cell viability (normal cells: up to ~ 94% dead ), while remote treatment indicated much weaker effect (up to ~ 10% dead, ~ 22% apoptotic, and ~ 2% preapoptotic cells). No effect of the discharge on DNA was observed. Comparison with the pulsed plasma jet results showed comparable efficiency only for remote treatment (up to ~ 10% dead).

*This work was supported by Slovak Research and Development Agency APVV 0134-12 and SK-RO-0024-12 grants.*

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# Enhancement of the Sterilization Efficiency of Argon Plasma Jet by Addition of $O_2$ and $H_2O_2$

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We have developed an atmospheric pressure non-equilibrium plasma jet generator (CAPPLAT)[1] and applied to the deactivation of *Bacillus subtilis* endospores[2]. In this paper, we investigate the effects of addition of the oxidants ($O_2$ and $H_2O_2$) to the plasma jet on endospore inactivation.

The plasma jet was generated by CAPPLAT which consists of two co-axial cylindrical electrodes separated by a dielectric silicone tube with a high voltage pulse power supply, Hiden SBP-10K-HF. The configuration of plasma torch and the experimental setup have been explained previously [2]. The dried agar sheet with *Bacillus subtilis* endspore ($1\times10^7$spore/ml,15µl) was exposed to the three kinds of plasma jet which were generated with the plasma gas of Ar 10slm + $N_2$ 100sccm, Ar 10slm + $N_2$ 100sccm + $O_2$ 500sccm and Ar 10slm + $N_2$ 100sccm + $H_2O_2$ plasma jet) at the 20 kHz applied voltage of +/-8k$V_{p-p}$ with 50% duty cycle. For the direct injection of $H_2O_2$ into plasma jet, bubbling method was adopted. For bubbling 150 sccm of Ar gas was used as a carrier gas. The procedures for revival of culture, collection of endospores then the preparation of agar disc, medium and other buffers, solutions, plasma exposure, collection of plasma treated spores, estimation of DPA (dipicolinic acid) and colony counting have already been explained in detail previously [2].

It was observed that the addition of the oxidants ($O_2$ and $H_2O_2$) enhanced the efficiency of the sterilization and particularly $H_2O_2$ was found more effective than $O_2$ (Figure 1). The amount of DPA, which is an indicator to confirm the breakage of cortex and leakage of protoplast, was not consistent (Figure 2). Most probably it is because of a huge range of byproducts. And even after a spore is etched and dead, the DPA in its cellular debris continues to be released.

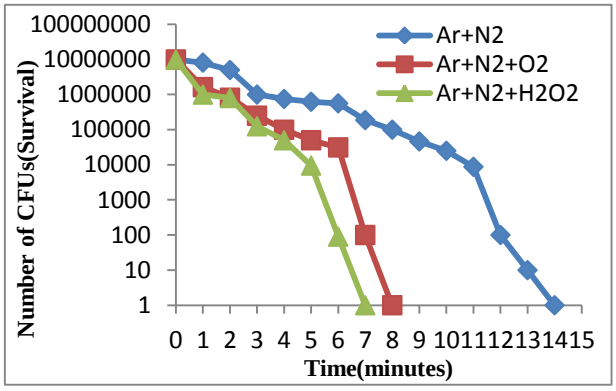


**Figure1:** *The number of CFUs as the function of the time of plasma discharge exposure*

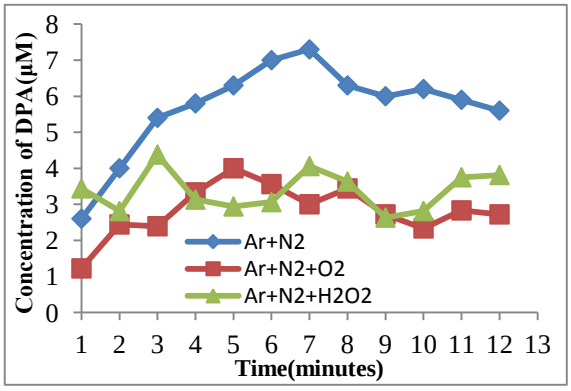


**Figure2:** *Released DPA during plasma treatment*

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# The Effect of Active Radical Production on the Plasma Degradation of Phorbol Esters in Bio-diesel Fuel industry

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Mechanical pressing of the Jatropha curcas (J. curcas) seed for oil extraction gives rich J. curcas oil, which can be used as the raw material for producing biodiesel. During its treatment processing, however, toxic waste water is produced and may be released to the environment, since Phorbol Esters (PEs) acting as a cancer promoter are contained in the seed and oil [1]. Gamma irradiation from radio-isotopes has been regarded as a more promising and efficient method than biological or chemical methods to degrade PEs. Recently, atmospheric pressure plasma is reported to have similar degradation effect [2]. Both methods produce active radicals such as hydroxy radical in the sample solution and they efficiently enhance the decomposition process of PEs.

For industrial application, however, this plasma source has many drawbacks. Among them, its running cost is not economical, since huge amount of expensive helium gas is required for plasma production. Argon gas is cheaper than helium and can become an alternative, although a breakdown voltage of argon is much higher than that of helium. Applied high voltage would enhance a rapid multiplication of electrons after breakdown and lead to the formation of streamers or a filamentary arc, which may induce the surface damage of treated samples. However, we succeed to produce Ar plasma with the help of corona discharge around a needle electrode under the lower applied voltage condition. The difference of both plasma parameters and decomposition effect of PEs between different working gases is under investigation.

In order to assist active radical production, the addition of water or alcohol [3] to the working gas is proposed. When these molecules exist in the discharge, they may dissociate themselves to produce radicals with the interaction not only of electrons but also of metastable. Quantitative study of this process is still an open question, and our study on it is just launched. The first result on this experiment will be reported at the conference.

This study is financially supported by Science and Technology Research Partnership for Sustainable Development (SATREPS) and Matsuda Yosahichi Memorial Foreign Student Scholarship, Osaka Prefecture University, Japan.

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# Mold sterilization of contaminated oil-on-canvas paintings via microwave atmospheric plasma pencil (MAPP)

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Filamentous fungi or molds can cause major damage on visual arts and artifacts. This study tests the feasibility of the microwave atmospheric plasma pencil (MAPP) in sterilization of mold-contaminated artifacts such as historical and prehistoric oil-on-canvas paintings. Efficacy of MAPP in inhibiting fungal growth is assessed using standard viable plate count methods and by determination of percent reduction and decimal reduction time value (D-value). Physical changes on the samples were determined through SEM. Efficacy using a conventional method (fumigation) were compared to the method using plasma.

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# Inactivation Acinetobacter Bacteria by Atmospheric Plasma

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This work presented here is on a preliminary study of the effect of atmospheric plasma treatment on Acinetobacter bacteria. Acinetobacter was chosen because it is a multidrug resistance property. Samples of Acinobacter were obtained from Hospital Tunku Fauziah, Kangar. Plasma was generated using Atmospheric Jet Plasma configuration. Helium gas was supplied and plasma was excited using AC current inverter. The main objective of this research was to inactivate the Acinetobacter by varying time.

The Acinetobacter bacteria were cultured in solid medium blood agar and incubated for 24 hours [1]. They were cultured in single cell colony to make sure maximum plasma exposure to each cell of sample. The exposure time was set at 1minutes, 10minutes, 15 minutes and 20 minutes. After the exposing to plasma, the bacteria was cultured again and incubated for 24 hours. The growth of bacteria on medium agar can be observed to confirm inactivation. Three samples were used for each parameter.

Observation of this experiment showed the bacteria growth in all control samples. There were no bacteria in growth in all samples for one minute and 10 minutes exposure. Five minutes exposure showed two sample bacteria growth in medium. This observation can also be seen at 20 minutes exposure. However, at 15 minutes of exposure there were bacteria growths for all three samples.

The result for this experiment is still inconclusive and further experiment need to be conducted. This is because at the stage of bacteria culturing bacteria the shape and size of the colony size are not consistent. This may affect the inactivation process because of the number of cells in the colony are larger and also the plasma exposure area are relatively smaller than the targeted colony.

Acknowledgements. The authors gratefully acknowledge Ministry of Higher Education Malaysia for Research Acculturation Grant Scheme (RAGS, Grant No: 9018-00035), Universiti Malaysia Perlis, and Hospital Tunku Fauziah, Kangar for providing research facilities.

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# Preservation of Growth Enhancement of Plants after Atmospheric Pressure DBD Plasma Irradiation

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Atmospheric pressure non-thermal plasmas have been widely employed in biological applications [1]. We have succeeded in enhancing growth of radish sprouts (*Raphanus sativus L.*) using a scalable dielectric barrier discharge (DBD) device [2]. Plasma irradiation for 180 s leads to the growth enhancement of radish sprouts in a long term of 7 days [3]. Here we evaluate preservation of the growth enhancement after plasma irradiation.

Plasma irradiation to seeds of radish sprouts was carried out using the scalable DBD device. Dry seeds of radish sprouts were set at 3mm below the electrode and arranged horizontally at 5 mm outside the electrode. After 180 s plasma irradiation, the seeds were cultivated on a plastic container with water in an incubator at 22°C and 80% relative humidity under dark condition. Their total length from their primary root to stalk was measured after 7 days' cultivation with 50 samples.

Figure 1 shows the length distribution of 7 days cultivated sprouts after plasma irradiation to seeds. Figure 1 shows the length distribution for untreated seeds (control) (a), for seeds cultivated immediately after plasma irradiation (immediate) (b), and for seeds cultivated 10 days after the irradiation (10 days) (c). The number of sprouts grown longer than 150 mm was the largest for immediate, the second for 10 days, and the smallest for control. It suggests that the effects of plasma irradiation to seeds tend to become weak by storing, while the effects remain for 10 days.

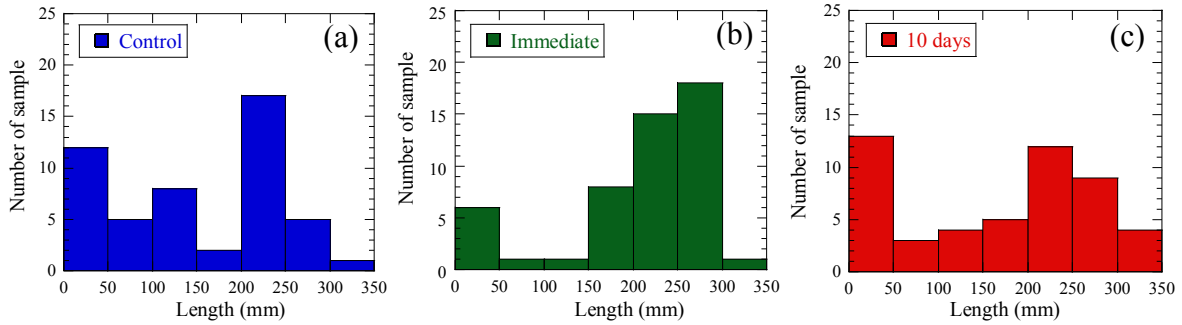


**Fig. 1:** *Length distribution of sprouts after plasma irradiation to seeds together with control.*

**Acknowledgement**
This work was partly supported by JSPS KAKENHI Grant Number 24340143.

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# Effects of ambient gas species for plasma irradiation to seeds on plant growth promotion

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In our previous work, we have found that atmospheric air dielectric barrier discharges (DBD) irradiation to seeds of plants have long term effects on their growth enhancement of plants [1-2]. In the air DBD plasmas, many kinds of reactive oxygen species and reactive nitrogen species are generated leading to complex interactions between plants and generated species. To clarify the effects of the individual species on the growth promotion, we have studied the effects of ambient gas species of the DBD discharges on the growth promotion.

Experiments were carried out using a scalable DBD device in chamber equipped with vacuum pump. Ambient gas in the chamber is replaced 3 times using the pump to purify the ambient gas. The discharge voltage and current was 9 kV and 0.2 A, respectively. After 180 sec of plasma irradiation to ten seeds of *Raphanus sativus L.*(radish sprout), the seeds were cultivated for 7 days with feeding pure water in an incubator at 22°C and 60% relative humidity under dark condition. Statistical significance of the total length was evaluated and analyzed by Tukey test.

Since one of important gas molecules for the growth promotion is NOx, we have examined plasma irradiation using $N_2$ diluted NO (900 ppm) gas as a first step of this study. Figure 1(a) shows growth curves for seedling after NO+$N_2$ plasma irradiation. Average length for seeds after plasma irradiation is similar to that for control for 3 days but grows rapidly after 5 days. Figure 1(b) shows a ratio of the length for plasma irradiation to that for control with a difference between the lengths for plasma irradiation. The difference increases with time after 3 days. The ratio increases to 1.6 after 7 days (Tukey test, P<0.1). Effects of other gas species will be discussed at the conference.

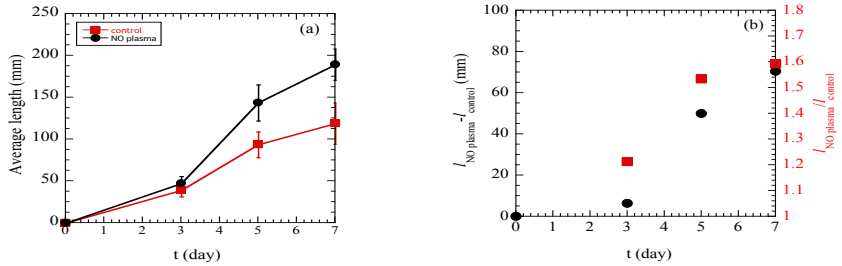


**Figure 1:** (a) Growth curve of plants and (b) a ratio between the length for NO plasma irradiation and control with a difference between the length for plasma irradiation and control.

## Acknowledgments

This work was supported by JSPS KAKENHI grant number 24340143 and 24108009.

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# Improvement of Growth Rate of Plants using Bubble Discharge in Water

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Discharges in water are one of the promising candidates to reduce the infection risk of plants in hydroponics because the discharges produce chemical active species such as atomic oxygen (O), ozone ($O_3$) and hydroxyl radical (OH), which work to inactivate the sundry germs [1]. In addition, the discharges also produce nitric acids which are working as fertilizer [2]. In this study, the effect of the discharge irradiation to the water on bacteria activity and growth rate of the plants were evaluated. In the experiments, *Fragaria* × *ananassa*, *Spainacia oleracea* and *Raphanus sativus var. sativus* plants were cultivated in pots filled with artificial soil, which included the use of chicken droppings as a fertilizer [3]. A magnetic compression type pulsed power generator was used to produce underwater discharge. The plasma irradiation times were set in range from 15 to 30 minutes per day over 28 days of cultivation.

Figure 1 shows the dried weights of cropped *Spainacia oleracea* at 28 days after cultivation. The dried weight increased to 0.170 and 0.209 g as a result of 15 and 30 minutes of discharge irradiation, respectively. These values correspond to 4.7 and 5.8 times incremental increases in comparison to that of the control group. These results indicate that the nitric acids are produced with the discharges and are absorbed by the roots as a fertilizer [3, 4]. Figure 2 shows the time history of the bacteria count in the drainage water of cultivated *Raphanus sativus var. sativus*. The bacteria count was found to increase from 3.5 to 4.5 Log-CFU at 28 days of cultivation without discharge irradiation. However, the bacteria count decreases by 2 Log-CFU with the application of plasma irradiation into the drainage water.

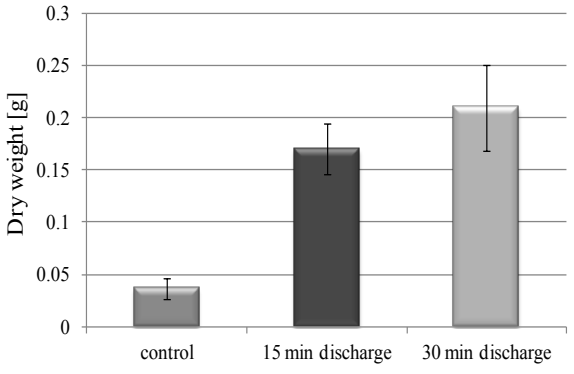


**Figure 1:** *Dry weight of Spainacia oleracea*

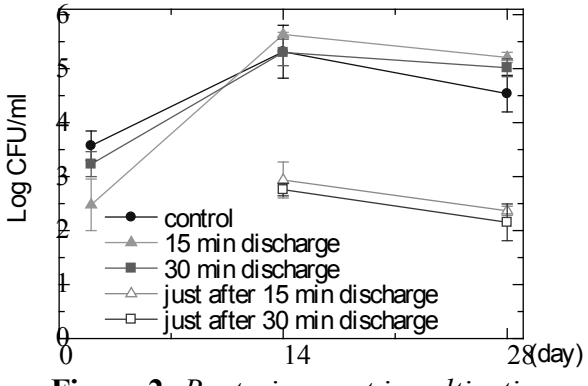


**Figure 2:** *Bacteria count in cultivation of Raphanus sativus var. sativus.*

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# Effects of Dielectric Barrier Discharge (DBD) Plasma on Seed Germination and Plant Growth

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Plasma technology has been widely used for sterilization, differentiation, and disease treatment. Recently, studies show that plasma has effects on enhancing seed germination and plant growth [1]. In spite of increasing number of studies about plasma effects, the interaction between plasma and plants has been rarely reported. In this study, we have analyzed the effects of nonthermal atmospheric pressure plasma on seed germination and growth of coriander(*Coriandum sativum*), a medicinal plant. Ar, air, and $N_2$ were used for plasma gerneration as feeding gases. Plasma was discharged at 0.62kV, 200mA, 9.2 W. Seed germination was increased over time when treated with $N_2$ based DBD plasma for exposure times of 30 seconds and 1 minute, everyday. After 7 days, about 80-100% of seeds were germinated in the treatment with $N_2$ based DBD plasma, compared to control (about 40%, only gas treated seeds). In order to elucidate the mechanism of increased germination, we have analyzed characteristics of changes in plant hormones and seed surface structure by SEM.

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP), No. 2010-0027963 and No. NRF-2013R1A1A3011245

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# Atmospheric pressure non-equilibrium plasma for the production of composite materials

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In the rapidly evolving field of tissue engineering, continuous advances are required to improve scaffold design and fabrication to obtain biomimetic supports for cell adhesion, proliferation, penetration and differentiation. Both electrospun fibrous scaffolds and hydrogels are widely used in this field since they well reproduce the structure of the extracellular matrix (ECM) of many biological tissues. Limitations of these two types of materials can be overcome through their combination, by developing composite structures [1] combining enhanced mechanical properties (provided by the fibrous components) and improved cell penetration (provided by the gel phase) in a superior ability to mimic natural ECM that is constituted by both a fibrous protein network and a hydrogel matrix.
In the present work, we develop new composite materials made of electrospun PLLA scaffolds and poly(amidoamine) hydrogels [2] with different degrees of crosslinking. In order to promote compatibility and good adhesion between the two materials, surface chemical reactions between hydrogels and PLLA mats are induced by inserting amino functional groups on electrospun PLLA mats by means of atmospheric pressure non-thermal plasma. Results will be presented concerning the exposure of PLLA substrates to the plasma region generated by a Dielectric Barrier Discharge at atmospheric pressure, driven by a HV Amplifier connected to a function generator operating with a microsecond rise time and operated in $N_2$. Surface and solid-state thermo-mechanical characterizations of plasma treated substrates and of resulting composite materials at different crosslinking degrees are presented. Results of mechanical tests highlight a high adhesion between hydrogel and plasma treated PLLA electrospun mats, underlining the opportunity to use atmospheric non-thermal plasmas to fabricate a composite starting from two materials otherwise physically incompatible.
Potential effects of nanofibrous-hydrogel were evaluated by investigating pluripotent stem cells (human-induced pluripotent stem cells and human embryonic stem cells) response. Preliminary results for proliferation and pluripotency markers showed successfully culture of pluripotent stem cells on hydrogel interfaces.

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# Aerosol-Assisted Atmospheric Pressure Dielectric Barrier Discharges on Polymer Surfaces for anti-microbial properties

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The formation of biofilms limits the life cycle of various technical filtration systems in medicine. There is the requirement that coatings, which decrease the biofilm growth, have to be very thin in order to prevent the original filter functionality. The atmospheric pressure plasma liquid deposition (APPLD) technology is a process, which allows creating ultrathin functional layers with anti-microbial behavior. Films made with APPLD can be deposited on all polyolefin foils to generate directly anti-microbial surfaces. Otherwise, APPLD modification is used for adhesion improvement for a subsequent anti-microbial modification by grafting hydrophilic polymer brushes in an Atomic Transfer Radical Polymerization (ATRP) process.

In the presented study, APPLD was performed on various polyolefin surfaces using the plasma treatment system CORLAB AS (kalwar). Selected model precursors e.g. polyethyleneglycole (PEG) were ultrasonically nebulized and deposited on low density polyethylene (LDPE) foils and polyethylene terephthalate (PET) foils. The aerosol is feed either directly into the filamentary plasma discharge or immediately after the plasma glow. The influence of the surface properties on the deposition parameters, such as plasma power and deposition time, was investigated. ATRP anti-microbial layers made from Polyhydroxyethylmethacrylate (pHEMA) were deposited on polyolefin foils which were pre-treated with APPLD to improve their chemical bonding. The properties of the PEG layers as well as of the layers made by ATRP were characterized concerning their morphological structure (profilometry, SEM, AFM), chemical composition (XPS) as well as their surface energy.

The anti-microbial effect of the layers was verified by inducing a reproducible microbial stress on the polymer surface with the presence of *steady state* (continuously) and *batch*-cultivated *Pseudomonas sp.* for 48 and 24 h, respectively. Afterwards, the biofilm formation was observed off-line via confocal laser scanning microscopy (CLSM). Depending on plasma intensity, foil speed and aerosol feeding position, deposition rates of the PEG layers vary in their morphology and homogeneity. The anti-micorbial layers of pPHEMA are very effective in both microbial experiments. The biomass decreases up to one third in relation to the untreated foils.

In summary, the presented ways to create anti-microbial coatings are very promising and the tests of various APPLD precursors and ATRP substances are ongoing.

# Direct covalent coupling of biomolecules to nanostructured plasma polymers

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Anchoring of proteins onto solid supports has become the focus of extensive research. Such systems are attractive in many biomedical applications including, but not limited to, development of biosensors, protein microarrays and biocatalysis. Often, covalent attachment of biomolecules is preferential as it may prolong their performance over extended periods of time. Traditional methods of biomolecule covalent immobilization include functionalization of surfaces and the use of chemical linkers. These methods, however, suffer from the need of several, often complicated, chemical pathways.
Our work was inspired by the recent reports on direct covalent binding of proteins to radical-rich polymeric surfaces [1]. It has been shown that radicals deliberately created in conventional polymers by Pulsed Ion Immersion Implantation may bind proteins very efficiently without impairing their functionality. Plasma polymer seem to be good candidates for this task as well since they are essentially produced by radical-based processes and usually, if not often, contain a certain amount of unreacted radicals.
In this work, ultra-thin films of hydrocarbon plasma polymers were fabricated by thermal vapor phase deposition of pol(ethylene) on silicon with subsequent argon plasma treatment. Thermal vapor deposition resulted in formation of 7 nm thick islands while argon plasma treatment led to redistribution of polymeric phase over the entire surface and to creation of radicals within the film. Thus, the surface with controllable nanostructure was produced. The film enabled also the direct covalent attachment of bovine serum albumin which was assessed by measuring the XPS N 1s signal of the protein-incubated samples after their vigorous washing with sodium dodecyl sulfate. The films were also tested for their interaction with cells. We demonstrate that such surfaces may serve as attractive platforms for research on the influence of nanostructure on biomolecule immobilization and cell adhesion.

Acknowledgments: the work was supported by the grant GACR 13-09853S.

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# Molecular Morphological Analysis of the Effect of Low Temperature Plasma on the Wound Healing of Skin

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The purpose of this study is to clarify morphologically the effect of low temperature plasma on the wound healing of skin. It is reported that the low temperature plasma is effective for hemostasis, promotes the wound healing of skin, and promotes the regeneration of epithelial tissue. Application of low temperature plasma to the treatment of inflammatory skin diseases is expected. On the other hand, so far almost all these studies were at light microscopic level, but at the electron microscopic level systematic study has not been performed yet. In this study we examined the ultrastructural morphology of wound healing skin by the transmission and scanning electron microscopy to elucidate the molecular mechanism of promotion of the wound healing by low temperature plasma.

We used our low temperature glow-like plasma, we call “SAKAKITA plasma”, which has been developed by us to achieve blood coagulation without thermal damages [1, 2]. The equipment to produce SAKAKITA plasma has the fully coated three kinds of electrodes with dielectric, and employed a dielectric barrier discharge with a peak-to-peak voltage of ~8 kV and frequency of ~60 kHz with small displacement current. The generated plasma prompted blood coagulation keeping thermal elevation less than 40°C without arc-like plasma formation [1]. Our plasma equipment is a new device for bleeding control.

We compared the effect of low temperature plasma on ultrastructural morphology of wound healing skin with that of electrocoagulation, high-frequency electrical coagulator. The morphological changes of cell and extracellular matrix were scarcely observed in the low temperature plasma irradiated skin, while those were observed in the electrocoagulator treated skin.

**Acknowledgments**
We thank Ms. Sachie Matubara and Ms. Tomoko Miura (Laboratory for Electron Microscopy and Department of Anatomy, Kyorin University School of Medicine) for technical assistance. This work was supported in part by Grants-in-Aid for Scientific Research on Priority Area (25108512 to Y. A. ; 21590454, 24590498, and 24108006 to Y. I.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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# Flow manipulation in thread-based microfluidics by plasma treatment of wool with various gas

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Recent progress in thread-based microfluidic devices shows portable and inexpensive field-based technologies enabling medical diagnostics, environmental monitoring, and food safety analysis.[1] However, capillary-driven liquid flow in a single thread is difficult to control, which is crucial in thread-based microfluidics.[2] Among others, hydrophobic wool thread is an appropriate candidate for the purpose of liquid flow control in a channel of thread-based microfluidics because it can readily tune its wettability by plasma treatment with various gas.

Thus, taking nature wool thread as a channel we demonstrate here that liquid flow manipulation such as micro selecting and micro mixing can be achieved by introducing a plasma treatment on wool thread. In addition to enabling flow control the treated wool channels consisting of all natural substances will be beneficial for biological sensing devices. We found that treated wools with various gas have different flow rate each other. We use atmospheric plasma with $O_2$, $N_2$ and Ar gas.

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# Surface-Modification Techniques of Thin Film Transistors and Capacitances by Plasma Deposition $SnO_xC_y$ for Improve Electric Conductivity of Biomedical Applications

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In this study, a novel room temperature type gas conduction device based on plasma deposition of tetramethyltin (TMT) and $O_2$ organically hybridized film followed by post treatment on the deposited film, and immobilized EDC/NHS-crosslinked protein or enzyme were developed for improving thin film transistors (TFT) and capacitances conductivity of biomedical applications. Photoelectric, chemical, and structural properties were monitored by using IV and CV electrical measurement system and UV/vis spectrophotometer, fourier transform infraredspectr oscopy (FTIR), contact angle analysis system and scanning electron microscope (SEM). The contact angle was observed to decrease from 37±4 ° to hydrophilic for SnO x Cy thin films.

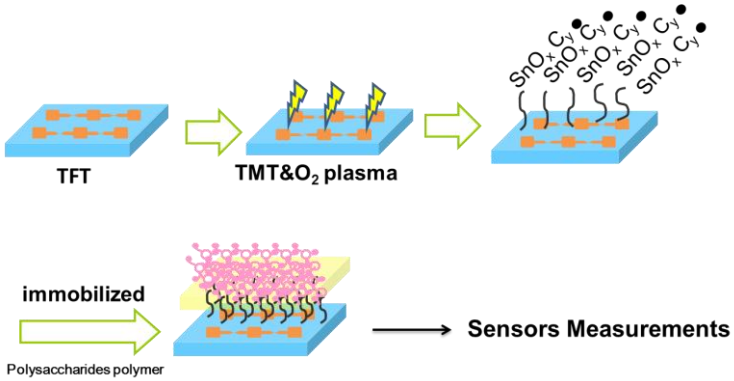


**Fig1. Experimental flowchart**

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# Preparation of Nylon/Chitin Membranes by Solution Casting and DBD Plasma Treatment for Wound Care Application

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Chitin, a structural polysaccharide in shrimp shell, is a copolymer consisting mainly of *N*-acetyl glucosamine unit with a minor component of glucosamine unit. Since *N*-acetyl glucosamine is also a monomeric unit in hyaluronic acid, an extracellular macromolecule that is important in wound repair [1], chitin may also be useful in promoting wound healing. However, utilization of chitin to produce wound dressing is difficult because chitin does not dissolve in common solvents and chitin film fabricated by solution casting using an organic solvent may be toxic to a wound. In this study, chitin was blended with nylon 6,6 in order to improve mechanical properties of chitin membranes prepared by using a mild solvent of calcium chloride-saturated methanol and casting to form nylon/chitin membranes. Nevertheless, the low compatibility between chitin and nylon leads to phase separation as evidence by SEM image. To overcome this problem, the nylon/chitin membranes were subjected to dielectric barrier discharge (DBD) plasma treatment in order to enhance the interaction between chitin and nylon. The effects of the blend ratios and plasma treatment time on morphology, physical, chemical, and mechanical properties were investigated by SEM, AFM, TGA, water contact angle, ATR FT-IR, XPS, Lloyd tensile tester. In addition, biodegradability test by using enzyme lysozyme and biocompatibility test using dermal skin fibroblast cell were also examined. It was found that after DBD plasma treatment, the FTIR spectra of nylon/chitin membranes showed the increment of intensity of the peak at the wavenumber of 1720 $cm^{-1}$ which refers to the carbonyl group. The formation of the new carbonyl groups might occur due to the interaction between active oxygen species generated by plasma and polymer molecules in the membranes [2]. The values of water contact angle decreased when plasma treatment time increased, indicating the higher hydrophilicity than the plasma-untreated one. SEM images of the plasma-treated nylon/chitin membranes show the distribution of the smaller size of chitin aggregates on the membrane surface. After immersion of the plasma-treated nylon/chitin membranes in lysozyme solution to hydrolyze chitin, porous structure, as a result of the removal of chitin, was observed on the surface of the membranes. Mechanical property of the nylon/chitin membranes decreased with the increasing of chitin content. From the TGA results, pure nylon completely degraded at approximately 420$^{o}$C whereas the nylon/chitin membranes degraded at a lower temperature of 405$^{o}$C. Finally, biocompatibility with human fibroblast cell was also investigated.

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# Observation of Skin Changes by Atmospheric Plasma Jet Irradiation

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## 1 Introduction

Recently, the medical applications of plasma became the new frontier of plasma field [1]. Various medical treatments using plasma have been developed all over the world. For medical application, a non-invasive treatment for the human body is required.
As the plasma source for biomedical applications, one of the most common could be plasma jet [2]. In this study, excised rat skin was used as the treatment target by plasma jet. Observation of the rat skin by plasma treatment was carried out.

## 2 Experimental Set up and Method

Plasma jet was applied to the sample rat skin. The experimental set up is shown in figure 1 and the experimental conditions are shown in table 1. Plasma jet electrode was energized at 5 kV and 16 kHz by a neon transformer as a power supply. The surface and cross section of the rat skin was observed by a digital microscope (MI-SST250). During experiments the temperature of the rat skin was measured with a thermo camera (TVS-200) when plasma jet was irradiated.

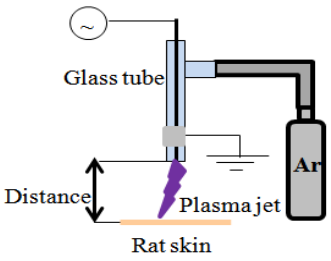


Table 1 Experimental condition

| | |
|---|---|
| Flow rate [L/min.] | 5.0 |
| Distance [mm] | 1.0 |
| Exposed time [min.] | 3, 5 |
| Process gas | Ar |

**Figure 1:** *Experimental set up*

## 3 Results

Color of the surface of sample rat skin changed slightly for treatment times ranging from 3 to 5 minutes. The observed spots where the color changed were about 0.1 ~ 0.3 mm in size. The temperature of rat skin was increased to 45 C° during the plasma jet treatment.

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# Micro-arc Oxidation Titanium and Post Treatment by Cold Plasma and Graft Polymer for Improving Biocompatibility

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Because of implants are used in contact with various tissues, it is need to have optimum surface compatibility with the host bone tissues and soft tissues[1]. Titanium and its alloys are considered have desirable properties, such as relatively low modulus, good fatigue strength, machinability, and corrosion resistance.[2] When implants of titanium in human body, usually lead to an oxidized, contaminated surface layer that is often stressed and plastically deformed, non-uniform and rather poorly defined[3].Therefore, in order to improve these problems, surface modification is often performed. In this study, a ceramic layer was coated on titanium substrate by micro-arc oxidized to enhance corrosion resistance and used plasma enhanced chemical vapor deposition of hexamethyldisilazane (HMDSZ) organic silicide films to introduce reactive group in surfaces. Then use UV-surface graft of acrylic acid and immobilize Chondroitin Sulphate by crosslinking EDC/NHS. The surface properties, morphology, phase, composition of the coating were measured by scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS) and X-ray diffraction (XRD) and water contact angle. The results of cell culture confirmed that the sample after immobilized Chondroitin Sulphate has the best biocompatibility.

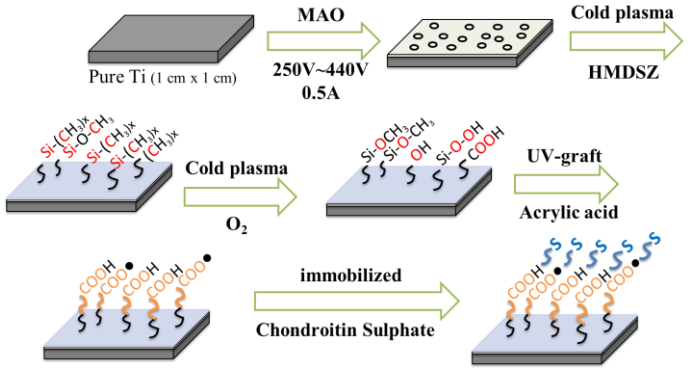


**Figure 1** *Experimental flowchart*

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# Atmospheric pressure plasma patterning of biocompatible substrates: comparison of localized treatment effectiveness with different plasma sources

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Recently, increasing efforts have been dedicated to atmospheric non-thermal plasma (NTP) local modification of biomaterials, creating patterns to control cell growth. In this work, a local modification of biocompatible materials has been performed with four atmospheric NTP sources: Dual Gas Plasma Jet, Dielectric Barrier Discharge (DBD), micro-plasma jet (Micropen), all driven by a generator producing pulses with a rise time of 9 ns and peak voltages in the range 7-20 kV into a 100-200 Ω load impedance, and commercial plasma jet.

To analyze plasma treatment localization with Dual Gas and commercial plasma jets, four quadrants were identified onto each sample and the plasma plume was oriented towards only one of these; water contact angle (WCA) measurements suggest that the distance between source outlet and substrate influences localization efficiency.

In the case of the DBD source, localization was obtained placing the grounded electrode below only one of the quadrants; only in the area above the counter electrode a drastic WCA reduction was measured.

To verify the localization of the Micropen treatment, the source was moved along a defined pattern on the substrate; the width of the surface modification induced by the plasma plume was estimated by WCA measurements to be smaller of 1 mm.

Furthermore, to verify the ability of cells to attach and grow on treated substrates, different kinds of cells (fibroblasts and epithelial cells) were plated on biocompatible substrates. The difference in the number of cells able to attach to treated or untreated substrates was determined 6 hours after cell plating, while the ability to replicate was determined 24 and 48 hours later.

# Plasma as a new odontoiatric tool to improve implants adhesion

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The development of new ceramic materials for dental applications has led to the introduction on the market of zirconia-based ceramics. Although unsurpassed for what concerns the combination of inertness, mechanical and optical properties, this class of ceramics presents clinical problems related to the achievement of suitable adhesion with the substrate, either synthetic or biological. Similar issues occur at the interface between fiber posts and luting cements, the failure of this bonding being the main cause of implants failure.

The present work focuses on plasma treatment of zirconia and fiber posts, usually adopted in prosthetic and implant dentistry, to improve the bonding strength of commercial fixing cements onto them.. The plasma source used in this work is a plasma jet able to work using air as a feeding gas and with the possibility to be handheld operated.

To test plasma improvement of the bonding strength on zirconia, several samples were treated before the application of the cement with the standard clinical procedure. As the chemical composition of commercial cements may widely vary, four different cements were investigated. The adhesive strength between zirconia and cylindrical molded cements specimens was tested after 24h storage in distilled water at room temperature, using the shear bond strength method (ISO 10477); results of these tests show an ample variability (from 40% increase to 7% decrease) depending on the tested cement. Water contact angle, SEM and FTIR analysis were also carried out in order to further investigate the effect of plasma treatment on zirconia.

Plasma treatment of fiber posts (Hi-Rem Post size #3, Overfibers) to enhance the adhesion with a self-etching cement used to fix them in the extracted teeth root canal was also investigated. The bonding strength was evaluated through push-out test on teeth sections after the curing of the cement. Results and microscope analysis show that plasma pretreatment enhance the bonding strength of the post-cement interface over the strength of the cement-dentin interface, where the failures were therefore induced during the tests.

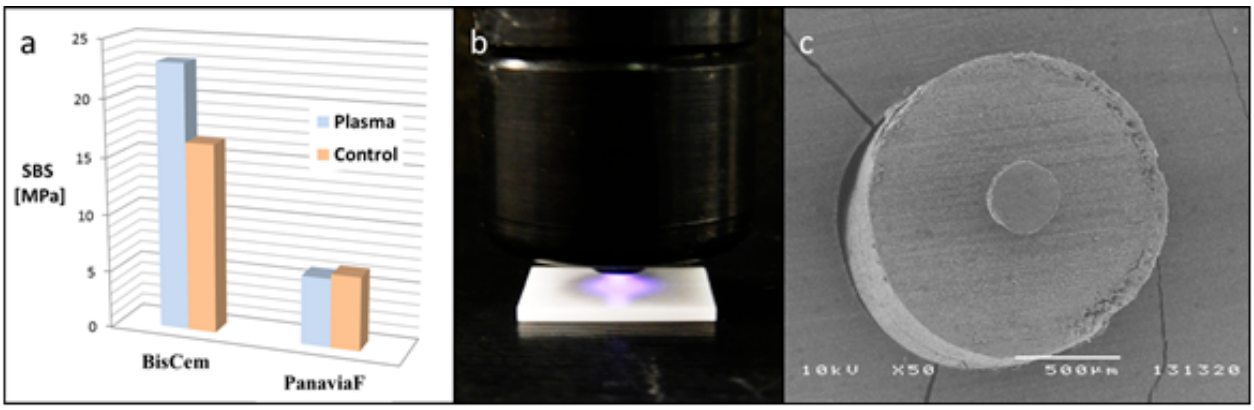


**Fig 1:** *(a) SBS result on zirconia, (b) Plasma pretreatment of zirconia, (c) post failure analysis*

# Influence of deuterium oxide generated through non thermal $D_2O$ plasma jet on biomolecule

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Over the past few years, the application of nontharmal jet plasma (NJP) in therapy has developed into various field of research. One promising new medical application of NJP is cancer treatment as well as modification of bio molecules through a various kind of reactive species generated through by plasma specially OH, $H_2O_2$, NO. However, growing demands of NJP in cancer therapy needs to further innovation and development of NJP source. Since heavy water (deuterium oxide, $D_2O$) has been seen to be an active in various cancer cells line *in-vitro* and *in-vivo*. Therefore by application of plasma with the combination of heavy water vapour (deuterium oxide) might enhance the potency of NPJ. Therefore in this work demonstrate that use of heavy water vapour in combination with nitrogen gas for the generation of plasma shows high amount of deuterium oxide instead of hydrogen oxide radical that was shown in the oxidation of DNA, carbonylation of protein and apoptosis in cancer cell through activation of apoptotic biomarker of related ATM, BAX and NOX gene expression.

Keywords: Non thermal Plasma jet (NJP), Reactive oxygen species (ROS), Nitric oxide (NO), Hydroxide (OH).

# Minimization and Localization of Cell Damage under Microplasma Irradiation for Gene Transfection

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On a unique gene-transfection technique using plasma irradiation, we have developed spatio-temporally stabilized microplasma irradiation system by employing small capillary tube having combined functions of the gas nozzle and the HV (high voltage) electrode. The outside and inside diameters of the capillary tube are 70 and 20 μm respectively. Using this technique, high transfection rate is realized with small damage to cells; the damage is localized to the microplasma irradiation spot, on the other hand, the transfection occurs around the spot. In this study, cell damage area is further minimized and localized by optimizing the shape of the GND (grounded) electrode.

Figure 1 shows schematics of electrodes and discharge images for (a) copper plate and (b) steel needle (∅0.5 mm) GND electrodes. It is found that the plasma irradiation spot shrinks with the needle electrode because of concentration of electric field. Driving voltage was a pulse-modulated (25 Hz, 1%) sinusoidal (24 kVpp, 20 kHz), discharge gap was 1 mm and duration was 5 s. The sample solution contained cells (COS 7) and plasmid (pCX-EGFP, 0.14 μg/μl).

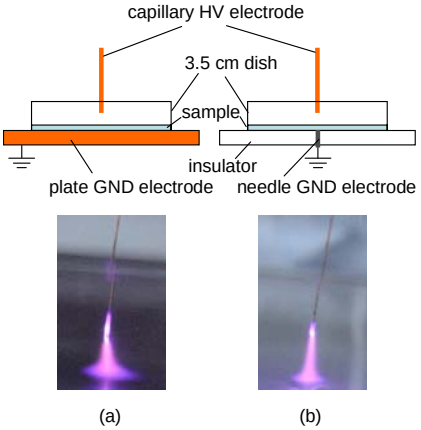


**Figure 1:** *schematics of electrodes and discharge images for (a) plate and (b) needle GND electrodes.*

Figure 2 shows (a) bright field and (b) fluorescence images after 24 h incubation after discharge irradiation with each electrode. From the bright field images, it is found that the area in which necrosis occurred drastically shrinked by using needle GND electrode. On the other hand, fluorescence images show that the area in which transfection occurred widely distributed in both cases. As a result, by using the needle GND electrode, the area ratio, (area in which transfection occurred) / (area in which necrosis occurred) increased by 3.5 times.

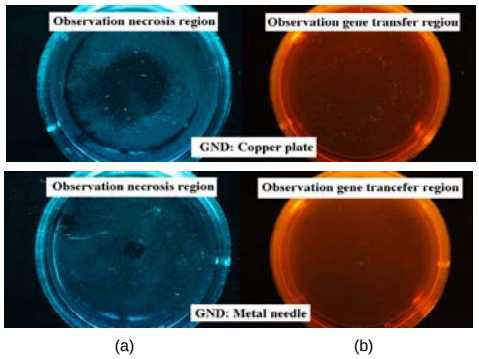


**Figure 2:** *(a) bright field and (b) fluorescence images after 24 h incubation after discharge irradiation with (upper) plate and (lower) needle electrodes.*

This work was partly supported by the Grant-in-Aid (25108509) from JSPS.

# Evaluation of DNA Damage Irradiated by Plasmas for Gene Transfection

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On a unique gene-transfection technique using plasma irradiation, we have developed spatio-temporally stabilized microplasma irradiation system by employing small capillary electrode. Using this technique, high transfection rate is realized with small damage to cells; the damage is localized to the microplasma irradiation spot, on the other hand, the transfection occurs around the spot. However, damage to DNAs has not been evaluated. In this study, conformation change of DNAs by plasma irradiation is evaluated.

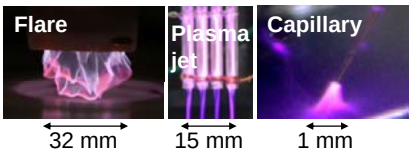


**Figure 1:** *Photos of plasmas*

Figure 1 shows three different forms of plasmas used in this study. The flare plasma was generated between two horizontally aligned electrodes with 3.2 mm distance and the plasma blew to the dish with working gas (He) flow. The applied voltage was pulse-modulated (25 pps, 1%) sinusoidal (20 kHz, ~10 kV) waveform. The flare swayed and irradiated the area of 32 mm in diameter on the dish. The plasma jet was generated on the tip of the four glass tubes with 1.25 mm of the inside diameter. A sinusoidal driving voltage (8.5 kHz, ~10 kV) was applied to the electrodes placed on the outside of the tubes. The microplasma was generated on the tip of the copper capillary HV (high voltage) electrode with the outside diameter of 70 μm. The driving voltage waveform was identical to that for the flare. This plasma was most stable and had smallest irradiation area (~1 mm in diameter). The sample plasmid DNA solution (pCX-EGFP, 3.6 μg / 120 μl) in the dish was irradiated by the above plasmas for 0.5–10 s. The DNA damage was observed by agarose gel electrophoresis and the damage degree was evaluated of the band intensity ratio of open and closed circular conformations.

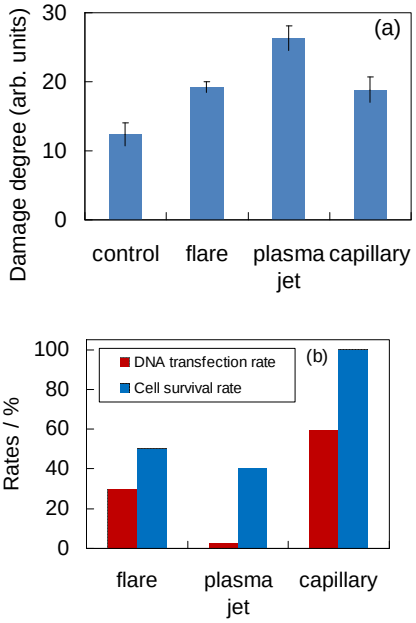


**Figure 2:** *(a) the DNA damage degrees and (b) the transfection and cell survival rates*

Figure 2 (a) shows the DNA damage degrees for each plasma source and (b) the transfection and cell survival rates measured under the same conditions. It is found that the damages to DNAs by the flare and the capillary are not so high compared to the control case by taking into account deviation. The capillary method realizes small damages to both cells and DNAs.

This work was partly supported by the Grant-in-Aid (25108509) from JSPS.

# Cell Activation using Micro-Spot Atmospheric Pressure Plasma Derived FGF-2/VEGF

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Using an *in vitro* model, we investigated the effect of the atmospheric pressure plasma irradiation to mice embryonic fibroblast cell line (NIH3T3 cell) and porcine aortic endothelial cells (POAEC). In the plasma exposure experiment for cell proliferation was inhibited in proportion to processing time. However, it was found that this inhibitory effect was suppressed by plasma irradiation and cells are rather on an increase trend. And, in comparison with the cell growth curve for the He gas flow group, the curve for the plasma irradiation group was shifted to the left. Doubling time of POAEC calculated from the multiplication curves were 28.8 h for the flow group and 24.0 h for the plasma irradiation group (Figure 1). We investigated expression analysis in the subsequent experiment with focus on factors related to angiogenesis, it was found that the transient overexpression of VEGF are observed in 24 h from the plasma irradiation (Figure 2). This proliferative effect is likely related to several growth factor releases due to plasma-induced ion/radical interaction.

This study was supported by a Grant-in-Aid for Scientific Research on Innovative Areas (No. 24108010) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

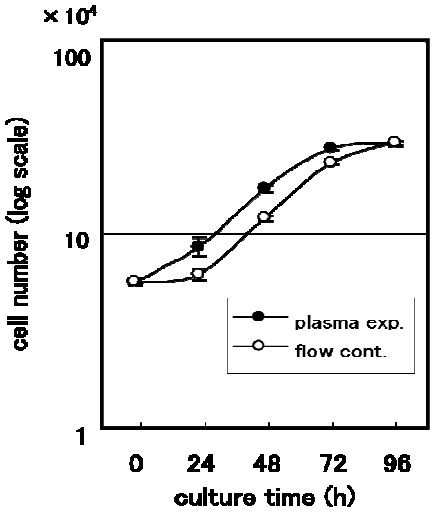


**Figure 1:** *Cell growth curve of plasma irradiation for POAEC.*

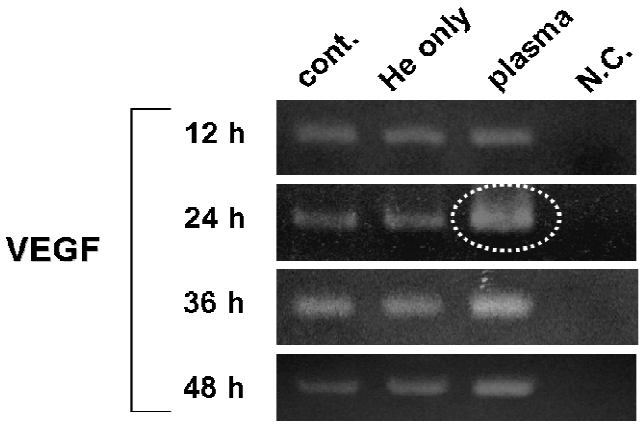


**Figure 2:** *Expression changes of VEGF after the plasma irradiation for POAEC.*

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# Plasma Gene Transfection with Surface Discharge

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On a unique gene-transfection technique using plasma irradiation with capillary electrode developed by the authors, it is found that the transfection area is widely distributed outside the plasma irradiation spot [1]. On the other hand, the damage to the cells concentrates at the irradiation spot because of vertical direction current flow. In this study, the authors attempt to realize high-rate and low-damage transfection with horizontal current flow by initiating surface discharge.

Figure 1 shows experimental setup for surface discharge irradiation. A half-wave rectified sinusoidal wave voltage (20 kHz) was applied between two needle electrodes. The discharge gap was set at 10 mm. In order to initiate surface discharge near the sample dish certainly, the only tips of the electrodes were exposed to the discharge area using epoxy resin insulator. The electrodes tips were placed 0.75 mm above the sample dish (cells: COS 7, DNAs: pCX-EGFP). After 24 h incubation following plasma irradiation, transfection rate was evaluated by fluorescence observation.

Figure 2 shows an example of the fluorescence image for the transfected cells under the condition of applied voltage 4 kV and discharge duration of 5 ms. It is found that several transfected cells are observed between the electrodes without necrosis nor fusion. On the contrary, only a few transfected cells were observed without the insulator between the electrodes. These results imply that the surface discharge contributes low-damage transfection. Analysis of the insulator effects on discharge propagation is under progress.

This work was partly supported by the Grant-in-Aid (25108509) from JSPS.

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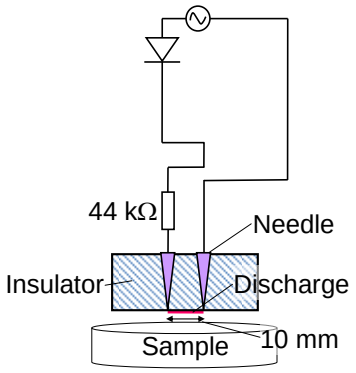


**Figure 1:** *Experimental setup*

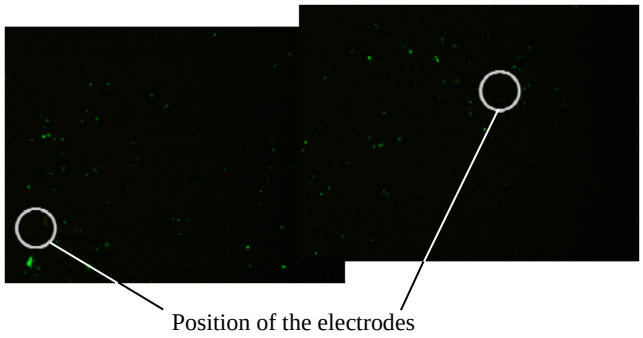


**Figure 2:** *Fluorescence images of the transfected cells*

# Measurement of electron temperature and 1s excited atom density by using collisional radiative model in nonthermal atmospheric Ar plasma jet

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Plasma has a large of applications such as in material processing and bio-medical fields. Currently, nonthermal atmospheric plasma is usually widely used in various research fields and industry. Especially, in bio-medical technology, this plasma plays a important role because this source generate to the radical chemical species which are important elements of bio-medical research. Plasma diagnostics techniques are very important to understand nonthermal plasma. We have built simple collisional radiative model about the excited and transferred process in Ar discharge atmospheric plasma jet. As using this model, we can get the electron temperature and 1s excited atom density ($1s_2$, $1s_4$ : resonance state, $1s_3$, $1s_5$ : metastable state) of Ar plasma[1]. In the experimental result, the electron temperature and Ar 1s excited atom density were about 1 eV and was ~ $10^{11}$ $cm^{-3}$, respectively. We have studied about the electron temperature and 1s excited atom density in accordance with distance from the electrode tip.

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# Mass spectrometric analysis of negative ion formation in atmospheric pressure corona discharges with point-to-plane electrodes

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Atmospheric pressure corona discharge has been used as an ionizer in a wide range of research fields such as analytical, atmospheric and plasma medical sciences and possible even commercial electric appliances. Despite substantial progress of applications using corona discharge, the elementary processes involved in ion formation occurring in ambient discharge area are not yet well understood. It has been reported that negative ion formation is rather complex compared to that of positive ions, and therefore that it is difficult to regulate the reproducible formation of specific negative ion species.

The negative ion formation in atmospheric pressure corona discharge is attributed to various different reactions involving electrons and common air constituents. Electron attachment reactions with $N_2$ and $O_2$ produce primary ions $O^-$ and $O_2^-$ and radicals N and O which are the precursors for the formation of neutral species $NO_x$ as discharge by-products. The primary ions move along the electric field lines between the electrodes. Simultaneously, they alter more stable ions through successive ion-molecule reactions with common air constituents and discharge by-products, referred to as "*ion evolution*". The progress of negative ion evolutions and resulting terminal ion formation are strongly dependent on the abundances of primary ions and neutral species $NO_x$ produced in the discharge area. It should be noted here that the abundances of such ionic and neutral species produced via the electron attachment reactions are regulated by the electron kinetic energy (*KE*) which is determined by the electric field strength (*E*) at the electron accelerated position, i.e., $KE = E \times \lambda_e$ ($\lambda_e$ is mean free path of electrons. 375 nm at 760 torr in ambient air). Thus, it can be expected that the electric field strength and resulting kinetic energy govern the sequential progress of negative ion evolution.

Here we have studied the formation mechanism of negative ions on the basis of thermochemical reactions to form various negative ions, electric field strength and the resulting electron kinetic energy, using an atmospheric pressure DC corona discharge system coupled with JMS-LCmate reversed geometry mass spectrometer (JEOL, Tokyo, Japan) and electric field calculation [1]. The experimental and theoretical results obtained suggested that the negative ion evolutions proceed along field lines established between the point-to-plane electrodes with arbitrary configurations and that the resulting terminal ion formation on a given field line is attributable to the electric field strength on the needle tip surface where the field line arises. The negative ions $NO_x^-$ ($NO_2^-$, $NO_3^-$ and $HNO_3^-$) and $CO_x^-$ ($CO_3^-$ and $CO_4^-$) were dominantly produced on the field lines arising from the needle tip apex region with the highest electric field strength, while the field lines emanating from the tip peripheral regions with lower field strength resulted in the formation of the $HO^-$ ion. Those fundamental studies made it possible to reproducibly generate various negative ions originating from ambient air only by varying corona voltage and needle angle to the plane, which in turn, contributed to the understanding of the effect of various ionic and neutral species formed in corona discharges on *E.coli* inactivation.

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# Experimental study of a discharge propagating in a dielectric capillary – Interaction of a plasma jet with a surface

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Plasma jets have a great potential for biological tissues and cells treatment [1,2]. They provide reactive species which interact stongly with surfaces. As these discharges can propagate in capillaries over long distances, the high voltage power supply can be far away from the region to treat. This is an important point for medical applications to avoid any electrical risk.

Recentely, using a low frequency sinusoidal power supply, we could point out a charge deposition on the capillary surface during the propagation of the discharge, and how this charge deposition affects the propagation of the following discharges in the tube [3]. In particular we were able to show a change in the discharge velocity during propagation when the capillary surfaces are not homogeneously pre-charged. We study here the propagation of a plasma bullet inside and outside a 1mm-diameter capillary tube.

The jet was put in contact with different surfaces (dielectric surface, metal plate, water of different conductivities). One of the surface effects observed was a discharge starting from the water surface and going towards the tube (Fig.1). Many shapes of capillaries were tested, as well as different gases (He and Ar). On these configurations, diagnostics used were fast imaging, electrical diagnostic, optical emission spectroscopy and laser absorption spectroscopy.

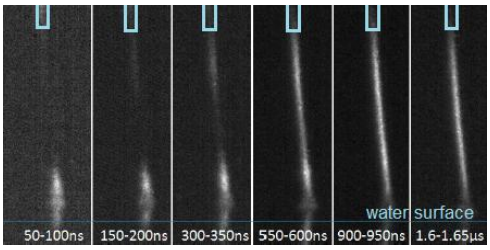


**Figure 1:** *50ns pictures of a He plasma jet coming out of the capillary (on the top of the pictures) and propagating in the open air. The jet impacts a water surface at 75mm from the tube. A back discharge starting on the water surface is observed.*

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# Study on the radical production in atmospheric pressure pulsed DBD plasma jets

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Applications of plasma discharges for bio-medical uses are rapidly growing. Atmospheric pressure plasmas have great effectiveness in bio-medical applications from coagulation or skin regeneration to sterilization. Atmospheric pressure plasma jets are one of the most common atmospheric pressure plasma sources, which have their unique virtue with better utility and concentrated treatment.

The radicals produced from plasma discharge have various roles in bio-system. It can enhance the cell proliferation and also cause cell death. However in the case of the direct treatment for bio-system, the heat from the plasma sources which can damage the bio-system is one of the main problems. Thus pulsed operation of plasma is suitable for bio-treatment, which can reduce the heat and enhance the radical production with better power efficiency. Also dielectric barrier in the jet source such as quartz tube or anodized surface serves stable glow discharge with low current which is related to the heat and bio-system damages.

In pulsed DBD plasmas, the radical production is dependent on the pulse rising time and the dielectric barrier properties. The dielectric barrier changes the relative capacitance of the plasma and it affects the plasma discharge and the radical production rate.

The experiment was performed by changing the dielectric materials and their structures and measuring the production of a few kinds of radicals. The radical production depending on the properties of dielectric barrier in atmospheric pressure plasma jets was analyzed. The result shows that the radical production in plasma tends to increase as the dielectric barrier thickness increases until the electric field for discharge is severely weakened.

# Study on Coloring Effect for Metal Surface using Atmospheric Pressure Plasmas

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Surface color for metal is added value to industrial applications. To obtain the surface coloring for metal, an anodic oxidation coating or a laser oxidation have been demonstrated[1]. However, these processes are required to preprocess for obtained oxidation on metal surface. Atmospheric pressure plasmas are generated dense oxidants as atomic oxygen, ozone, and hydroxyl radicals[2]. Thus, we proposed demonstrate the surface color for metal using irradiation of atmospheric pressure plasma.

The atmospheric pressure plasma is generated with a dielectric barrier discharge, which consists of an inverter power supply and electrodes covered by a dielectric of alumina. To sustain the plasma, the dielectric barrier discharge is introduced buffer gas of helium. A sample plate as oxygen-free copper is set on the dielectric. To measure the excitation temperature and the generated radicals in the plasma, visible and near-infrared spectrometers are used. After the plasma irradiation, composition ratios for the surface and the depth were observed with the X-ray photoelectron spectroscopy (XPS) and the glow discharge spectroscopy (GDS), respectively.

Figure 1 shows the surface color variation of the copper plate after plasma irradiation. As shown in Fig.1, the surface color changes from that of the natural color to orange, purple, silver and gold during the irradiation. As the result of analysis for chemical composition of copper plate by GDS, it indicates that the surface color depends on the thickness of the oxidation on the sample. From the results of XPS, CuO, $Cu_2O$, and $Cu(OH)_2$ are generated on the sample surface.

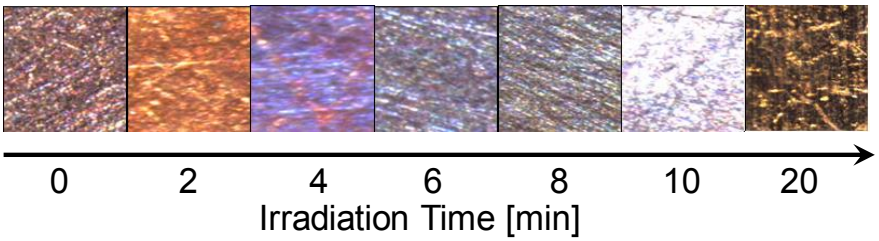


**Figure 1:** *Color variation of copper plate as a function of irradiation time of the atmospheric pressure plasma*

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# Correlations of in-line analytical investigations of atmospheric pressure plasma processes with surface analysis

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Due to the excellent ability to be integrated in technological processes, atmospheric pressure plasma discharges are an often used tool to modify polymer surfaces. Chemical changes of the outermost polymer surface layers are obtained by this short, intensive plasma treatment [1-3]. Tailor-made surface properties will be achieved by defined changes in the treatment atmosphere depending on supplied gases, aerosols or also precursors within a carrier gas. Besides the pre-treatment of polymer foils by DBD, plasma jet systems are used for applications in biology or dermatology, nowadays [4, 5]. It is in general interest to correlate plasma parameters with the resulting surface modification and there is a great demand from foil-producing and foil-converting companies to find parameters for inline process control.

The aim of the presented investigation is to find correlations between the reactions in the gaseous phase and the resulting surface properties and evaluate the gained information as a process controlling tool. First, different atmospheric pressure plasma devices are compared and evaluated concerning their efficiency of surface activation on different types of polymers. Simultaneously, optical emission spectroscopy (OES) is applied to study the reactive species in the plasma. The surface modification of the different polymer surfaces is analysed in particular by the determination of the polarity by contact angle measurements. Additionally, X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM) are applied to investigate and evaluate the plasma modified surfaces. By means of peeling tests, the adhesion behaviour of the plasma treated polymer surfaces is tested.

Beside the high intensity of the nitrogen atomic emission, the intensity of the weak oxygen emission peak can be used as parameters for plasma characterization and it can be correlated with surface properties like oxygen content determined by XPS and the polar component of surface tension. Therefore, the peak intensity can be used for inline monitoring of the plasma parameters and the resulting surface modification.

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# Development of ECR Microwave Antennas for the Production of Streaming Atmospheric-pressure Plasma

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A microwave plasma device consists of a continuous-wave 2.45 GHz, 2 kW magnetron attached to waveguide components, has been used to generate Argon plasma jet at atmospheric pressure for bacterial inactivation application. The presence of UV emission during Ar plasma exposure is confirmed by line spectra observed in the wavelength range from 200 to 400nm. There are other observed spectral lines of species like Ar I at 521 nm and Ar II at 426 nm which indicate the ion bombardment to the cell wall leading to the structural damage of the cell [1]. Bacterial inactivation by atmospheric-pressure plasma jet can be achieved by the synergistic effects of plasma particles, UV radiation and heat. The present results have shown the dominant role in the inactivation of *B. subtilis* is played by the plasma particles.

To further investigate ion transport under collisional condition, a device that holds Electron Cyclotron Resonance (ECR) plasma in a magnetic field has been designed and being tested under a reduced gas pressure environment. Microwave is supplied through a coaxial cable and transmitted into space by a spiral antenna. The microwave antenna structure is water cooled to prevent overheating of the connection between the coaxial cable and the antenna. A ring-shaped gas injection unit with nozzles is located in front of the antenna to produce a directional flow of a plasma.

Currently, the ECR microwave antenna system is being modified in such a way the device can produce streaming atmospheric-pressure plasma. Ring shaped gas nozzle structure will be incorporated into a microwave antenna immersed in magnetic field. The effect of the magnetic field is investigated in an extremely high collision rate condition. The results of comparative study with other antenna structures with respect to potential applications to biomedical fields are discussed.

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# He Plasma Plumes in Different Surrounding Gases

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Since the plasma jets generate plasma plumes in surrounding air, the diffusion of surrounding air affects the plume propagation. However, the existing knowledge on the effect of surrounding gases on the plasma plume propagation is very limited with no previous experimental evidence on the possible effects of nitrogen or oxygen surrounding gases. This knowledge is becoming more and more important because of the recently demonstrated effects of the plasma-generated reactive species (e.g., $N_2$ and $O_2$ derived) in various nanoscale doping, oxidation and reduction techniques.

Here, we report on the effects of the $N_2$ and $O_2$ surrounding gases on the propagation of the plasma plume. It is found that an interesting and unusual feather-like plasma plume can be generated when $N_2$ is used as surrounding gas, as shown in Fig. 1. A combination of nanosecond-precision high-speed photography, electromagnetic measurements, and numerical modeling is used to relate the quantized plasma bullet propagation and the He density distribution to the formation of the unusual feather-like diffuse plasma plume structures. The He concentration on the axis at the starting point of the feather-like plume is ~0.85 of the maximum value and is independent on the He flow rates [1]. High-speed optical imaging reveals that dim diffuse plasmas emerge just behind the bright head of the plasma bullet at the starting point of the feather-like plume, as shown in Fig. 2. As these structures develop away from the nozzle exit in surrounding nitrogen rather than in oxygen-prone atmospheres, it indicates the possible crucial role of seed electrons and Penning ionization. These results can be used to tailor the exposure of various surfaces of living cells and nanomaterials to the reactive species in the emerging applications of non-equilibrium atmospheric-pressure plasma jets in medicine and nanotechnology.

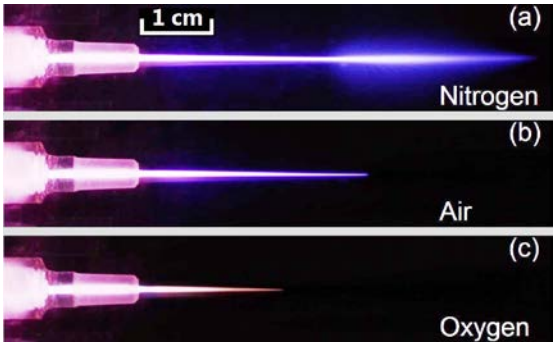


**Figure. 1:** *Photographs of the He plasma jet propagating in ambient (a) $N_2$, (b) air, and (c) $O_2$.*

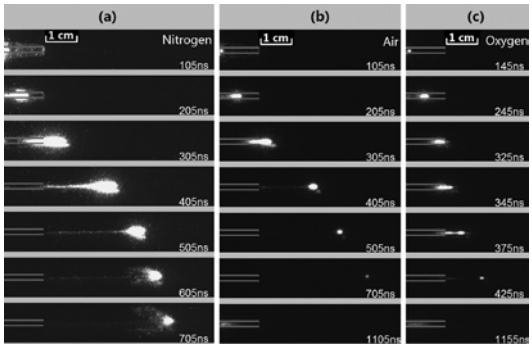


**Figure. 2:** *High-speed optical imaging of the He plasma jet propagating in ambient (a) $N_2$, (b) air, and (c) $O_2$.*

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# Characteristics of Reactive Particle Production in Atmospheric Pressure DBD Plasma Jet

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Atmospheric pressure non-equilibrium discharge plasmas have been widely employed in biomedical applications because such plasmas induce little thermal damage to biomaterials. Dielectric-barrier-discharge (DBD) plasma jet is the most common atmospheric pressure discharge system for biomedical application. In this study, we report the spectroscopic properties of DBD plasma jet in order to investigate the characteristics of reactive particle-production. Furthermore, we carried out absorption spectroscopy to directly evaluate number density of reactive O atoms.

The He atmospheric DBD plasma jet was ignited in a quartz tube wrapped with copper metal strips of 45 and 15 wide as the power and ground electrode, respectively. The distance between the power and ground electrode was 7 mm. The outer and inner diameter was 6 and 4 mm, respectively. The power electrode was connected to 5 kHz positive pulse voltage with an amplitude of 6 - 10 kV. The flow rate of the helium gas was 3 - 10 slm. Plasma bullet traveled from the outlet of quartz tube at velocity of 30 - 70 km. We observed emission spectrum from the plasma bullet under the open-air condition. O 777 nm line was clearly detected in addition to He lines. As can be seen in Fig. 1 (a), the O emission intensity $I_{O:777\ nm}$ rapidly decreases with increasing z at gas flow rate of 3 - 5 slm, where z represents the distance away from quartz tube outlet. The $I_{O:777\ nm}$ also increases linearly with discharge frequency up to 5 kHz, and then is saturated. This means that number density of reactive O atoms is controlled by the discharge frequency.

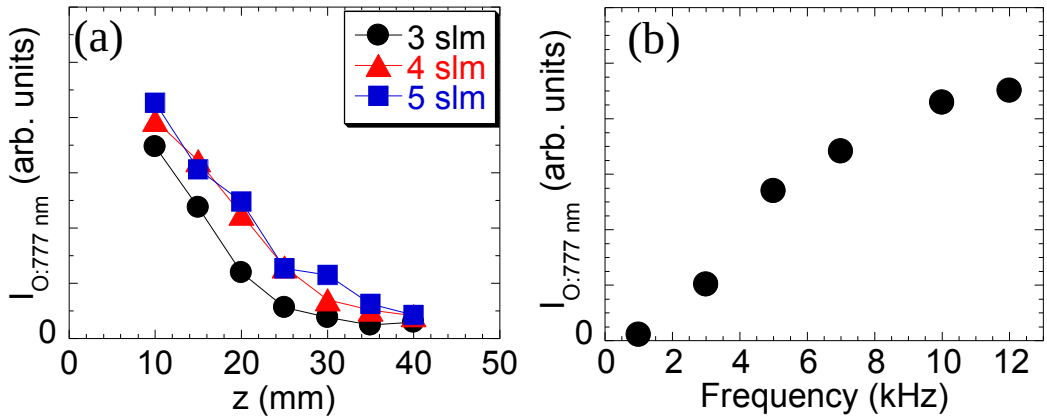


**Figure 1:** *Dependence of the emission intensity of O atoms on (a) z distance and (b) discharge frequency. z represents the distance away from quartz-tube outlet.*

Acknowledgements
This work was partly supported by The Grant-in-Aid for Scientific Research on Innovative Areas “Plasma Medical Innovation”(24108003) from The Ministry of Education, Culture, Sports, Science and Technology (MEXT).

# Atmospheric pressure plasma jet for separated and combined treatment with plasma generated reactive species and photons

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Atmospheric Pressure Glow Discharges (APGD) are effective sources of reactive particles, photons, ions or electric fields and it was shown many times that direct or remote treatment of biological substrates can lead to local inactivation of microorganisms or to positive changes in the wound-healing process. However, due to the complexity of the plasma-substrate interaction, only limited and mostly qualitative understanding of this interaction is available. Particularly difficult is to identify, which plasma components are dominating the treatment and whether there are synergistic effects among them.

Here we present the geometry, operation conditions and examples of applications in fundamental research of a remote microscale Atmospheric Pressure Plasma Jet (μ-APPJ), where only the plasma effluent is interacting with the substrate. This source has been modified in such a way that either only the reactive species or only the plasma generated photons can be used for the treatment, allowing the separation of both effects or the study of synergistic processes between them (Fig. 1).[1,2] Fluxes of reactive species are quantified with mass spectrometry or laser absorption spectroscopy and the qualitative changes in the plasma spectrum in the range 50-300 nm are measured.

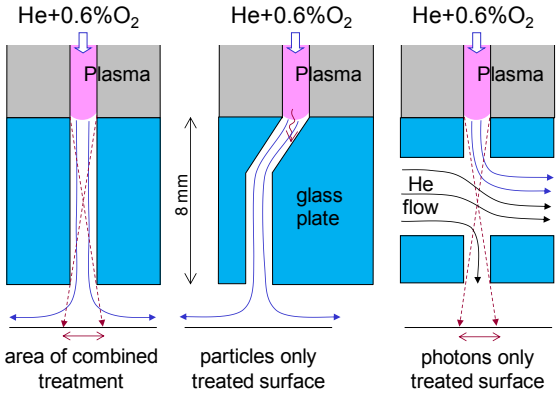


**Figure 1:** *The separate or combined treatment of the substrate with (V)UV photons and/or particles as enabled by different extensions of the μ–APPJ plasma channel.*

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# Modification of hydroxyapatite and polystyrene surface for cell culture by low-pressure plasmas

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It has been known that biocompatibility and bone regenerative capacities of porous hydroxyapatite can be significantly improved by plasma surface treatment [1,2]. Plasma surface treatment is also often used to improve biocompatibility of polystyrene cell-culture dish surfaces, which can affect adhesion and therefore proliferation/differentiation of cells cultured on them. For example, establishment of safe and stable culture processes for cells obtained from a patient's own body are highly desired for regenerative medicine and plasma surface modification of cell-culture materials may play an important role for the development of such processes.

In this study, we have examined plasma surface modification of biomaterials and the relation between chemical characteristics of plasma treated biomaterial surfaces and reactions of living cells to them. Analyses of plasma treated porous hydroxyapatite and polystyrene surfaces were performed with X-ray photoelectron spectroscopy (XPS) and Fourier-transform infrared spectroscopy (FT-IR) Figure 1 shows a FT-IR spectrum of a plasma modified polystyrene surface. It has been found that immobilization of extracellular matrices strongly depend on surface conditions of biomaterials treated by plasmas.

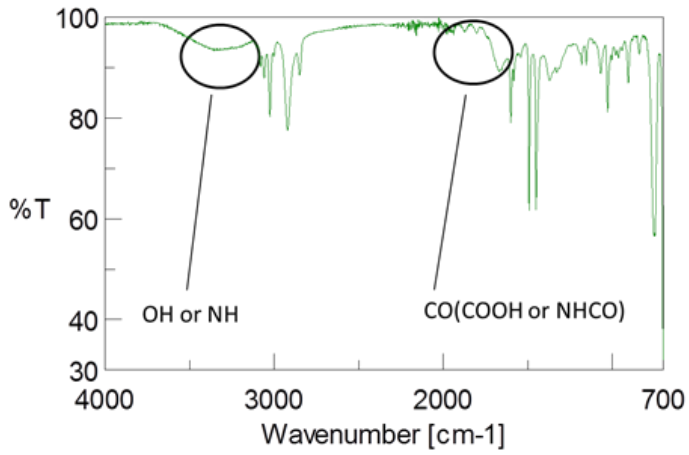


**Figure 1:** *FT-IR spectrum of a polystyrene surface with amino and carboxyl groups.*

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# Defect formation of lipid bilayer membrane by dielectric barrier discharge irradiation and comparison with chemical treatment

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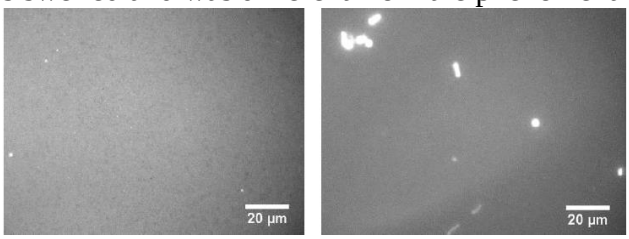
Basic study of the mechanism of plasma medicine on living cell has been studied [1]. This study aims to elucidate the interaction between plasma and cell membrane by treating artificial lipid bilayer of cell membrane [2] with dielectric barrier discharge (DBD). Lipid bilayer has a lateral diffusivity, and it was observed with fluorescence microscopy.

DOPC (dioleoylphosphatidylcholine) and Rb-DOPE (rhodamine B-dioleoylphosphatidylethanolamine) were chosen as a material of lipid bilayer. Lipid bilayer membrane was formed by the vesicle fusion method [2]. Lipid bilayer was treated with the DBD apparatus. The lower grounded electrode of the apparatus has a dent, in which lipid bilayer on substrate was mounted. Plasma was generated in Ar gas ambient between the parallel plate electrodes by applying low frequency (~ 15 kHz) high voltage for 120 s.

After the plasma irradiation, a defect in the form of patchy pattern on the lipid bilayer was observed. Fluorescence recovery after photobleaching technique (FRAP) showed that this defect did not move on substrate. In order to understand the mechanism of defect formation, we tested heat and chemical treatments without plasma irradiation. When lipid bilayer was heated up to 70°C, some white spots whose fluorescence intensity was higher than other area were seen on the lipid bilayer after heat treatment. The white spots were also observed when $H_2O_2$ solution was added to lipid bilayer, as shown in Figure 1. These results indicate that a part of lipid bilayer was swelled and was different from the phenomena by plasma irradiation.




(a) (b)

Figure 1: *Microscope photographs of lipid bilayer membrane: (a) before and (b) after $H_2O_2$ addition*

This work has been partly supported by the EIIRIS Project from Toyohashi University of Technology (TUT); JSPS KAKENHI Grant Number 24360108 and 25630110; and MEXT KAKENHI Grant Number 24110708.

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# Interactions of Atmospheric Pressure Non-equilibrium-Plasma with Organic Materials through Gas/Liquid Interface

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Medical and biological applications of plasma recently have shown remarkable progress with worldwide attentions [1]. In plasma medical application, it is important to understand plasma interactions with organic materials. In our previous works, plasma interactions with soft materials including biomolecules have been performed on the basis of surface analysis in terms of physical and chemical interactions using plasmas sustained with low-inductance antenna (LIA) modules at low-pressure (0.13-13 Pa) [2,3]. Based on these scientific knowledge, the present study extends further to investigate interactions of atmospheric-pressure He plasma with organic materials. In this study, plasma interactions with organic materials through gas/liquid interface have been investigated by examination of molecular-structure modification of organic materials in liquid due to irradiation of atmospheric-pressure plasma.

Generation of atmospheric pressure He plasma has been performed using micro follow cathode type plasma source. The atmospheric pressure He plasma sustained at a DC voltage of 800 V. He gas flow rate was 0.4 slm. Plasma interactions with organic materials through plasma/liquid interface were investigated using methylene blue (MB) in aqueous solution. Concentration of MB in aqueous solution is 10 mg / l.

To investigate atmospheric-pressure He plasma interactions with organic materials in liquids through the gas/liquid interface, atmospheric-pressure He plasma treatment was carried out using MB aqueous solution. Figure 1 shows the FT-IR spectra of MB in aqueous solution with and without plasma treatment. The FT-IR spectra of MB in aqueous solution without plasma treatment show the peaks attributed to the molecular structure of MB as shown in Fig. 1, while, the FT-IR spectra show the peaks attributed to partially degraded MB molecules after 5 min treatment degraded almost completely.

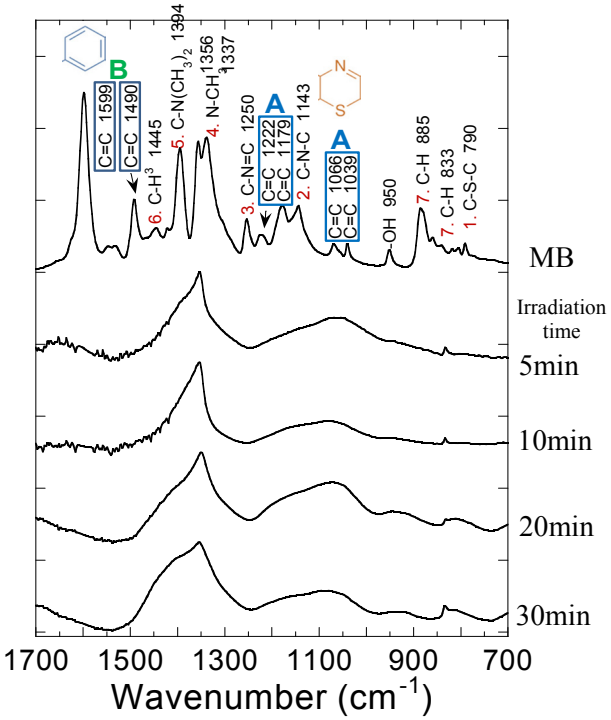


**Figure 1:** *FT-IR spectra of MB in solution irradiated with and without plasma.*

**Acknowledgements**

This work was partly supported by The Grant-in-Aid for Scientific Research on Innovative Areas "Plasma Medical Innovation" (24108003) from The MEXT, Japan.

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# Electron Spin Resonance Study of Plasma-Activated-Medium

**Kenji Ishikawa[1], Naoyuki Kurake[1], Hiromasa Tanaka[1], Takashi Kondo[2], Kae Nakamura[1], Hiroaki Kajiyama[1], Fumitaka Kikkawa[1], Masaaki Mizuno[1], Keigo Takeda[1], Hiroki Kondo[1], Makoto Sekine[1], Masaru Hori[1]**

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## 1. Introduction

Cancer is the first cause of death in Japan and thus cancer therapy is noticible an ugent requirement. Recently atmospheric pressure plasmas (APP) has actually applied to use therapeutic method for practical cancer therapy. Our group has reported some effects on anti-proliferative activity against cancer cells such as carcinoma and glioma by indirect plasma-activated medium (PAM) exposure. [1-3] Although the APP consists of various active particles such as charged species, neutral species, radicals, energetic photons and so on, a mechanism on induction of apoptotic death has not been clarified so far. Therefore it is required to elucidate how reactive species generated inside the PAM.

In this study, we focused on clarification of generation of reactive species such as radicals in the PAM. We have detected elecron-spin-resonance (ESR) signals originating generation of radicals in the PAM. From analysis of kinetic behavior of the ESR signals, we will discuss about anti-proliferation effects on the basis of experimental results for chemical modication on the PAM.

## 2. Experimental

3 ml of cell culture medium (D-MEM+FBS+P/S) in a microplate with 6 well was irradiated by the APP for 3 min. First, HO* radical having highly chemical reactivity was trapped by using spin-trapping agent of 5,5-dimetyl-1-pyrroline-N-oxide (DMPO). Radical densities were analyzed by referencing signal arisen from stable radical; 2,2,6,6-tetramethyl-4-hydroxy piperidine 1-oxyl (TEMPOL).

## 3. Results and discussion

After the plasma treatment of medium, ESR signals from OH adduct were observed clearly and its concentration was about 11.7 μM. This signal decayed exponentially with reaching the initial value of 1.0 μM for 10 min. The amount of OH adducts and temporal behaviors on the PAM were apparently different compared with plasma activated water activated identically by the APP. Therefore, the set of reactive species involving HO* at least in the PAM may contribute on the effect of anti-proliferation of cancer cells.

Preliminarily we will discuss about generation mechanism of active species in the cell culture medium by exposing reactive species such as O atoms. On the basis of experimental evidence of the generated chemical species, we propose the effect of free radicals generated by the plasma on the biological response under exposure of the plasma activated medium.

## Acknowledgements

This work was partly supported by a Grant-in-Aid for Scientific Research on Innovative Areas, “Plasma Medical Innovation” (No. 24108001) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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# Reaction of Amino Acid and Protein in Water Induced by Electric Discharge at Argon / Aqueous Solution Interface

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Proteins make up about 20% of human body. Recently, discharge plasma treatment for cell, bacteria and living tissues has recieved attention in medical application [1] [2]. However, their mechanism and reaction have not been clarified yet. This work focused on chemical reaction of amino acid and protein induced by electric discharge at gas-liquid interface and assessed the possibility of biomedical application.

The schematic diagram of experimental apparatus is shown in Figure 1. The experiments were conducted in a batch type reactor made of stainless steel. The inside of reactor contained amino acid or protein solution as a cathode. The cylindrical copper electrode of 1.00 mm diameter as an anode was set at 3 mm above the liquid surface. A voltage was applied to the anode using an MPC (Magnetic Pulse Compressor) charged by DC power supply.

Aqueous solution containing 10 g/L glycine under argon was utilized as the media for reaction. Conversion of glycine in liquid phase was analyzed by HPLC and products were identified using MALDI-TOF/MS. After treatment by DC pulse discharge plasma, dehydration was promoted in aqueous solution producing peptide of glycine. However, after 1000 of pulse discharge, conversion of glycine was fallen immediately. It indicated that oligomers were decomposed and produced monomer of glycine. In other words, reaction of amino acid shifted to hydrolysis with increasing pulsed discharge.

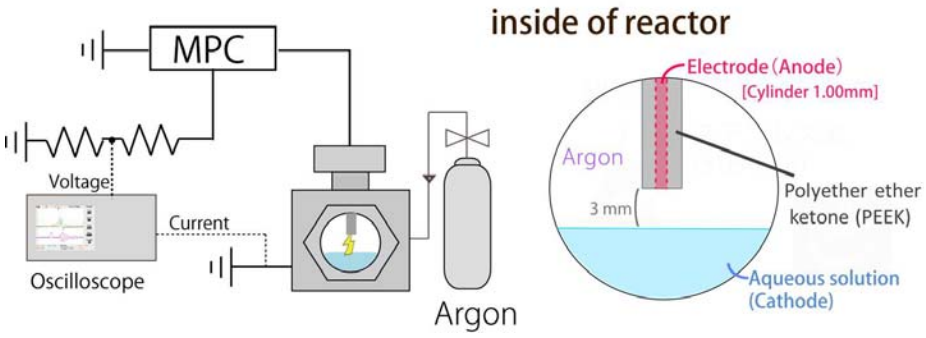


**Figure 1:** *Schematic diagram of experimental apparatus*

**Acknowledgement**
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# Characteristic measurements of a plasma flare of medical equipment using a low temperature plasma

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Hemostasis equipments are indispensable in many surgical procedures such as gastrointestinal surgery. High-frequency electrical equipment is being used as typical blood coagulation method. The hemostasis equipments currently being used for controlling the bleeding of cappilaries by heat (cauterization). This is one of the effective procedures, but it induces tissue damages, and causes post-operative disorders. Therefore, minimally invasive hemostasis prodedures that minimize the tissue damages have been studied. Argon plasma coagulator has been used for an endoscopic surgery which is categolized in cauterization methods. Recently, a medical equipment using a low temperature plasmas has been tried to treat the stop bleeding [1,2]. It was reported that the plasma flare defined as the visible region from the equipment to the treated area accerelates blood coagulation. Moreover, the histpathological obserbations show that tisse damage was not found around the treated area.

The purpose of this study is to mesure characteristics of the plasma flare around the surface of the treated material for understanging the blood coagulation phenomena. Especially, visivle and near-infrared spectroscopy, and Schlieren mesurements have been conducted. Spatial profiles of major emission lines between the equipment and the treated area along the helium gas flow are measured. As a reference, spatial profiles of those without target materials also mesured. It was found that each emission intensity ratio between the cases with and without target materials is different, and depends on the location. The results using Schlieren imaging indicate that the mixture of helium gas with an ambient air containing moisture affects on the spatial dstributuin of excited particles. At the conference, detail results will be presented.

**Acknowledgements**
This work was supported by Grants-in-Aid for Scientific Research on Priority Area (24108006) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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# Role of non-thermal dielectric barrier discharge (DBD) plasma for wound healing application

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Plasma technology is being developed for a range of medical applications including wound healing [1], which is a complex process requiring the collaborative efforts of various tissues and cell lineages. However, the behavior of contributing cell types in healing phases, including proliferation, matrix synthesis, migration, and contraction, as well as the signals controlling the cells activities at a wound site, are not fully understood after plasma treatment [1, 2].

In this study, we investigated the *in-vitro* response of, two of the most important wound healing contributing cell types, normal human dermal fibroblast (NHDF) and normal human epidermal keratinocyte (NHEK) after plasma exposure. We optimized the plasma treatment and incubation time for the cell proliferation and cell migration. Live-cell imaging system was used to access the migration rate. To evaluate the effect of plasma on cell migration supportive cell components, actin, staining was executed. For further investigation of plasma effect on wound development, keratin expression; re-epithelization marker and collagen synthesis; one of the major matrix components in healing phases was accessed. In addition, to explore the signaling mechanisms that contribute to the cell migration and proliferation on plasma exposure, intracellular reactive oxygen species (ROS) were detected and optical emission spectrum of plasma device was taken.

The results of this study indicate the increased cell proliferation and migration rate, as well as increased keratin expression and collagen synthesis on mild plasma treatment. Furthermore, the mechanisms for cell migration, proliferation and cell adhesion after plasma treatment and planning for *in-vivo* future studies will be discussed.

**Key words:** Plasma treatment, Cell migration, Fibroblast, Keratinocyte, Wound healing

**References:**

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# Ozone concentrations in the plasma volume and the surrounding of a plasmamedical dielectric barrier discharge source operated in ambient air

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Low-temperature plasmas operated with ambient air are known to be sources of significant amounts of ozone [1]. In the interaction with biological systems, ozone as a strong oxidizing agent can lead to complex behaviors. While, e.g., germ reduction in the context of wound healing can be regarded as a health promoting effect, ozone can also lead to undesirable effects on the respiratory tract whereas limit values of 0.1 ppm (daily maximum 8-hour mean) have been defined by the WHO [2]. As a consequence, the characteristics of ozone concentrations have to be carefully analyzed for plasmamedical sources operated with or in contact with oxygen containing gases.

Therefore, the UV absorption method was applied to measure mean ozone concentrations in the discharge volume of a dielectric barrier discharge (DBD) over a wide range of operating parameters. Concentrations as high as some 100 ppm can be determined, while only in some $10^{-2}$ m distance to the discharge volume, ozone concentrations decrease below the WHO limit value through dilution with neutral gas. Ozone concentrations depend primarily on the interelectrode distance, whereas this parameter is shown to influence the mean electron energy of the discharge. This effect can subsequently affect the dissociation rate of molecular oxygen and thus the formation of ozone. Finally, in view of applications of DBD sources in dermatology, ozone concentrations were compared for operation against metallic counter electrodes as well as on porcine skin.

The financial support by the German Federal Ministry of Education and Research (grant no. 13N11190 and grant no. 13N11185) is gratefully acknowledged.

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# Tuning of low temperature plasmas ejected in open air for biomedical applications from diagnostic and modeling tools

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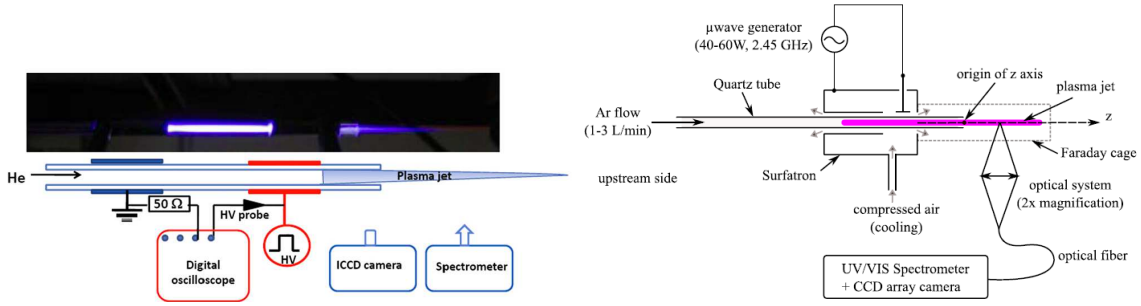
Many biomedical applications of low temperature plasmas are nowadays under intense investigations more particularly in the case of decontamination for bacteria or biofilm inactivation, in the case of tissue and biomaterial engineering and also in the case of medicine for therapeutic purposes [1].
The needed plasma active species as for instance radicals, charged particles, long lived excited species, UV photons and also electric field self generated by the plasma can be very different from one application to another. Therefore, it is necessary in a first step from investigations involving both plasma physicists and biomedical researchers (micro-biologists, biochemists, cancer biologists, physicians etc.) to select the most efficient plasma active species for a given application. Then in a second step, it is possible from plasma engineering and technology approaches based on a judicious choice of set of operating parameters (kind of power supply as pulsed or DC voltage or microwave (MW) source, gas composition, reactor design, etc.) to develop the most adapted plasma device to the studied biomedical application.
From specific plasma device setups (displayed in the following figures and developed in our laboratory [2] and [3]), some illustrative examples are given to show how to quantify the evolution of the main plasma active species from electro-hydro-kinetic modeling and experimental diagnostics.




*Figures: Low temperature plasma jet by a pulsed voltage DBD setup using Helium gas flow (left side [2]) and by a MW surfatron [3]) setup using Argon gas flow (right side).*

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# Visualization of OH radical interactions in living cells by adding $D_2O$ in non-thermal plasma jet

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The various effects of non-thermal plasma on living cells are frequently reported. However, hydroxyl radical remains several questions cells due to the short lives time and no exact way to confirm the effect of hydroxyl radicals has been existed except some molecular works indirectly supporting their suggestions. In order to observe the acting mechanism of hydroxyl radicals on cells directly, OD radical from $N_2/D_2O$ plasma jet treated *E.coli* in both $H_2O$ and $D_2O$ solution, and incorporation of deuterium into cells were visualized by use of ToF-SIMS and NanoSIMS.

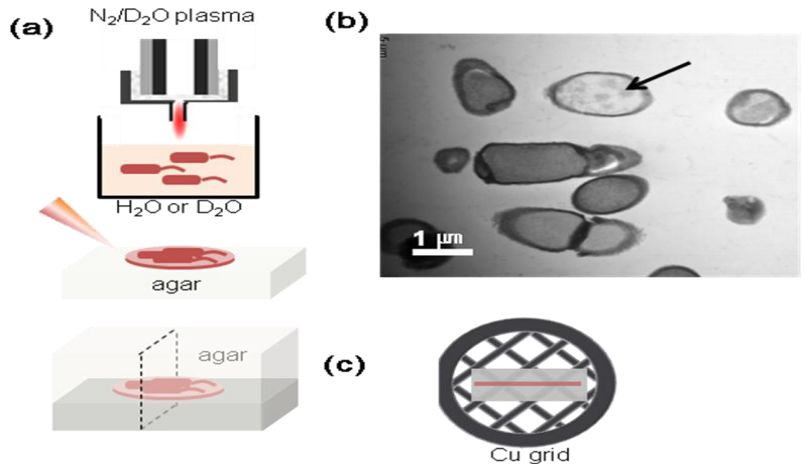


**Figure 1:** *Bacteria specimen preparation. (a) Bacteria are treated by $N_2/D_2O$ plasma jet in $H_2O$ or $D_2O$ water, embedded in agar gel. (b) Magnified TEM image showing normal and damaged bacteria (marked with arrow) together, and (c) prepared for TEM specimen*

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# An atmospheric-pressure cold plasma jet device with a multi-microchannel structure

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So far various techniques of plasma jet have been developed and applied to a variety of purposes [1][2]. In this paper, we propose a multi-microchannel plasma jet device having a simple structure and fabricate it by a simple process and test the performance.

This device is composed of microchannel layer and electrode layer. The layers were fabricated with Polydimethysiloxane (PDMS) by soft-lithography process as shown in Fig. 1. The microchannel layer has 10 microchannels and the height and the width of each microchannel is 100 and 500 μm, respectively. The electrode layer has two buried stainless steel electrodes. The diameter of the stainless steel electrode is 300 μm. In order to generate plasma jet we used AC bias voltage at 20 kHz and nitrogen gas. Gas was blown through the microchannel array and the bias was applied to the stainless steel electrodes. We observed plasma jet generated at 3.6 $kV_{p-p}$ for 4 lpm of nitrogen gas flow as shown in Fig. 2.

In conclusion, we fabricated the proposed simple device to generate plasma jet at atmospheric-pressure and the device operated successfully.

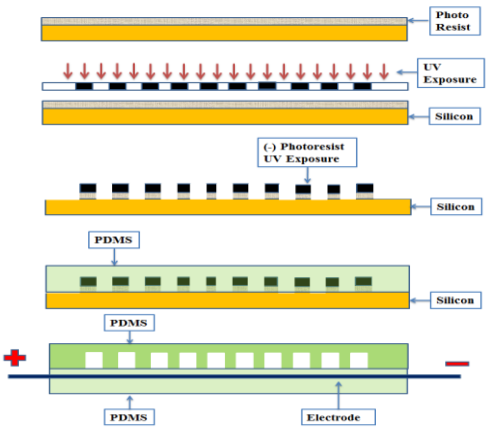


**Figure 1:** *Fabrication process*

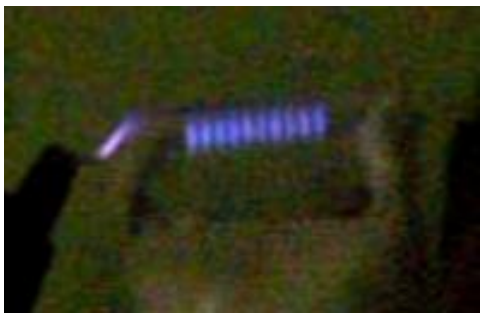

**Figure 2:** *The photo image of plasma jet*

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# Non-equilibrium atmospheric pressure plasma (NEAPP) generates oxidative injury

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Non-equilibrium atmospheric pressure plasma (NEAPP) is developed in the field of processing nano-scale materials. NEAPP contains ultraviolet radiation (UV), electron and reactive oxygen species (ROS), which include hydrogen peroxide, singlet oxygen, nitric oxide and hydroxyl radical. Although NEAPP is an established industrial technology in material science, the properties against biological materials are not fully understood. To clarify the molecular evidences of oxidative damage by NEAPP, we examined products of lipid, protein and nuclear acid, after irradiation of NEAPP. Formation of conjugated diene in linoleic acid and linolenic acid were generated by NEAPP irradiation. 2-thiobarbituric acid reactive substances (TBARS) in phosphatidylcholine and VitaminE-stripped corn oil liposome, were also generated by NEAPP irradiation. Irradiation of NEAPP against rat liver elevated formation of TBARS, 4-hydroxy-2-nonenal (HNE)-modified protein and acrolein-modified protein by immunohistochemistry. Cyclobutane pyrimidine dimers, which are formed by UV-induced DNA damage, were increased after irradiation of NEAPP. Irradiation of NEAPP against plasmid induced double strand break and elevated the formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG). These *in vitro* studies disclosed that NEAPP caused oxidative injury to lipid, protein, and nuclear acid, by irradiation time-dependent manner. These results would be helpful to assign the biological applications of NEAPP.

# Power Supply in Non-Thermal Plasma Generators for Biological Applications

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Dielectric Barrier Discharge (DBD), Gliding Arc Discharge (GAD) and Atmospheric Pressure Plasma Jet (APPJ) reactors with their supply systems have been discussed from the point view of their characteristics, possibility to control power to the discharge and efficiency. Non-thermal plasma reactors require specially designed, efficient and reliable power systems. Taking advantage of the transformer's cores nonlinearity, simple, reliable, low cost and efficient power systems, especially suitable for industrial applications, can be constructed [1]. Main disadvantage of transformers' power suppliers is the relatively narrow range of voltage and/or current control. AC/DC/AC inverters can operate both in the sine voltage or sine current regime. In the voltage regime inverter is suitable to supply DBD reactor while the current regime is most suitable to supply GAD reactor. Taking into account the electromagnetic compatibility, power supply system of GAD reactor with nonlinear transformers seems to be the better solution than electronic power inverter. Electronic modules of AC/DC/AC inverter are sensitive for GADs' origin interferences, that could propagate both through conduction and radiation and at some conditions can even destroy the supplier. The use of electrical resonance allows for higher power supply efficiency and higher power density factor of the power electronic converter. Resonant power supply system topology for DBD reactors allows continuous resonant frequency tracking and operation in reactor short–circuit state. An additional benefit is the property of transistor synchronization with the resonant capacitor voltage instead of the widely used method of distorted reactor current waveform synchronization. Precise identification of the APPJ power system parameters, together with an indication of parasitic elements allow constructing efficient systems for the treatment of the heat-sensitive surfaces. The main elements of the RF APPJ power system are: air transformer with a high leakage of magnetic flux and high-voltage capacitor to provide the voltage resonance in the high voltage side. The maximum voltage is located in close proximity to the point of resonance. This voltage can be used to supply APPJ. On the other hand, due to the resonance, output voltage of such a current source is too small to maintain conditions necessary to generate non-thermal plasma.

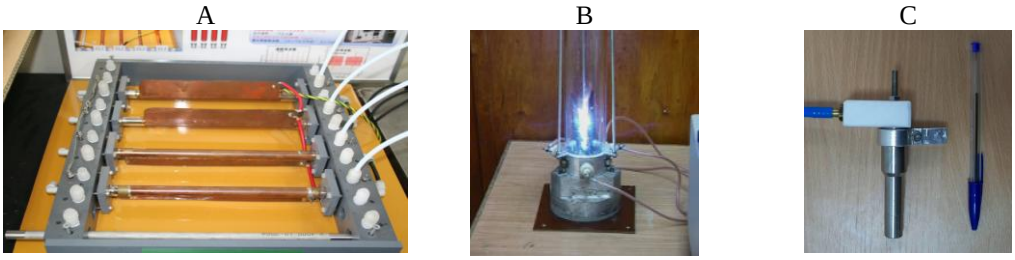


**Figure 1:** *Multielectrode soil treatment DBD reactor (A), electrical discharge in GAD reactor (B), APPJ reactor (C).*

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# Therapeutic effects of gases formed in hot air plasmas and medical applications of graphene-based polymer materials.

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Therapeutic effects of different plasma sources including plasmas generated gaseous nitric oxide (gNO) has been utilized for various clinical applications. Our previous studies [1-2] indicated that exogenous gaseous nitric oxide (gNO) flow produced by air-plasma generator ‘‘Plason’’ acts beneficially on the wound healing Classical thermodynamics confirmed by compositional analysis of the thermodynamical equilibrium of air in a plasma state shows that at a temperature lower than 2000°C the concentration of NO in the gas does not exceed 1%. Increasing the plasma temperature increases the NO concentration up to its maximum (~5%) at a temperature of 3500-4000°C. Slightly less than 4000°C is the gas temperature in the air-plasma generator ‘‘Plason’’. But this device is not exclusive, and alternative sources of plasma-generated NO may also be used for medical applications. An alternative device for production of NO-containing gas mixture employs microwave discharge technology for producing plasma having a desired composition (i.e., about 2,000 ppm of NO). Another device for production of NO-containing gas employs magnetically stabilized gliding arc discharge technology for producing plasma flow for medical use (again, containing about 2,000 ppm of NO).

A number of biological synergistic actions of gNO with $H_2O_2$ and gNO with $O_2$ species have been shown in previous publications, such as apoptosis induction and intensification of tumoricidal and bactericidal activity [3]. In the present paper we have calculated the concentration of all stable gaseous species generated in hot air plasmas. Therapeutic effect of plasma gas from these sources could be also intensified by the synergy of gNO with other species.

Some new promising medical applications of vacuum ultraviolet plasma modified graphene based polymer materials will be also demonstrated in this presentation.

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# Characterization of propagating ionization waves in atmospheric plasma discharges

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The phenomenon of propagating ionization waves at atmospheric pressure, which is often referred to as plasma bullets, is well described in recent publications [1]. Nevertheless, numerous open questions remain. This field of research is especially interesting for biomedical applications, where simple discharge types are needed, that allow direct treatment of biological tissue. This study aims to contribute to the understanding in this field of research.

The device used is a dielectric barrier plasma jet, which is basically a quartz tube in which plasma is excited by application of a high voltage (HV) pulse to an electrode wrapped around the outer wall of the tube. Various gases are flushed through the tube, namely Helium, Argon, and Neon. The discharge originates from the region where the electrode is attached. From this point, the ionization wave starts to travel through the tube in both directions, regardless of the applied gas flow. Out of the open end, a so-called effluent is ejected several centimeters beyond the tube.
HV pulses are produced in a custom-made generator, which allows studies with independent tuning of relevant parameters. Repetition rate of HV pulses is tunable from a few hundred Hz up to about 25 kHz. HV amplitude is adjustable up to 22 kV of positive or negative polarity. The current through the discharge can be influenced by variation of wall thickness of the quartz tube.

The discharge is characterized with different diagnostic methods, namely current-voltage characteristics, short-time photography, and optical emission spectroscopy [2].
Current-voltage characteristics are measured with a current monitor and a HV probe, both connected to an oscilloscope.
Short-time photography is performed with an intensified CCD camera which has a minimum exposure time of 3 ns. By using a delay generator between the pulse generator and the camera, pictures are taken of the temporal evolution of a plasma bullet.
With optical emission spectroscopy, various important plasma parameters are measured and calculated, such as electron density, electron temperature, gas temperature, and densities of gas molecules. All diagnostics are applied to the different discharge regions of interest.

We show results for different gases and electrical parameters, which causes considerably deviating discharge properties. A transition from a bullet-like behavior towards a more filament-like discharge is observed and is influenceable to a certain degree. Variations are also noticeable in effluent properties, such as varying length and electric current through the discharge. The results can help to design a discharge for specific biomedical applications.

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[2] N Bibinov *et al* 2007 *Meas. Sci. Technol.* **18** 1327

# Discharge characteristics of an atmospheric pressure cold plasma jet for medical applications

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Atmospheric pressure cold plasmas are an attractive technique for application to future medical equipment including a blood coagulator. They can overcome problems of occurring carbonization, vaporization and deep tissue injuries in using previous medical electrical equipment such as an argon plasma coagulator (APC), high-frequency electrical coagulator, ultrasonic wave equipment and laser. Recently, we developed the technology of blood coagulation using an originally-designed nonthermal plasma jet which is based on DBD using helium or argon gas [1,2].

In this work, the discharge characteristics of the plasma jet have been experimentally studied. An optical emission spectroscopy and an infrared camera are used to investigate neutral gas temperatures in plasmas and temperature distributions in applications, respectively. Its thermal properties are compared with an APC apparatus (ERBE Elektromedizin GmbH, Germany) [3]. These studies show that our plasma jet has a high level of nonequilibrium property and permit a minimally invasive plasma with very low temperature below 40°C for blood coagulation. In addition, visible and near-infrared optical emission spectroscopies, Schlieren imaging, and current measurements are carried out to estimate the characteristics of the plasma jet as a plasma coagulator. These latest experimental results will be introduced in this presentation.

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# Investigation of Singlet Oxygen ($^1O_2$) and OH radical in Bacterial Sterilization

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In recent years, atmospheric non-thermal plasmas have attracted much attention as sterilization method for heat-sensitive objects like a medical device made of plastic because of low temperature and non-toxic[1]. It is considered that sterilization effect of plasma is contributed by active species such as $^1O_2$ or OH radical which are high-oxidative. Kinds and amount of these active species depend on plasma gas species. However, conventional plasma sources have limitation in usable plasma gas species, therefore these plasma sources have little options of usable active species. To solve this problem, we designed and used multi-gas plasma jet source (PCT-DMFJ02, PCT). The plasma source can generate stable plasma at atmospheric pressure using various gases.

In this study, amount of $^1O_2$ and OH radical in various gas plasmas and contribution of these active species to sterilization effect were investigated. Using Electron Spin Resonance (ESR), amount of $^1O_2$ and OH radical generated in each gas plasma were measured. Liquid solution (200 µl) containing spin trapping reagents was irradiated with plasma. The distance between the outlet of plasma and the liquid surface was 6 mm. The gas flow rate was 1 slm. As shown in Fig. 1, amount of $^1O_2$ and OH radical were the most in carbon dioxide plasma and in nitrogen plasma respectively. Sterilization effect on *S. aureus* by various gas plasmas was estimated. As shown in Fig. 2, carbon dioxide plasma and nitrogen plasma were more effective, so the result that amount of $^1O_2$ and OH radical relates to sterilization effect was yielded. To investigate the contribution of these active species to sterilization effect, scavenger was added in the suspensions of *S. aureus*, then irradiated with various gas plasma. As a result, sterilization effect was lost. This indicates that both of $^1O_2$ and OH radical contribute largely to sterilization effect. The details of the results of these experiments will be presented.

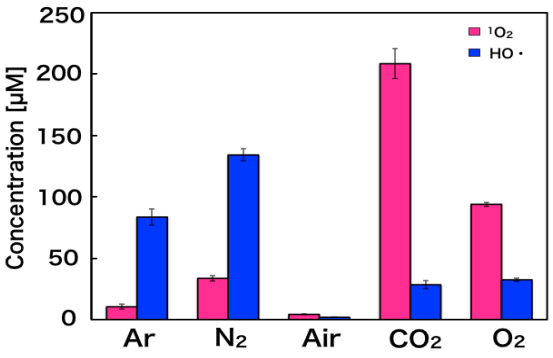


Fig. 1: Amount of the $^1O_2$ and OH radical

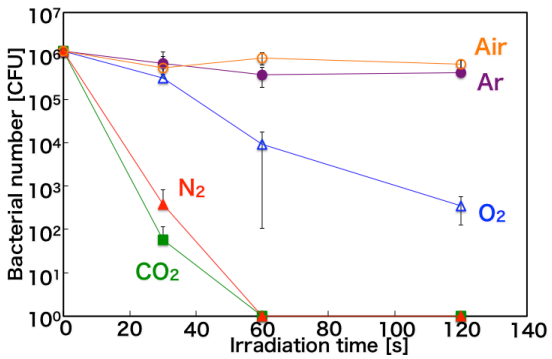


Fig. 2: Sterilization effect of various gas plasma

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# Selective reactive species production in a µs helium plasma gun discharge

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The ability to tailor reactive species production with cold atmospheric pressure plasma jets could be an effective way to maximize the efficiency of medical plasma treatments, minimizing in the same time potential collateral damages with undesirable species. The plasma source used in this work is a µs pulsed plasma gun (PG) [1]. The PG is a coaxial dielectric barrier discharge reactor with quartz capillary, flushed with helium and powered by µs voltage pulses in the kHz regime. The PG, with specific T-shaped quartz capillary geometry, allow to mix nitrogen or oxygen directly in the helium buffer gas through the inner hollow electrode (direct mixing) or downstream in the capillary between the plasma reactor and the target (downstream mixing). These two mixing configurations lead to different discharge kinetics and consequently reactive species production. As an example, the fig.1 shows two time integrated spectra of the plasma plume impacting on a grounded copper target (first approach of treatment condition on a conductive organism). Two drastically different plume emission spectra are produced when operating the PG with either 10% of nitrogen in the direct mixing configuration or 10% of oxygen admixture in downstream mixing configuration. In the first case, NO γ system is clearly visible with very weak atomic oxygen line. In the second case, NO γ system is quenched while intense atomic oxygen line (777.2 nm) production is measured. It must be pointed out that 10 % of O2 mixing is only possible in downstream mixing configuration, and seems to have in addition a stabilizing effect on the plume movements. Such $O_2$ mixing in direct configuration results in the discharge extinction in the plasma reactor. Much more detailed analysis of plasma tailoring with the PG, have been studied by time and space-resolved optical emission spectroscopy (OES) in the plasma plume region for different gas mixing configurations with or without metallic grounded target in front of the discharge. The effect of applied voltage, pulse repetition rate variations are documented in relation with species production. The impact of gas mixing configuration on reactive species production is expected to allow for a better understanding of the processes involved in the observed plasma effect and significant improvement of plasma jet efficiency in various treatments of culture cells or living tissues [2].

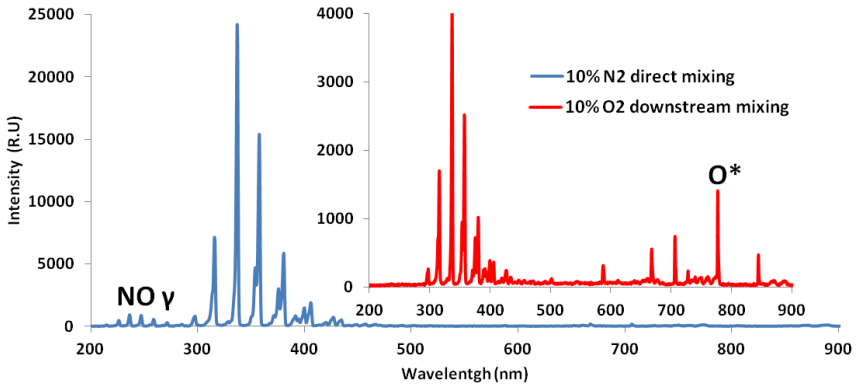


**Figure 1 :** *Emission spectra of the plasma plume on metallic grounded target in distinct gas mixing configuration with 10 % N2 (direct mixing) and 10% of O2 (downstream mixing) in helium buffer.*

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# The study of atmospheric pressure plasma to induce p53-mediated apoptosis through ROS generation in human lung cancer cells

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Recent studies have shown that atmospheric pressure plasmas are a possible candidate in cancer therapy. In the case of biological structure damage induced by plasma treatment, the primary role is played by reactive oxygen species (ROS), UV photons, charged particles and electric fields. Among them, reactive oxygen species (ROS) such as superoxide (O2-), hydrogen peroxide (H2O2), and hydroxyl radical (OH) is known to induce oxidization of DNA, proteins, or lipids and regulate cell signaling pathways involved in apoptosis. We confirmed that non-thermal atmospheric pressure plasma jet induce oxidative stress in lung cancer cells (A549). It was observed that Intracellular ROS generation was increased by helium plasma in DCF-DA assay. To enhance the intracellular ROS generation in cells, the plasma-induced ROS production controlled by additive oxygen gas. In cell cycle analysis, plasma treatment resulted in the alteration of the cell cycle that contributes to the induction of apoptosis in A549. Plasma-treated cells showed cell cycle arrest at G2/M phase that increased, a consequence that is known to be caused by DNA damage. In addition, we investigated that p53 is involved in plasma-induced apoptosis pathway in A549 cancer cells. As p53, tumor suppressor gene, is essential for cell cycle arrest and apoptosis induction. After 2hours plasma treatment, we confirmed p53 protein expression by the western blot analysis.

# The Effect of Differing Cold Plasma Composition on Glioblastoma Cell Viability

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## Abstract

Previous research in cold atmospheric plasma (CAP) and cancer cell interaction has repeatedly proven that the cold plasma induced cell death [1, 2]. It is postulated that the reactive oxygen species (ROS) and reactive nitrogen species (RNS) play a major role in the CAP cancer therapy [3]. In this study, we seek to determine a mechanism of CAP therapy on glioblastoma cells (U87) through an understanding of the composition of the plasma, including treatment time, voltage, flow-rate and plasma-gas composition. In order to determine the threshold of plasma treatment on U87, normal human astrocytes (E6/E7) were used as the comparison cell line. Our data showed that the 30 sec plasma treatment caused 3-fold cell death in the U87 cells compared to the E6/E7 cells. All the other compositions of cold plasma were performed based on this result -- plasma treatment time was maintained at 30 s per well while other plasma characteristics such as voltage, flow rate of source gas, and composition of source gas were changed one at a time to vary the intensity of the reactive species composition in the plasma jet, which will finally have various effect on cells reflected by cell viability. We defined a term "plasma dosage" to summarize the relationship of all the characteristics and cell viability:

$D \sim Q * V * t$,

where $D$ is the entire "plasma dosage" applied to the cells; $Q$ is the flow rate of the feeding gas (helium); $V$ is the output voltage; $t$ is the treatment time.

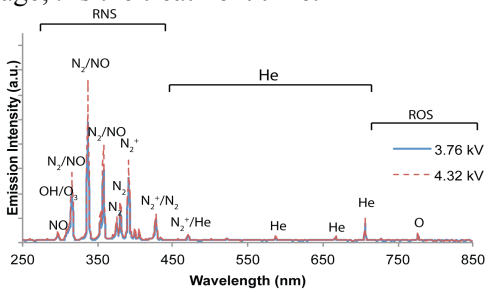


**Figure 1** *Typical spectrum of helium plasma jet (output voltage at 3.76 kV and 4.32 kV)*

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# LIF Imaging of Sodium Atoms in Atmospheric-Pressure Miniature Gas Flow DC Glow Discharge in Contact with Sodium Chloride Solution

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Recently, atmospheric-pressure nonequilibrium plasmas (APNEPs) have been widely studied with the intention of applying them to biological and medical fields. In our previous work, the spatial distribution of hydroxyl radical density in the gas phase of APNEP in contact with electrolyte surface was studied by two-dimensional laser-induced fluorescence (LIF) spectroscopy. In this work, we investigated the spatial distribution of sodium atom density to obtain further understanding on the interaction between APNEP and the electrolyte surface.

The DC glow discharge was obtained between a stainless-steel nozzle anode with an outer diameter of 0.7 mm and the surface of 1% sodium chloride (NaCl) solution as an electrolyte cathode. The gap distance between the anode and the cathode was 4 mm. The discharge current was controlled using a high-voltage DC power supply in the constant current mode. The working gas, helium, was fed through the nozzle via a mass flow controller. The excitation light for the LIF measurement was generated using an optical parametric oscillator tuned at a wavelength around 589.0nm, which is one of the D-lines of the atomic sodium spectrum. The excitation beam was arranged to be a vertical sheet beam to illuminate the gap space below the nozzle anode. The fluorescence image at the same wavelength was captured using a gated ICCD camera via an interference filter.

Figure 1(a) shows the LIF image of sodium atoms, which was observed at a helium flow rate of 70 sccm and a discharge current of 40 mA. The bottom of the figure corresponds to the surface of the sodium chloride solution. This figure shows that the peak of the sodium LIF intensity is separated from the electrolyte surface. This suggests that sodium atoms are not emitted from the electrolyte surface to the gas phase directly, but are produced in the bottom part of the plasma column. Figure 1(b) shows the LIF image of hydroxyl radicals at the same discharge condition. Comparing with Fig. 1(b), sodium atoms are localized in the region near the electrolyte surface. To obtain more accurate spatial distribution of the sodium atom density, the quenching effect of the laser-produced excited state should be considered.

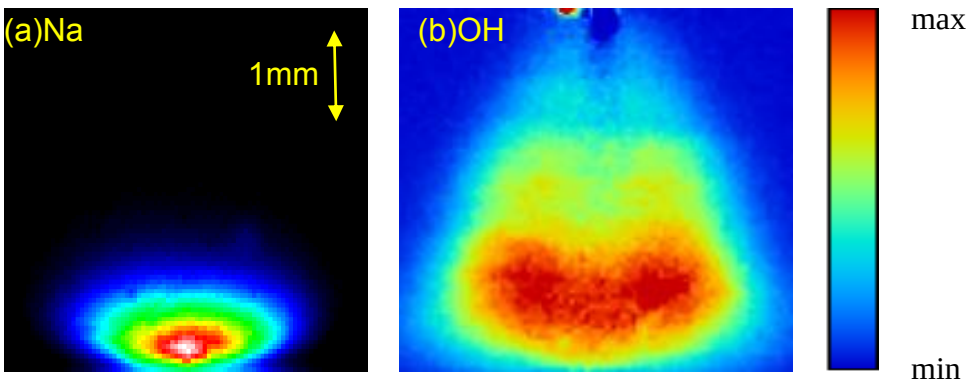


**Figure 1:** *LIF Images in the gas phase of helium dc glow discharge in contact with sodium chloride solution electrolyte cathode: (a) sodium atoms and (b) OH radicals.*

# Measurement of OH radicals in RT-APPJ using laser-induced fluorescence

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OH radicals play an important role in various applications of room temperature atmospheric pressure plasma jet (RT-APPJ) [1]. So the measurement of absolute density of OH radicals in RT-APPJs is very necessary. In this paper, the time and spatially resolved OH radicals density of a RT-APPJ is measured using the laser-induced fluorescence (LIF) technology [2]. For RT-APPJs, because of the gas flow, the OH density at a given position is different from the case without the gas flow. An OH density decay model, which including the main loss processes of OH and gas flow effect, is present. Based on the model, the simulation results have a very good agreement with the OH decay behavior measured by laser-induced fluorescence (LIF). The absolute OH density of the RT-APPJ is obtained when the best fit is achieved as shown in Figure 1(a), which is about $2.0\times10^{13}$ $cm^{-3}$ at 5 mm away from the plasma jet nozzle and 1 μs after the discharge. The OH density reaches to maxima when $H_2O$ concentration in helium gas flow is about 130ppm with the applied voltage of 8kV, frequency of 8 kHz and pulse width of 800ns as shown in Figure 1(b). In addition, in order to control the OH density, the effect of voltage polarity, applied voltage magnitude, pulse frequency, pulse width on the OH density is also investigated and discussed.

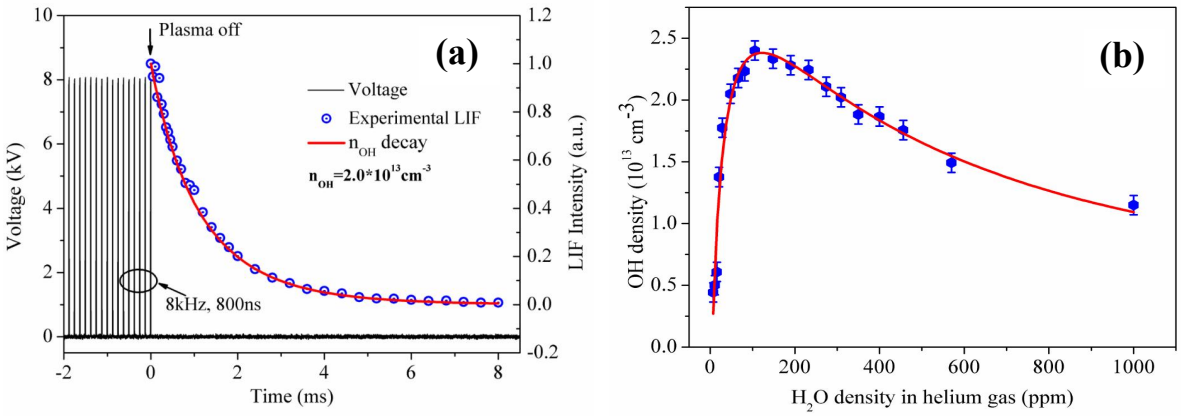


**Figure 1: (a)** *Characteristic decay curve of OH LIF signal .The blue ring is the experimental LIF signal which decayed with time evolution. The red line is the simulation result using the improved OH radicals decay model.* **(b)** *Relation between OH density at 5 mm away from the nozzle and H2O density in helium flow.*

Acknowledgments: This work is partially supported by the National Natural Science Foundation (Grant No. 51077063, 51277087), Research Fund for the Doctoral Program of Higher Education of China (20100142110005).

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# Thomson Scattering Measurements of Atmospheric Plasmas Contacting with Ionic Liquids

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Non-equilibrium plasmas in atmospheric pressure have received much attention for their economic and scientific potential. Since these plasmas are easily generated in atmosphere, they have many opportunities to be generated contacting with water or other liquid electrodes. Therefore, understanding of atmospheric plasmas contacting with liquids is important for expanding plasma applications. In order to understand plasma characteristics, precise measurements of their electron density ($n_e$) and electron temperature ($T_e$) are indispensable. For the measurements of $n_e$ and $T_e$ of plasmas, laser Thomson scattering (LTS) has been considered to be one of the most reliable method. LTS can give local values of $n_e$ and $T_e$ in plasmas with high spatial and temporal resolutions. In addition, it can measure these parameters with small perturbations compared to other conventional methods, such as probe methods. Therefore, if LTS is successfully applied to plasmas contacting with liquids, it can be a powerful method to diagnose them. Under such background, in this research, LTS has been applied to atmospheric plasmas contacting with liquids.

In order to obtain sufficient signal-to-noise (SN) ratios of LTS signals, Thomson scattering signals from a few thousands of laser shots are needed. This is because a cross-section of Thomson scattering is too weak and energy of the probing laser should be limited less than 10 mJ to avoid laser perturbations [1]. When a conventional pulsed Nd:YAG laser, whose repetition rate is 10 Hz, is used as the probing laser, more than a few minutes are needed to obtain LTS signal with enough SN ratios. Therefore, for precise measurements, experimental condition should be kept same for at least a few minutes. However, when water or solutions are used as a liquid material, the condition may be easily changed because of a evaporation and a heating of liquid materials. In order to avoid the evaporation and keep a same condition during the LTS measurements, ionic liquids, whose vapor pressure is negligible, were used as a liquid material. Ionic liquid has been already used as a electrode material for several type of plasmas [2]. In addition, non-thermal atmospheric pressure plasmas, whose duration are very short, were used as plasmas for the LTS measurements [1]. Because their heat flux are very low, it is expected to be easy to keep the temperature of the liquid material same for a long time.

In the experiment, discharge plasma produced by nano-second pulsed current was produced between a needle and a plane-shaped liquid electrode with 3 mm gap. A peak voltage and a peak current of the plasma were 3 kV and 8 A, respectively. At a time after 40 ns from a start of the discharge, $n_e$ and $T_e$ at a center of the discharge were $1.4\times10^{22}\mathrm{m}^{-3}$ and 2.8 eV, respectively. Results of the LTS measurements show that $n_e$, $T_e$, and diameter of the discharges with the liquids were clearly different from those without the liquids.

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# Investigation of Chemical Species Production Rates in Aqueous Solution Irradiated by Non-equilibrium Atmospheric Pressure Jet

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To date much attention has been given to the plasma-liquid interactions for biomedical applications since high reactive species can be supplied under low temperature condition to enhance the chemical reactions in liquid. Low frequency (LF) plasma jet is one of the convenient tools to produce and supply reactive species under atmospheric pressure condition with low cost. There are other kinds of atmospheric pressure plasma jets (APPJs) such as a dc-driven plasma jet, an rf(~MHz) plasma jet and a microwave(~GHz) plasma jet. However the fundamental characteristics of these APPJ's chemical reactivity are still inadequate due to so many operational parameters.

To develop APPJ for bio-applications, it is very important to investigate the chemical reactivity between plasma and liquid, and the relationship between plasma-mediated reactive oxide species (ROS) generation rate in the liquid phase and intracellular ROS generation as well as apoptotic cell death, since biomedical cells exist under liquid environment. [1] To progress knowledge of the fundamental chemical and physical processes occurring in liquid, it is necessary to obtain quantitative estimates of reactive species generation in aqueous solution by APPJ irradiation.

A variety of techniques are suitable for the detection of ROS such as electron spin resonance (ESR) spin trap and chemical probe techniques. Since ESR technique requires a rather complicated measurement system, we have used a chemical dosimetry technique based on a terephthalic acid (TA) to detect hydroxyl (OH) radical. TA reacts with OH radicals to form 2-hydroxyterephtalic acid (HTA), that gives a bright stable fluorescence at 425 nm for analyzing using an excitation wavelength of 315 nm[2]. A High Performance Liquid Chromatography (HPLC) is used to measure concentrations of nitrite ion($NO_2^-$), nitrate ion($NO_3^-$) and ammonium ion ($NH_4^+$).

In this study, we have investigated plasma-liquid chemical reactivity focusing on the effect of 1) Irradiation distance, 2) Gas species, 3) Irradiation area, and 4) Plasma source (dc, LF and MW plasma jet). It is realized that the OH generation rate in the liquid drastically decreased as increasing irradiation distance. Schlieren images showed spatial and temporal variations of flow structure such as a laminar and a turbulent flow, indicating an influence of surrounded atmosphere along APPJ flow direction. The influence of the oxygen addition to the primary operation gas (He) was investigated to enhance OH generation rate in the liquid. It was found that OH generation rate in the liquid had a maximum when the small amount of oxygen (~1%) was added to the He gas. Details will be presented in the conference.

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# Molecular structure of microorganisms measured by multiplex coherent anti-Stokes Raman scattering microspectroscopy

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## 1. Introduction

Various stimuli or stresses cause responses of microorganisms such as activation, functional depression, and cell death, depending on the dose or flux of factors produced from plasmas. Reactive oxygen species (ROS) induces Redox reactions in metabolism or cell membrane**,** denaturalization of DNA or protein, and some biological responses. A coherent anti-Stokes Raman scattering microspectroscopy(CARS), which is non-linear Raman scattering spectroscopy with high sensitivity, is attractive method to monitor the change in molecular structure of microorganisms. CARS also permits nondestructive molecular signal without any labeling. In this study, we developed system of multiplex CARS and analyzed molecular structures of the microorganisms.

## 2. Experimental setup

Two lasers were used to generate a coherent anti-Stokes frequency beam, which can be enhanced by resonance. Picosecond laser beams with 1064 nm and supercontinuum (450 to 2000nm) were employed as the pump and the Stokes beams, respectively. There is no need of scanning the laser wavelength to obtain CARS spectra because supercontinuum light has ultrabroad band spectra. The pump and Stokes laser beams were collinearly overlapped and tightly focused into a sample using an objective lens on the microscope. The emitted CARS signal is measured with a spectrometer and CCD.

## 3. Results and Discussion

Fig. 1 shows a CARS spectrum of a budding yeast cell (*Saccharomyces cerevisiae* W303a). Vibrational spectra were obtained in the fingerprint region (between 1800 $cm^{-1}$ and 800 $cm^{-1}$), which exhibits many skeletal vibrations that are highly sensitive to molecular structure. The CARS signals at 1655 $cm^{-1}$ and 1440 $cm^{-1}$ were assigned to the superposition of the C=C stretch of lipid chains and the amide I mode of proteins and CH bend mode, respectively. The band at 1602 $cm^{-1}$ originates from mitochondria and its intensity sharply reflects the metabolic activity of mitochondria. The Vibrational spectra structures of a budding yeast cell were successfully observed with multiplex CARS.

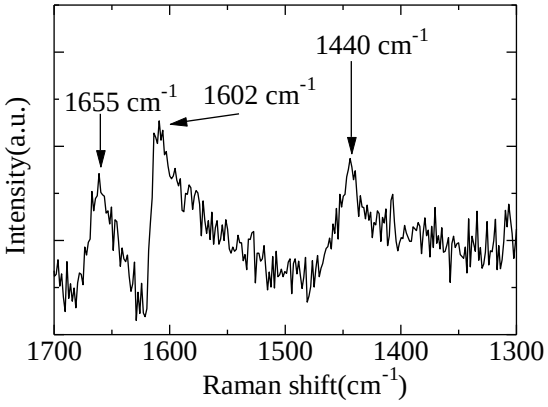


**Figure 1**: *CARS spectra of budding yeast cell in fingerprint region.*

# Numerical simulation of Fenton reactions in water exposed to an atmospheric-pressure plasma

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The numerical simulations of chemical reactions and diffusion of reactive species in water exposed to an atmospheric-pressure plasma (APP) have been performed based on reaction-diffusion equations. When a living tissue is exposed to a low-temperature APP, there is almost always a liquid layer, such as blood, lymph, or other body fluids, that separates the gas phase and the tissue. Therefore chemically reactive species such as OH, NO and $O_3$ generated by the plasma in the gas phase are transported through the liquid before reacting with the tissue surfaces [1]. In this study, we focus on effects of Fenton reactions as a body fluid contains iron ions.

The simulation model used in this study is a one-dimensional system of a gas and a liquid that contact each other through a flat gas-liquid interface. The initial condition of the liquid is assumed to be pure water (pH=7) with dissolved oxygen and nitrogen in equilibrium with air at 1 atm. The gas phase species generated by plasma discharge are assumed to enter water at their thermal velocities. The outward flux of chemical species from water to the gas phase is determined from Henry's law. The gaseous species entering water are assumed to be dissolved into water without any barrier. The model incorporates 37 species and 111 chemical reactions in water at room temperature. The governing equations for chemical reactions and diffusion in the liquid phase are reaction-diffusion equations. If the system consists of $N$ species that are represented by $X_i$ with $i = 1,2,\cdots N$, the equations hold;

$$\frac{\partial[X_i]}{\partial t} = D_i \frac{\partial^2[X_i]}{\partial z^2} + \widetilde{R}_i \qquad (1)$$

where $[X_i]$ represents the concentration of $X_i$ in water, $D_i$ is the diffusion coefficient of $X_i$ in water, and $z$ is the coordinate representing the depth in water. The source $\widetilde{R}_i$ represents the chemical reaction term determined from rate equations.

In the presence of $Fe^{2+}$ ions in liquid, OH radicals may be generated from Fenton and Fenton-like reactions;

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + \cdot HO_2 + H^+$$

OH radicals generated by these reactions are considered to play an important role in biological systems. In our simulation we have evaluated, based on known rate constants, how the distributions of reactive oxygen species (ROS) and reactive nitrogen species (RNS) would change in the presence of uniformly distributed $Fe^{2+}$ ions in pure water.

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# Modeling of the behavior of reactive oxygen species in a liquid water layer of interest for plasma medicine

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In recent years, there is a growing interest in the use of low-temperature atmospheric pressure plasmas for biomedical applications. Until now, however, very little is known about the plasma-induced processes occurring on the surface of living organisms, although some fundamental work has already been carried out [1, 2]. In this respect, computer simulations can provide insight, which is difficult to obtain experimentally. At present, very few modeling investigations have been performed to study the interaction processes of the reactive plasma species with biomolecules at the atomic level [3-5].

It is known that most bio-organisms, including bacteria, are coated by a liquid film surrounding them, and there might be many interactions between plasma species and the liquid layer before these species reach the surface of the bio-organisms. Therefore, it is essential to study the behavior of the reactive species in a liquid film, in order to determine whether these species can travel through this layer and reach the biomolecules, or whether new species are formed along the way. In this work, we investigate the interaction of reactive oxygen species (i.e. O, OH, $HO_2$ and $H_2O_2$) with water, which is assumed as a simple model system for the liquid layer surrounding biomolecules [6]. Our computational investigations show that OH, $HO_2$ and $H_2O_2$ can travel deep in the liquid layer and are in principle able to reach the bio-organism, whereas the O atoms are rapidly transformed into OH radicals. Moreover, O, OH and $HO_2$ radicals react with water molecules through hydrogen-abstraction reactions, whereas no H-abstraction takes place in the case of $H_2O_2$.

This study is important to gain insight in the fundamental operating mechanisms in plasma medicine in general, and the interaction mechanisms of plasma species with a liquid film in particular.

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# Tissue Distribution of Indium After Repeated Intratracheal Instillations of Indium-Tin Oxide in Hamsters

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**Objectives:** From several case reports and epidemiological studies concerning indium-tin oxide (ITO) exposed-workers, the potential occupational exposure to ITO has attracted much attention [1] [2]. Although findings of lung lesions were already reported in animals and humans, it is not clear that progress of indium distribution in the body after exposure of ITO particles via respiratory tracts. The aim of this study was to clarify the tissue distribution of indium after instilled ITO in the lung of hamsters intratracheally.

**Methods:** Male Syrian golden hamsters were intratracheally given 3 mg/kg or 6 mg/kg of ITO particles, containing 2.2 mg/kg or 4.5 mg/kg of indium, twice a week, for 8 weeks. Control hamsters were given vehicle of distilled water only. The hamsters were euthanized serially from 8 weeks up to 78 weeks after the final instillation. The distribution of indium in the lung, liver, kidney spleen and serum were determined.

**Results:** The lung indium contents in the 2 ITO groups gradually decreased up to 78 weeks. Biological half-time of indium in the lung was almost the same; 142 weeks in the ITO 3 mg group and 124 weeks in the ITO 6 mg group. However, indium concentration in the lung, liver, kidney spleen and serum among the 2 ITO groups gradually increased up to the end of the observation period.

**Conclusions:** The present results clearly demonstrate that body burden clearance of indium is extremely slow and when repeated intratracheal instillations were given to hamsters.

**Acknowledgements**: This study was funded in part by the Grant-in-Aid for Scientific Research on Innovative Areas (24108009) from MEXT.

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# Atmospheric Chemical Reaction by Air Activation Apparatus Using Corona Discharge and UV Lamp

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Air activation technique was developed using corona discharge and a UV lanp [1]. In this study, we report the chemical reation in air with this apparatus.

The developed apparatus consist of two pairs of corona electrodes and a UV lamp. The first corona electrodes produces negative ions. The negative electrode is situated separately from the positive electrode, and the produced negative ions are accelerated towards the positive electrode. Thus the ionic wind is generated. The ionic wind flows to the UV lamp, generating ozone. It is introdueced to the second pair of corona discharge electrodes. The electronic shower is irradiated to the ozone which has a long lifetime, resulting production of excited molecules with a short lifetime. The excited molecules produces a wide variety of atmospheric chemical reaction.

The apparatus was set in a 48L transparent container with PMMA panels. Products sre examined by gas detector tubes or sensors. Reaction gases were introduced to monitor the reaction.

As a result, the apparatus produces less amount of ozone in comparison with other types of air activation technique. The corona discharge by the second pairs of electrodes destroyed the ozone and produces reactive oxidant were produced. $N_2O$ production was also observed. After the introduction of reaction gases, dissociations of methylmercaptan and ethylene were observed. It was revealed that the reactive oxidant with a short lifetime produced by this technique is useful for air activation

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# Poster Presentations

## Tuesday, May 20

# Visually non-contact argon plasma jet on microliter water-dropped wound accelerates wound healing

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**Aim:**It was reported that visually non-contact argon plasma jet on 2-mm acute full-thickness wound accelerated healing through promoting the late stage of inflammation, reepithelialization and wound contraction [1]. In this research, we investigated the effect of the same type of plasma source on water-dropped 4-mm wound of mice. **Methods:**The mice were classified into three groups: plasma wound group (PW), plasma - water-dropped wound group (PWW) and control (C). In PW, wounds were treated using cold plasma once daily for 1 minute, and then covered with hydrocolloid dressing; wounds in PWW had 20-40 microliters of distilled water dropped on them,were treated using plasma once daily for 1 minute and then covered with hydrocolloid dressing; wounds in the control group were left to heal under hydrocolloid dressing. Daily macroscopic evaluation was conducted for 14 days. Thermal properties of treated groups (PW and PWW) were recorded during their plasma treatment using an infrared thermal camera. On days 3, 7, 11 and 14, wound tissue was harvested for histological evaluation. General staining was applied. **Results and Conclusion:**It was found that there was a difference in thermal properties between PW and PWW. During the inflammation stage, day 1 to 5, in the PW,the local temperature of the wound surface under plasma treatment was lower than that of its surroundings, but in the PWW group, the opposite was found. Using macroscopic evaluation, it was revealed that the wound healing in PWW was faster than that in PW and C.From the histological data, it was found that the percentage of nerve-like substance near the wound surface on day 3 in PWW was higher than in PW and C. Dropping water on a wound may modify the mechanism of plasma-wound interaction, by which it may improve the late inflammation phase and the early granulation phase through nerve-like sprouting. Such mechanism may also influence the change of heat transfer pattern on the wound surface during plasma treatment.

**Acknowledgments**
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Reference:
[1] Nasruddin et al. 2013. Cold plasma on full-thickness cutaneous wound accelerates healing through promoting inflammation, re-epithelialization and wound contraction. Clinical Plasma Medicine. ***Accepted***

# Atmospheric Pressure Plasma for Nail Fungus Treatment

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Onychomycosis is a common fungal infection of the nail with significant barriers to successful treatment [1]. The prevalence of onychomycosis is estimated to affect 10% of the world's adult population [2], particularly the elderly and patients with immunodeficiency diseases such as HIV, diabetes and circulatory disorders. Contributing to treatment difficulty is the challenge of getting topical antifungal agents through the nail. Systemic oral medications have a higher cure rate, but cost and toxic side effects (e.g. liver problems) are both serious concerns.

Nonthermal plasma in air generates antimicrobial reactive oxygen and nitrogen species at near-room temperature. Reactive plasma species, at the moderate concentrations and doses found in nonthermal plasma streams, appear to cause little to no permanent damage to living tissue, while being capable of efficiently destroying microbial cells. Our study focuses on using atmospheric pressure plasmas to treat onychomycosis. Initial tests of 20 minute exposures using plasma jets ($He/O_2$ : 0.995/0.005) show ~2 log reduction of *E. coli* colonies cultured underneath a bovine hoof disk (as a model nail) by treating the upper side of the disk. Nitrites, $H_2O_2$ and small amounts of $O_3$ were detected in adjacent liquid following plasma exposure. Acidified nitrite has been reported to render nail antifungal by forming S-nitrosothiols in the nail [3], suggesting air plasma may be a promising way to create and deliver therapeutic antifungal compounds to infected nails. We report results correlating $He/O_2$ plasma jet and air surface microdischarge (SMD) plasma-generated species with model bovine nails coated with a model fungus. In particular, we report the relationship between the nail thickness and treatment protocol with the diffusion of reactive species into and through the model nail.

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# Treatment of Cardiac Disease by of Atmospheric Pressure Plasma Inhalation

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In our previous research, we performed experiments involving direct irradiation of tissues and cells using an atmospheric-pressure plasma source [1]. Especially, we examined saturation pulse oxygen ($SpO_2$) in the blood under plasma inhalation at atmospheric pressure using a rat heart failure model of myocardial infarction (MI).

A schematic diagram of the experimental setup for the plasma inhalation [2] is shown in Figure 1. The coaxial plasma source that connected silicon tube has a 1-mm-diameter tungsten wire inside a glass capillary that is surrounded by a grounded tubular electrode. The AC/DC amplifier provides a high voltage for helium (He) plasma generation. The rat myocardial infarction (MI) model used here involved ligating the left coronary artery to induce ischemia in the coronary artery of left ventricle of the heart, the performance of which was requested to a private sector research institute, the Institute for Animal Reproduction (Ibaraki, Japan). Rats used in the MI model (n = 6; initial body weight range = 202.4-224.1 g) were anesthetized with 5% sevoflurane, nitrous oxide (4 L/min) and oxygen (3 L/min) using an anesthesia device with a mechanical respirator.

$SpO_2$ as a function of the duration of plasma inhalation is shown in Figure 2. When He gas was inhaled, no changes in $SpO_2$ were observed (86-88 %). Conversely, $SpO_2$ was observed to increase (from 83 %→97 %) during the plasma inhalation period, which suggests that this method can be beneficial in restoring heart function following cardiac ischemia. Additionally, *in vivo* blood pressure decreased from 89/81 to 73/60 mmHg in the abdominal aorta during plasma inhalation. The inhalation of plasma flow including ion and radical was performed immediately preceding endocardial ischemia, and could potentially ameliorate the observed decrease in the volume of blood flow due to the onset of decreased cardiac function.

This study was supported by a Grant-in-Aid for Scientific Research on Innovative Areas (No. 24108010) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

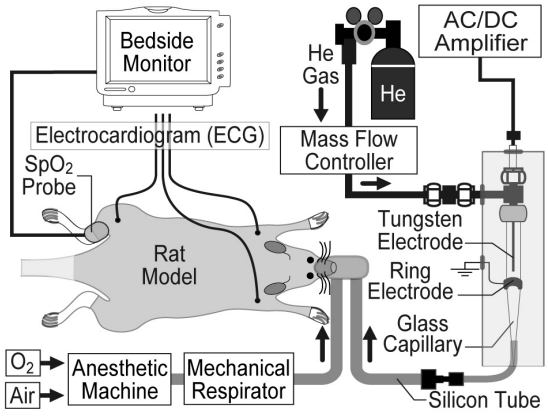


**Figure 1**: *Schema of experimental setup.*

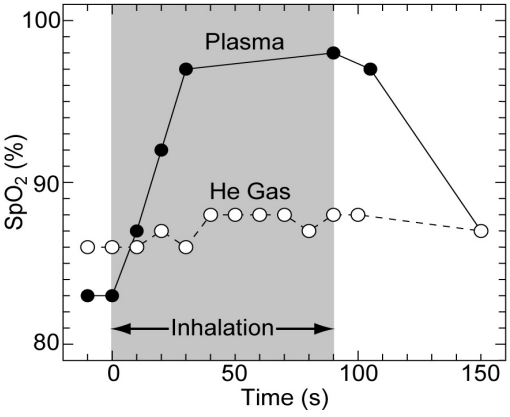


**Figure2**: *$SpO_2$ as a function of the duration of plasma inhalation*

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# Plasma inactivation of biofilms formed ex vivo within a root canal by the causative agent of pulpitis

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The aim of the work was to evaluate efficiency of the non-thermal plasma as bactericidal agent affecting biofilms formed in vitro and on walls of the root canal.

The multiple antibiotic resistant strain *Staphylococcus epidermidis* isolated from the case of pulpitis was used. Biofilms formed in vitro on the plastic surface and ex vivo at walls of the root canal were treated with plasma torch with the diameter of about1-2 mm formed by argon:air (9:1) mixture eradiated with 100 kHz electromagnetic field. Bacterial viability was determined by plating and by differential Live/Dead labeling.

The dose-dependent decrease in counting of living bacteria in biofilms was demonstrated for both plastic and root wall grown bacteria. The three-step kinetics of bacterial killing was observed that suggested differential rates of destruction for bacterial subpopulations characterized by different physiological statuses and/or different positions within the biofilm. Total elimination of up $10^9$ CFU/sample was reached at exposition of 240 s.

Obtained results proved a potency of the non-thermal plasma effectively destroyed bacterial biofilms within root canals.

# Research for Regenerative Medicine Using Micro-spot Atmospheric Pressure Plasma Source

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Atmospheric pressure plasma is applied in the medical field for coagulation, sterilization, and treatment for diabetic gangrene. Direct plasma irradiation has recently been reported to promote wound healing [1]. The aim of the present study was to clarify the mechanism by which wound healing is promoted by plasma irradiation. In our previous study, we reported that plasma treatment could promote angiogenesis [2]. In this study, we measured nitric oxide (NO), as this is known to stimulate macrophage activation, angiogenesis and regeneration of epithelium, in order to investigate whether NO is generated by plasma treatment and plays a causal role in plasma medicine.

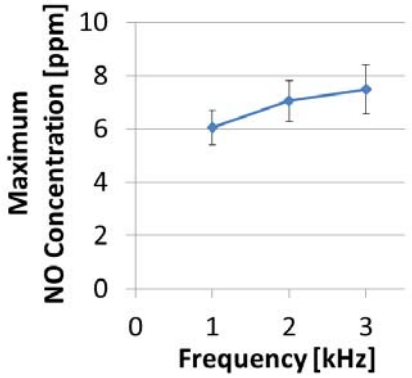


**Figure 1:** *NO generation by plasma (X +/- SD of 5 measurements)*

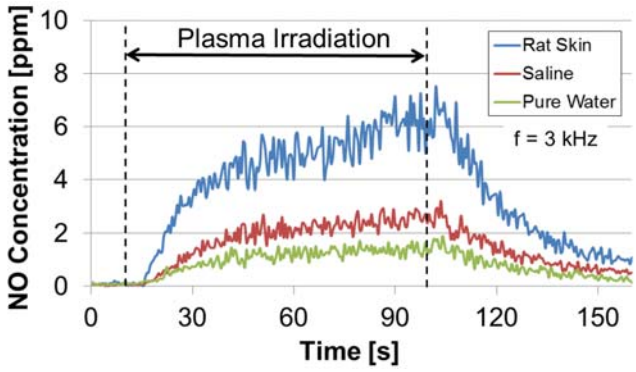


**Figure 2:** *Irradiation to differential targets*

The maximum NO concentrations (plasma irradiation time: 90 s) at each frequency are shown in Figure.1. We showed that plasma could generate NO, but no substantial change was seen in the maximum NO concentration. Previous reports indicated that a NO concentration more than 500 ppm for was needed for “NO-therapy,” we found only a very low concentration NO (less than 10 ppm) was generated by our plasma treatment [1]. However, by changing the plasma irradiation target, the NO concentration on rat skin was much better than that produced by pulp immersed in saline and pure water (Figure.2). Thus, the difference in NO concentration may have a huge effect on the targets.

These experiments were conducted to verify whether the promotion of wound healing by plasma irradiation is due to NO generated by plasma. The concentration of NO generated by our plasma was low, but plasma has the ability to improve the transfer of materials into cells. Thus, we need to investigate the intravital NO concentration and the transfer efficiency of the active species generated by plasma into cells.

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# Cell death and cytokine release induced by surface plasma in immortalized human keratinocytes (HaCaT)

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Background: The use of non-thermal atmospheric-pressure plasma in dermatology, especially the effectiveness of wound healing, has shown a great promise as viable clinical application, even though its responsible component and mechanism is not yet very clear. The immortalized human keratinocytes (HaCaT) is a most commonly used cell model in the study of interaction between material and skin. It has been shown that the cell membrane with its embedded proteins may be the first target to plasma treatment in a series studies of a DBD plasma source treated HaCaT. And it also influences the expression of surface molecules that play an important role in promoting cell-cell or cell-matrix adhesion[1] [2].
Objective: To ensure a safe application, it is essential to understand basic interactions between plasma and human skin cell and to observe the series changes in cell structure and function. This study focused on measurement of cell viability, apoptosis, cell cycle and cytokines released by plasma treated HaCaT.
Method: In this study, HaCaT cells with PBS in a certain volume were directly treated by a surface DBD plasma generator in air generated with a peak voltage of 10 kV at 10 kHz. The treatment time was 0 - 5min. After the treatment, cells with fresh medium were cultured for a certain time, and then using the method of CCK - 8 to measure cell viability, Annexin V FITC/PI staining to determine cell apoptosis with a flow cytometry, the quantitative sandwich enzyme immunoassay technique to assay the secretion level of IL-1, 6, 8 and VEGF in culture supernatants released by HaCaT cells.
Results: Plasma used in this study was observed at 4h and 24h after the treatment to have inhibitory effect on cell viability as treatment time extended. At the same time, apotosis cells increased significantly with a treatment time of more than 3min whereas the increase within 2min was modest. We evaluated 4 cytokines in cell culture supernatants from HaCaT cells exposed to 0-5min plasma irradiation. Plasma treatment time longer than 2min induced the release of all the four cytokines, IL-1, 6, 8 and VEGF, but when the treatment time is more than 3min, levels of cytokines decreased as the number of living cells decreased.
Conclusion: The results suggested that surface DBD plasma in air led to cell death and induced the release of several cytokines in human keratinocytes. Plasma irradiation may be associated with multiple physiological events in human skin. Much more work should be done in the future on the effect of cell function in plasma treated HaCaT cells.

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# Effect of non-equilibrium atmospheric pressure plasma in cancer initiating cells

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Recently, medical applications of non-equilibrium atmospheric pressure plasma (NEAPP) have been reported for its effect on cancer therapy [1] [2]. Tumor cells with tumorigenic potential are limited to a small population, called cancer-initiating cells (CICs), in several tumors, such as leukemia, breast, brain, and colon cancers. CICs efficiently efflux anti-tumor chemicals, resist radiotherapy, degradate reactive oxygen species (ROS) and have high aldehyde dehydrogenase (ALDH) activity [3]. CICs are believed to be cause of cancer recurrence or metastasis. A small population of cancer cells had high ALDH activity. Clinical cases with many ALDH high-expressing cells showed a poor prognosis. ALDH-positive cells were supposed to have the character as CICs in uterine endometrioid adenocarcinoma [4]. Therefore, using ALDH as the target of CICs, we examined the effect of NEAPP on human uterine endometrioid adenocarcinoma cells (Hec-1) and poorly differentiated human gastric carcinoma cells (GCIY). By NEAPP, Hec-1 and GCIY cells fell into apoptosis and the tendency of high ALDH-expressing cells decreases was observed. Further, NEAPP might have anti-tumor effect in both ALDH-high and -low cells. These results suggested that NEAPP might control the CICs.

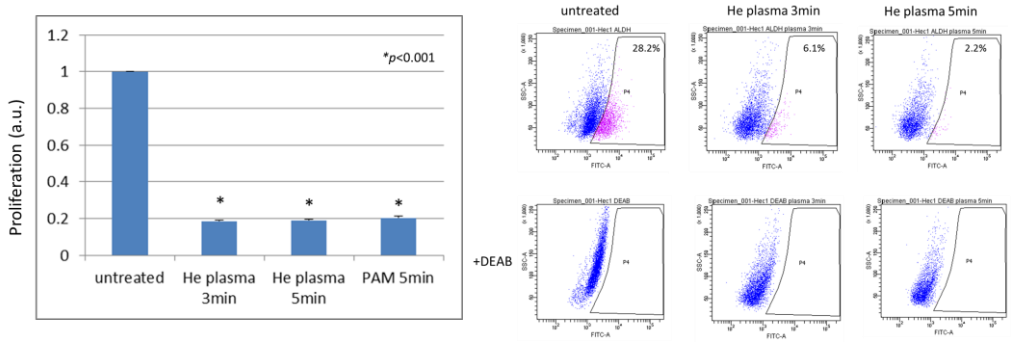


**Figure 1:** *Anti-tumor effect and ALDH activity in Hec-1 cells by NEAPP*

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# The Effect of Cold Plasma Treatment on Cancer Stem Cells

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Intratumoral heterogeneity challenges existing paradigms for anti-cancer therapy. We have previously demonstrated that the human embryonic stem cells (hESC)-derived cellular microenvironment in immunocompromised mice, enables functional distinction of heterogeneous tumor cells, including cells which do not grow into a tumor in conventional direct tumor xenograft platform. We have identified and characterized six cancer cell subpopulations each clonally expanded from a single cell, derived from human ovarian clear cell carcinoma of a single tumor, to demonstrate striking intratumoral phenotypic heterogeneity that is dynamically dependent on the tumor growth microenvironment. These cancer cell subpopulations, characterized as cancer stem cell subpopulations, faithfully recapitulate the full spectrum of histological phenotypic heterogeneity known for human ovarian clear cell carcinoma. Each of the six subpopulations displays a different level of morphologic and tumorigenic differentiation wherein growth in the hESC-derived microenvironment favors growth of CD44+/aldehyde dehydrogenase positive pockets of self-renewing cells that sustain tumor growth through a process of tumorigenic differentiation into CD44-/aldehyde dehydrogenase negative derivatives. Strikingly, these derivative cells display microenvironment-dependent plasticity with the capacity to restore self-renewal markers and CD44 expression.

We have shown previously the effect of cold atmospheric plasma as a potential novel therapy for the treatment of cancer. In addition it was demonstrated that cancer cells are more sensitive to the effects of plasma than normal cells. This offers a promising alternative to conventional therapy with their harmful side effects. Intratumor heterogeneity is evident already at the level of CSCs so multimodal anti-cancer therapeutic strategies are needed to eradicate the tumor. CSCs are more resistant to conventional therapies than nonCSC populations. CSCs are responsible for tumor recurrence and metastasis so it is important to find ways to eradicate them. Therefore any method capable of destroying the CSCs will be of great benefit in treating cancer. We therefore decided to test the effect of cold plasma on 3 different ovarian cancer stem cells first in vitro and then in vivo.

Our invitro results showed all three cells were sensitive to plasma to varying degrees. The C12 cell line was very sensitive to the plasma treatment whereas the other two ( C13 and C13diff) to a lesser degree. We are now testing the effect of plasma treatment of these cells in vivo.

# Pediatric skin inflammatory reactions (urticaria) increases the risk of developing new-onset depression - a database study

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Urticaria caused acute or chronic inflammatory reactions. Inflammatory reactions in peripheral plasma have been demonstrated to increase stress.[1-3] The stress might also be increased by the symptoms of urticaria; however, this association was not clear. We aimed to firstly analyze the risk of depression after urticaria using a nationwide population-based study. We used the Taiwan Longitudinal Health Insurance Database. Patients who had histories of any urticaria or depression before the study period were not included. A total of 6,742 adolescents (aged 13 to 18 years) hospitalized for a first-attack urticaria episode from 2006 to 2009 were recruited as a study group, together with 20,226 matched non-urticaria enrollees as a control group. Each patient was prospectively traced for one year to identify the occurrence of depression. Cox proportional hazards models were used to compute the risk of depression between the study and control groups, making adjustments for the subjects' place of residence and sociodemographic characteristics. Depression-free survival curves were also analyzed. Finally, the risks of depression were analyzed between different age groups. We found that sixty-three (0.9%) adolescents with urticaria and 61 (0.3%) non-urticarial control subjects suffered a new-onset episode of depression during this period. The stratified Cox proportional analysis showed that the crude hazard ratio of depression among adolescents with urticaria was 1.86 times (95% CI, 1.25-2.98) that of the control subjects without urticaria. Patients who aged 16 to 18 years, with history of asthma were more likely to suffer from depression (both $p<0.05$). Finally, urticaria was determined to be a risk factor for depression only in adolescence and not in patients aged <13 years (n=6,745) or those aged between 19 and 24 years (n=7,185).In conclusion, individuals who have a first-attack of urticaria during adolescence are at high risk of developing depression.

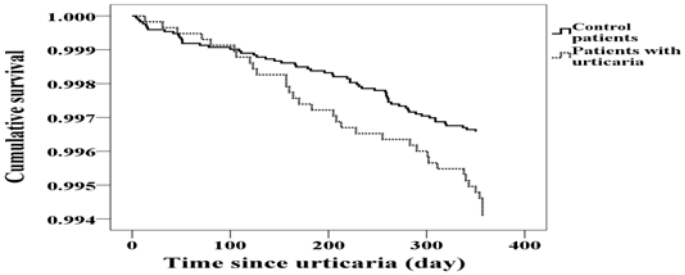


Figure 1 Depression-free survival curves

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# NTP Antitumor Soft Treatment: Evidence of a Triggering Effect?

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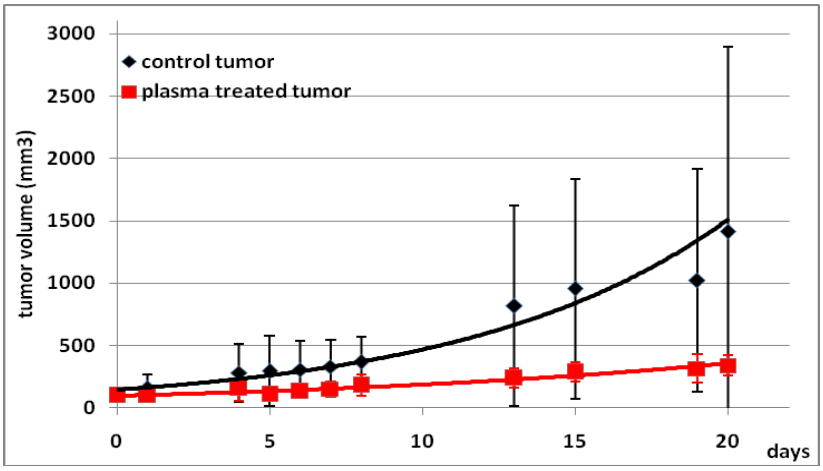
Intensive ongoing research in the field of anticancer treatment points to the atmospheric non-thermal plasma (NTP) as a new potential mean to attack tumors from a distinct innovative approach. The antitumor effect of NTP has been clearly shown *in vivo* on murine models with various cancer types (glioblastoma, colon, pancreas). Although the mechanism is far from being fully understood, the therapeutic effect is admitted. To date, the literature reports mainly induction of apoptosis, effect which is not restricted to cancer cells and probably masks other aspects of the NTP for cancer therapy.

Our work focuses on "**soft**" application allowing highlighting interesting effects as recently shown [1] on blood vessel parameters: blood flow and tissue oxygenation. With this aim to avoid the deleterious impact of plasma on cells and tissues, various plasma conditions were established to allow a daily treatment without skin burn or damage. Plasma Gun helium NTP used in this work not only leads to the production of reactive species, but also generates transient electric fields. Immuno-competent Balb/c mice were used to study the proliferation of orthotopically implanted 4T1 breast carcinoma. Mice were double grafted. One tumor was used to apply a specific treatment condition whereas the second tumor of the same mouse was used as control tumor (untreated). Preliminary data show a regulation of tumor proliferation regardless the variations among the treatment conditions applied (connected or not to the ground, treated through H2O impregnated compress or directly, 2 kHz *vs* 200 Hz). This observation is in favor of a **triggering effect**, evidenced for the first time, of the plasma fraction which similarly affects the tumor proliferation (treated group figure A) as compared to untreated tumor (contra-laterally grafted on each mouse). This comparable reduction of tumor proliferation obtained whatever the plasma soft treatment conditions, with a significant reduction of standard deviations in all cases, **clearly indicates a tumor growth regulation**. Taking into consideration the recent vessel normalization based-cancer treatment, the NTP effect should be further investigated in view of blood vessels structure and function (blood flow) as well as tumor hypoxia compensation to confirm a possible NTP-based adjuvant approach for cancer treatments. These results suggest a new way to consider the plasma and its therapeutic delivery in NTP-based tumor therapy.




***In vivo* study of 4T1 breast carcinoma proliferation with distinct treatment conditions (see text)**

[1] G. Collet, E. Robert *et al*, PSST **23** (2014) 012005

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# Role of Radicals on Cell Viability

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Reactive oxidation species (ROS) produced by atmospheric pressure plasma are widely-accepted to play a key role for promoting biochemical reactions on plasma medical applications: disinfection, wound healing, dental caries and cancer tissue treatment [1]. In this work, low-temperature plasma irradiates cellular medium with perpendicular dry-gas flow from glass tube. The humidity distribution as various radical sources is measured using laser-induced fluorescence (LIF). The humidity in the vicinity of the medium surface widely varies with the gas flow rates. In addition, Radical doses of O, NO, and OH in beam height of approximately 100 μm are measured by LIF. These doses are controlled by $O_2/N_2$ ratio and the dry-gas flow rate (i.e. humidity on the medium surface). Finally, viability of B16F10 cells is determined by MTT assay with varying the proportion of these radicals.

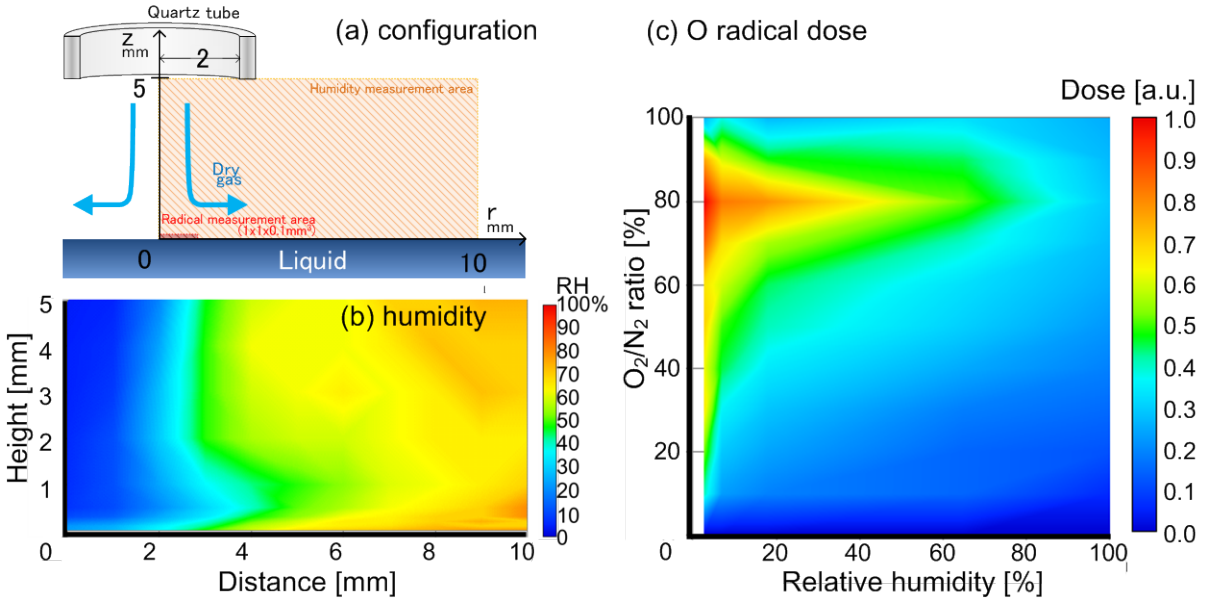


**Figure 1:** *(a)configuration and (b)humidity distribution, and (c)O radical dose on the surface.*

Acknowledgments: I would like to express my gratitude to Prof. Kazunori Kataoka and Assistant Prof. Yutaka Miura. This work was supported by Grant-in-Aid for Scientific Research on Innovative Areas and JSPS Research Fellow.

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# Development of method for analyzing eukaryotic cellular responses to atmospheric pressure non-thermal plasma using yeast knockdown collection

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Recently, sterilization and medical application using atmospheric pressure non-thermal plasma (APNTP) have been developed. An atmospheric pressure plasma jet (APPJ) is one of the APNTP. It generates several chemically reactive species such as charged particles, excited species and radicals. Especially APPJ can treat subjects without thermal loading, so it is expected that the APNTTP can be applied in the fields of medicine and biology. It is generally considered that hydroxyl (OH) radical and other long lifetime factor(s) injected in aqueous media play an important role in plasma medicine. For example, it was reported that the plasma-exposed medium killed not astrocyte normal human brain cells, but glioblastoma human brain tumor cells through induction of apoptosis [1].

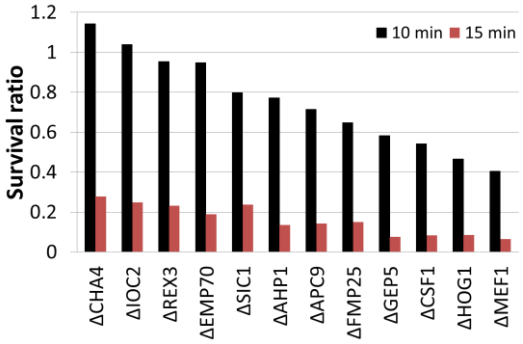


**Figure 1:** *The typical results in this study*

However, cellular responses induced by exposure to APNTP and plasma-treated water are still unclear. To elucidate the cellular responses induced by exposure to APNTP, we are trying to use the yeast knockout collection (YKO) which contains over 6,000 gene-disruption mutants as a unique tool for the functional analysis of the yeast genome. This enables identification of essential genes for survival after the plasma exposure. However, it is difficult to expose the APPJ to each mutant and to apply a standard agar plate culture method for the calculation of the survival ratio. Here, plasma-treated water was used to enable high throughput analysis, and a colorimetric method was used to obtain survival ratio in a short time. Here, 35 mutants were tested and we found apparent decrease in survival ratio for several mutants.

This work was partly supported by Grant-in-Aid for Scientific Research on Innovative Areas "Plasma Medical Innovation" (24108005) from MEXT, Japan.

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# Antimicrobial activity of a low power inductively coupled plasma source at safe levels for eukaryotic cells

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The widespread use of antibiotics has led to emergence of multi-drug resistant organisms especially in healthcare settings. While bacterial contamination is most common in trauma and burn wound care, patients with extensive burns are particularly susceptible to opportunistic pathogens; the bacterial species most frequently isolated from wounds are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Escherichia coli*, *Acinetobacter baumanii* and *Klebsiella pneumoniae*.

An effective patient treatment by antibiotic use is not always achieved and this fact prompts to look for new treatment alternatives. Plasmas as a source of reactive species, radicals, UV radiation, heat and charged particles are a promising technology to reduce bacterial load in chronic wounds, as demonstrated by *in vitro* tests [1] and also clinical trials [2].

Low power inductively coupled plasma sources integrated with a quenching device (cold ICP) for the efficient production of reactive species at atmospheric pressure have been recently developed for potential biomedical applications [3-4].

The aim of this work is to evaluate the antibacterial activity and possible associated cytotoxicity of cold ICP treatment on both prokaryotic and eukaryotic cells in a realistic *in vitro* model able to represent a wound environment. An ICP torch, supplied by a 1 kW-13.56 MHz power generator and operated with argon/air mixtures, was used and coupled with a quenching device with suitably designed air injection ports and orifice for the gaseous effluent exiting the source at a biocompatible temperature.

In a first step, plasma operating conditions (power, gas type and flow rates, sample distance, etc.) for efficient load reduction in case of Gram-positive and a Gram-negative bacteria have been defined, testing different concentration of *S. aureus* and *E. coli* in suspension; in a second step, the level of cytotoxicity to eukaryotic cells under the same plasma operating conditions has been analyzed by the alamarBlue® assay (Invitrogen), on kidney epithelial cells (Vero) and lung fibroblasts (HEL 299). Both bacterial and eukaryotic cells were treated in identical small volumes (50 µl) of physiological solution (NaCl 0.9%): bacteria were suspended in the liquid, while adherent eukaryotic cells were just covered by the liquid, in order to reproduce, in a two-step processing procedure, a realistic wound environment; in fact, the wound surface is not completely dry and plasma inactivation of bacteria can strongly depend on the solution in which bacteria are treated. Moreover, eukaryotic cell viability has been evaluated 4 h and 24 h after plasma exposure, to better assess the cytotoxic effect in both the short and long term.

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# Treatment with low temperature atmospheric pressure plasma enhances cutaneous delivery of epidermal growth factor by regulating E-cadherin-mediated cell junctions

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The skin's barrier system not only defends against antigens and harmful substances, but also hinders the permeation of medicines and cosmetics into the dermis. Many strategies have been developed to enhance the absorption ability of skin, including the use of chemicals and skin ablation devices. However, their cost and inconvenience highlights the need for a novel, safe method for increasing skin penetration. In this study, we examined the effect of low temperature atmospheric pressure plasma (LTAPP) on the efficiency of drug penetration through the skin, as well as its mechanism of action. HaCaT human keratinocytes and the dorsal skin of hairless mice were subjected to LTAPP treatment, and the cellular and tissue gene expression, morphological changes were monitored. The skin barrier function after LTAPP treatment was tested using an eosin or human epidermal growth factor (EGF) patch on the skin of an anesthetized mouse. We found that the LTAPP treatment immediately modulates the expression of E-cadherin in skin cells and led to the loss of cell-cell contacts. The LTAPP treatment of mouse skin also reduced the expression of E-cadherin and prevented junction formation in the skin cells within the tissue, leading to enhanced absorption of the hydrophilic agents, eosin and EGF, through the mouse skin. The reduction in E-cadherin expression and reduced skin barrier function were completely recovered within 3 hours after the LTAPP treatment. Taken together, this study shows that LTAPP can induce a temporal decrease in the skin barrier function by regulating E-cadherin-mediated cell-cell interactions, leading to the enhanced transdermal delivery of drugs and cosmetics.

**Acknowledgement**
This study was supported by the Basic Science Research Programs through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (20110013205, NRF-2013R1A1A2064760) and a grant of the Traditional Korean Medicine R&D Project, Ministry for Health & Welfare, Republic of Korea (B11003911110000200).

# Non-thermal atmospheric pressure plasma inhibits invasion of thyroid cancer cells : Involvement of cytoskeletal modulation and MMP change

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**Background and Objectives:**Plasma is known as the fourth state of matter,which is partially or completely ionized gas including mixture of electrons and ions. Advances in plasma physics make possible to use non-thermal atmospheric pressure plasma (NTAP) in cancer research. However, previous studies including author's focused on mainly apoptotic cancer cell death by NTAP as a mechanism of potential cancer therapy.In this study, we investigated the effect of NTAP for inhibiting of invasion or metastasis and by which mechanism plasma induced anti-migration and anti-invasion properties in human thyroid papillary cancer cell lines (BHP10-3 and TPC1), for the first time.**Materials and Methods:** Wound healing, pull-down and trans-well assays demonstrated that NTAP reduced cell migration and invasion activities. In addition, NTAP induced cell morphologic change with cytoskeletal rearrangement, which is detected by scanning electron microscopy and immunocytochemistry. Then, The activity of matrix metalloproteinase (MMP)-2,-9 and uPA was examined by gelatin zymography and RT-PCR.The levels of FAK, E-cadherin were detected by western blot analysis and TUNEL assay. **Results:**NTAP inhibit the invasion and metastasis of BHP10-3 and TPC1 cell lines by decreasing MMP-2, -9 and uPA activities and led to the cytoskeletal rearrangement which are regulated by FAK.NTAP decreased FAK, E-cadherin and the activity of MMPs/uPA system. **Conclusion:** These findings suggest novel mechanism of the anti-cancer effect of NTAP and aid in the developmentof new therapeutic strategies for local invasive and metastatic cancer.

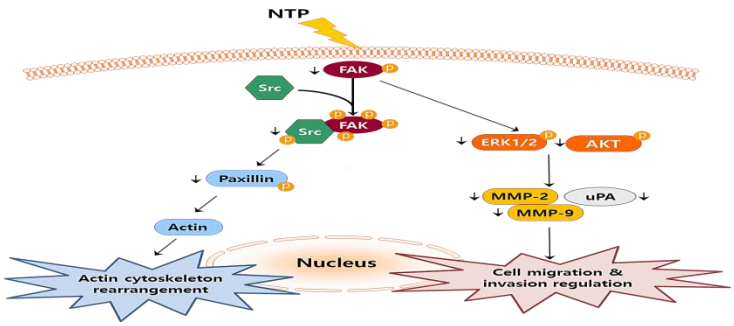


**Figure 1:** *NTP inhibited cell migration and invasion in BHP10-3 and TPC1 cells*

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# Effects of Plasma on Lens Epithelial Cells

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A secondary loss of acuity and a major cause of post-operative visual loss - posterior capsular opacification (PCO) can develop after cataract surgery in approximately 20% of eyes up to 5 years [1]. Posterior capsular opacification is caused by the remaining lens epithelial cells in the capsular bag after the cataract surgery. The remaining cells can re-colonize posterior lens capsule which was previously cell-free and therefore obstruct the visual axis contributing to the light scattering. The key factors responsible for the formation of PCO are the proliferation and the migration of lens epithelial cells.

We investigate the effects of the plasma generated by the plasma needle on proliferation and migration of lens epithelial cells. Lens epithelial cells have already been investigated for the mechanical stress when cell contractions are observed [2,3]. The similar experimental setup was used for the plasma studies. Plasma needle is known to abundantly generate radicals [4] and the effects on proliferation and migration of human periodontal ligament mesenchymal stem cells is described elsewhere [5]. Preliminary results show that in the case of lens epithelial cells plasma affects both proliferation and migration for the given range of parameters and experimental conditions. Further optimization is needed to modulate and control the observed effects as a logical step towards the assessments of more practical applications.

This work has been supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, project numbers III41011 and ON 171037 as well as Slovenian Research Agency (ARRS) and Slovenian Human Resources Development and Scholarship Fund (AdFutura).

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# A New Generation of Biocompatible Pulse-discharged Plasma by Marx Generator and its Application on the Biomolecules

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Characteristics of pulse-discharged plasma in liquid and its biological applications to proteins are investigated by making use of high voltage Marx generator. The Marx generator has been consisted of 5 stages, where each charging capacitor is 0.5 μF to generate a high voltage pulse with rising time of 1 μs. We have applied an input voltage of 6 kV to the each capacitor of 0.5 μF. The high voltage pulsed plasma has been generated inside a polycarbonate tube by a single-shot operation, where the breakdown voltage is measured to be 7 kV, current of 1.2 kA, and pulse width of ~ 1 μs between the two electrodes of anode-cathode made of tungsten pin, which are immersed into the liquids. For the investigation of the influence of pulsed plasma on biological proteins, we have exposed it onto the proteins such as hemoglobin and myoglobin. The structural changes in these proteins and their analysis have also been obtained by circular dichroism (CD) and ultra violet (UV) visible spectroscopy.

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# Effective Region of Atmospheric Pressure Plasma on Transfection

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Recently, gene transfection using atmospheric pressure plasma (APP) has attracted attention because there is a possibility to solve some problems that occur in other transfection methods by this technique [1]. However, the mechanism of the gene transfection using the plasma has not been clarified and the conditions of the plasma irradiation are not improved. Therefore, we try to find the factors of the plasma which enhance the transfer with revealing the optimum condition and the effective region of the plasma on transfection toward developing highly-efficient and minimally-invasive gene transfection method using the controlled APP [2].

Schematic of an experimental setup is shown in Fig. 1(a). We generate atmospheric pressure plasma using low frequency (LF) (frequency: 10 kHz, voltage: $\boldsymbol{V_{p\text{-}p}}$ kV) with He gas flow, which is irradiated to the bound cells covered with genes during the time ($\boldsymbol{t_d}$). In this experiment, preliminarily, we used fluorescent YOYO-1 ($\boldsymbol{c}$ μM) instead of the genes and observed its fluorescence. By the fluorescence image, we distinguished whether the cells were transfected.

Figure 1 shows (b) typical bright image and (c) typical fluorescence images of YOYO-1 with liquid height $\boldsymbol{h}$ as a parameter after direct plasma irradiation for $\boldsymbol{t_d}$ = 1 s, $\boldsymbol{V_{p\text{-}p}}$ = 7.8 kV, $\boldsymbol{c}$ = 5 μM. We define the liquid height $\boldsymbol{h}$ as the distance between the bound cells and liquid surface. When the distance is shorter, the effect of atmospheric pressure plasma on transfection is significant. Especially within the distance of 1.3 mm, most of the cells are transfected as shown in Fig. 1(c). It is clarified that effective region of the plasma is limited to the vicinity of the liquid surface and the elements of the plasma which are critical for transfection are shielded by the liquid. These results indicate the electric field effects of charged particles are the most critical for transfection.

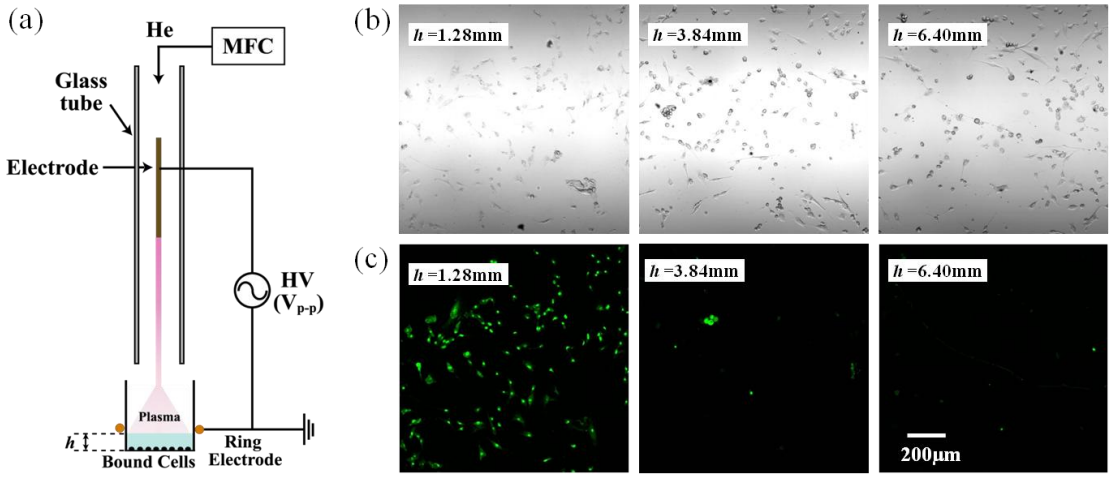


Fig. 1: (a) Schematic of an experimental setup for atmospheric pressure plasma irradiation, (b) typical bright image and (c) typical fluorescence images of YOYO-1 with liquid height $\boldsymbol{h}$ as a parameter after direct plasma irradiation for $\boldsymbol{t_d}$ = 1 s, $\boldsymbol{V_{p\text{-}p}}$ = 7.8 kV, $\boldsymbol{c}$ = 5 μM.

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# The High Significance of Hydrogen Peroxide in Cold Atmospheric Pressure Plasma treated Human Blood Immune Cells

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Plasma medicine gives way to many potential applications in diseased tissues, often related to human skin. For this, investigations have primarily focused on plasma effects of skin cells including, fibroblasts, keratinocytes[1], or endothelial cells. Immune cells are omnipresent in most tissues but have been widely neglected in plasma medicine so far. They not only elicit specific immune responses against pathogens and tumor cells but also regulate inflammation, which is central in healing and regeneration. It is hypothesized that reactive oxygen and nitrogen species (RONS) mainly mediate effects of plasma on cells. High concentrations of RONS induced by plasma lead to apoptosis, also in human blood cells [2].

We investigated human peripheral blood $CD4^+$ T helper cells after cold atmospheric plasma treatment with an atmospheric pressure argon plasma jet. It was operated at a sinusoidal voltage signal of 2–6 kV pp with a frequency of 1 MHz using 3 standard liter per minute of argon gas (purity 99.999 %). Recently, our group found that this jet produces hydrogen peroxide ($H_2O_2$).[3] We therefore investigated its effects on cells with regard to viability and intracellular oxidation. We used catalase and to plasma-treatment concentration-matched controls of $H_2O_2$ to monitor for effects of the peroxide alone. After staining with six redox-sensitive dyes (DCF, *Mitotracker*, *Superoxide sensor*, APF, HPF, $C_{11}$-Bodipy) similar fluorescence increase was observed in plasma- or $H_2O_2$-treated cells. Dye-dependent, addition of catalase also diminished most or all of the probe oxidation. Interestingly, residual membrane and cytosol but not mitochondrial fluorescence was measured in plasma treated cells supplied with catalase. This suggests that plasma-created species may be too short lived to cross the cellular and mitochondrial membrane. Thus, $H_2O_2$ had a dominant but not exclusive role in intracellular oxidation by plasma. Plasma and concentration-matched $H_2O_2$ treatments gave an equivalent reduction in viability and this was completely abrogated if catalase was added prior to plasma exposure. To our knowledge, we show for the first time the dependence of plasma-mediated cytotoxicity to a single molecule in eukaryotes. These results will help clarifying how physical plasma-induced oxidative stress relates to wound relevant cells.

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# Bacteria show increased susceptibility against common available antibiotics and no resistance by repetitive atmospheric pressure plasma application

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**Motivation:** Non-thermal atmospheric pressure plasma is well known for its antimicrobial effects. Bacteria can be inactivated by direct plasma exposure or indirectly by exposure to plasma-treated liquids [1]. However, bacteria are able to develop resistance against antibiotics and toxic environmental conditions [2]. Therefore, during the clinical daily routine different antibiotic agents or methods need to be combined to guarantee an effective therapy.
The goal of our study was to test if bacteria develop resistance against directly or indirectly induced plasma effects. Furthermore, in case of a possible combination therapy of atmospheric pressure plasma together with bactericidal agents, it was also investigated if plasma influences (either in a positive or negative way) the bacterial susceptibility against common antibiotics.

**Methods:** Studies have been conducted with *Escherichia coli* (*E. coli*) K12. With the plate count method inactivation kinetics have been determined for direct plasma exposure and exposure of plasma-treated sodium chloride solutions to bacteria spread on agar plates or suspended in solution, respectively. Both direct and indirect exposures have been used in re-exposure experiments to estimate if bacteria develop resistance against subsequent plasma treatments. An antimicrobial susceptibility test using test leaflets impregnated with different antibiotics was performed to study if plasma treatments increase or reduce the bacterial sensibility against common available antibiotics.

**Results:** Directly applied plasma effectively inactivates *E. coli* but did not induce any bacterial resistance. When the same strain was exposed to plasma-treated sodium chloride solutions the bacterial burden was effectively reduced below the detection limit. Again, no evidence for bacterial resistance was observed. Moreover, direct plasma treatment of an *E. coli* suspension led to higher sensibility against common available antibiotics.

**Conclusion:** Direct and indirect plasma treatments are effective methods of decontamination. The plasma-enhanced sensibility of bacteria against common antibiotics makes plasma to an attractive potentially add-on therapy for critical infected or chronic wounds. However, more detailed studies are still nesseary.

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# Second degree burn wound healing on mice stimulated by $N_2$ /Ar micro-plasma exposure

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Second degree burn may reach the epidermis and partial dermis layers. Several methods and techniques have been applied to manage such burn injuries, such as different kinds of dressings, pharmacotherapies and plasma treatment. The latter has been increasingly studied. In this work, non-thermal $N_2$/Ar micro-plasma was applied to enhance healing on the second degree burn wound mice through the wound area reduction. Six wounds were created in the dorsal of each mouse (6 mice in total) by solid aluminum bar with 5 mm in diameter (46 g) and an average temperature of 70± 2℃. The parameters for micro-plasma exposure for burn wound on mice were chosen: excitation at 13 W (< 40°C) and addition of 0.5% $N_2$ in Ar, corresponding with relatively high NO peak intensity. $N_2$/Ar micro-plasma was utilized to expose upon the burn wound achieved mice in three groups: (1) immediately after the burn achievement, (2) continuous exposure until post-burn day 2, and (3) continuous exposure until post-burn day4. Dressing and gas flow exposure were also conducted as well for the references. The burn wounds were assessed every day to examine the wound size. Until post-burn day 18, the mice were sacrificed for H&E staining. The wound area reduction rate was higher for the cases of $N_2$ /Ar micro-plasma exposed wounds than those of gas flow exposed and dressing ones, while the control group exhibited the lowest wound area reduction rate. From this study, non-thermal $N_2$ /Ar micro-plasma is presumably effective for the stimulation of newly-born cells growth and proliferation [1] and burn wound healing on mice.

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# Energy Source Effects of Non-thermal Plasma Jet on Skin Cancer Cells in Artificial Tissue Scaffold

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This research studied the effects of various pulsed power direct current energy sources in atmospheric pressure nitrogen gas indirect plasma jet on skin cancer cells in an artificial tissue scaffold configuration. Plasma jet and dielectric barrier discharge have been previously shown to induce apoptosis in breast cancer cells and produce preferential killing of lung cancer over healthy cells from plasma treatment [1] [2]. The effectiveness of indirect plasma jet on reducing the skin cancer cell viability was observed. Various plasma energy sources were tested to determine the effect of electronic input parameters on cells. Cells are configured in a scaffold to create an *in vitro* artificial skin tissue and simulate real skin. Plasma density and temperature, energy input, gas output temperature, and reactive species production are measured. The effects to the cell's deoxyribonucleic acid (DNA) is quantified using circular dichroism (CD) and Raman spectroscopy methods [3].

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# Bacterial biofilm response to argon plasma treatment

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Biofilms are the predominant mode of growth for bacteria. They are cell clusters encapsulated by an extracellular matrix attached to a surface. It is now widely recognized that more than 60% of all infections are caused by biofilm-forming bacteria. These biofilm infections can become resistant to treatment with traditional antibiotics and often develop into a chronic state.

A number of studies show very promising results for atmospheric-pressure plasma (APP)-mediated killing and removal of biofilms, including pathogenic bacteria such as *Pseudomonas aeruginosa*. However, the mode of action of APP and resulting bacterial response is not fully understood.

Here we show the successful removal of *P. aeruginosa* PAO1 biofilms using the 'kinpen 09' argon non-thermal plasma jet (1). Biofilms were allowed to form for 24 h on glass coupons in a CDC biofilm reactor (2) before plasma treatment of 0, 1, 3, 5 or 7 min. Bacterial cells were then either removed from the coupon for cell counting (CFU) or stained with LIVE/DEAD® *Bac*Light™ viability kit for visualization using a confocal laser scanning microscope.

A 7 min plasma treatment led to a 3 log reduction in viable cells compared to the untreated control. Interestingly, only a few surviving, attached cells could be observed with confocal microscopy and LIVE/DEAD staining (Fig. 1) for this treatment time. Bacterial survival rates after plasma treatment depended on the age and thickness of the biofilm.

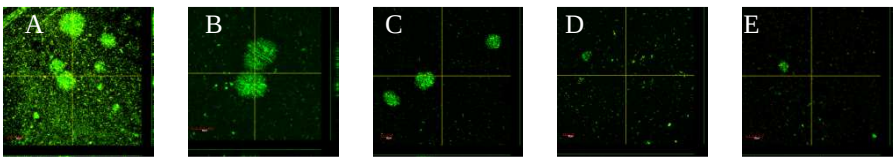


**Figure 1:** *24 h P. aeruginosa biofilms treated with argon plasma for 0 min (A); 1 min (B); 3 min (C); 5 min (D) and 7 min (E)*

To investigate a possible bacterial resistance mechanism to plasma treatment, a lawn of *P. aeruginosa* cells was spread onto agar plates and exposed to argon plasma treatment. Surviving bacterial colonies were selected after incubation. The stability and inheritance of possible resistance traits are currently under investigation using repeated plasma exposure and DNA sequencing methods.

**Acknowledgments:** We would like to thank Debra Birch from the Microscopy Unit, Faculty of Science, Macquarie University (Sydney, Australia) for help with biofilm imaging.

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# Antibacterial performance of magnetron sputtered $TiO_2$ thin films deposited at varying discharge current and deposition time

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This study investigates the antibacterial activity of different $TiO_2$ thin films deposited on glass substrates using a Compact Planar Magnetron (CPM) plasma sputtering device at various discharge currents and deposition times. The deposition was performed using a 99.5% pure titanium target and an argon:oxygen plasma discharge for 2 hours, 3 hours and 4 hours deposition times.Antibacterial activity test was performed using a modified protocol used by Liao,*et al.* [1]. The films were irradiated with UV for 1.5 hours afterwhich a bacterial suspension of *Escherichia coli* was made in contact with the films and incubated for 2 hours at 37°C. The films were then washed and the washed bacterial suspension was plated on Tryptone Soya Agar (TSA). Bacterial colonies were then counted. Fig. 1 shows the bacterial colonies formed on TSA plates. Based on a standard bacteria reduction criterion[2], samples *G*, *E* and *I* have powerful bactericidal effect with % reduction of 71.33%, 86.67% and >99.97%, respectively. Samples *C* and *F* have 60.67% and 30.00% reduction, respectively, which makes sample *C* an expressive bactericide while sample *F* has a low bactericidal effect. The rest of the samples have very low bactericidal effect.An untreated glass surface (sample *J*) was used as control.

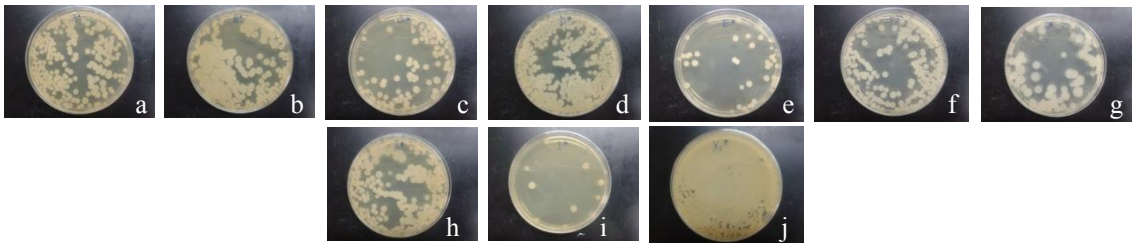


**Fig. 1**: Bacterial colonies formed on TSA plates from washed bacterial solution from the surfaces of $TiO_2$ coated glass deposited using different discharge current and time: (a) 15mA, 4h (b) 12.5mA, 4h (c) 10mA, 4h (d) 15mA, 3h (e) 12.5mA, 3h (f) 10mA, 3h (g) 15mA, 2h (h) 12.5mA, 2h (i) 10mA, 2h and (j) untreated glass.

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# Transcriptional profiling in human keratinocytes in response to non-thermal plasma and identification of transcription factor for regulating differential gene expression

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Non-thermal plasma exhibits many biomedical useful properties such as antimicrobial and stimulating activities. Non-thermal plasma treatment induces changes in redox balance in cells, and causes various cellular responses. To elucidate its effects on human keratinocytes, we investigated the transcriptional profiles of plasma-treated HaCaT cells using cDNA microarray analysis and identified hundreds of transcripts that exhibited significant changes in expression (Fig.1).

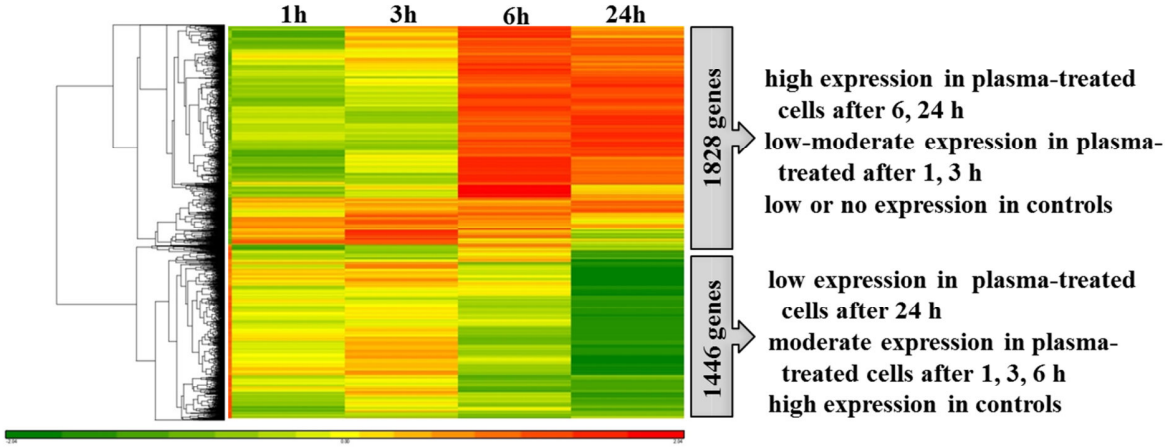


**Fig. 1** Differentially expressed genes in keratinocytes 1, 3, 6, and 24 h after plasma treatment [1].

These transcripts were then classified into several categories according to their functional roles e.g. metabolism, transport, redox homeostasis/antioxidant activities, regulation of transcription, and signal transduction. We identified potential transcription factors for gene regulation in plasma-treated cells. The nuclear erythroid-related factor 2 (Nrf2) plays a key role in regulation of genes, which encode detoxifying enzymes and antioxidant proteins, and functions in cellular defense against imbalances in redox homeostasis. We found Nrf2-dependent signaling in plasma treated HaCaT and primary NHEK cells.
Our microarray analysis has provided useful information for determining the genetic regulatory network affected by non-thermal plasma, and this approach will be useful for elucidating molecular targets for therapies for impaired wound healing in which redox balance was negatively influenced.

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# Dielectric barrier discharge devices tailored to specific skin treatments

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Cold atmospheric pressure plasmas (CAPs) have shown great potential in skin disinfection and wound healing [1]. Now the next step should be taken in developing CAP devices tailored to specific skin treatments. To allow fast and effective treatment of larger skin surfaces, larger plasma sources that can follow the shape of the skin are preferred.

Therefore, two types of dielectric barrier discharge (DBD) plasma devices are being developed. Both are flexible and will be used to decontaminate skin. One is aimed at not harming the intact skin whereas the second DBD should stimulate healing of wounded skin.

These DBDs will be characterized by extensive plasma diagnostics, including mass spectrometry [2] and electrical field measurements and modeling [3]. Together with the biological experiments on bacteria, skin cells and skin this should lead to optimal configurations and settings for the specific application of these plasma devices as well as more insight into the mechanisms of the effects of CAPs on bacteria and skin cells.

Acknowledgment
This work is part of the program Koers Noord, which is funded by the Samenwerkingsverband Noord-Nederland (SNN).

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# The effect of atmospheric pressure plasma to angiogenesis

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In tumor progression, two very important processes occur, namely epithelial-mesenchymal transition (EMT) and angiogenesis [1]. For the efficacy of APP for the cancer treatment, its effects on other cell types must be investigated. In this study, we applied APP on human dermal fibroblasts (HDF) and human aortic endothelial cells (HAECs) in a moderate plasma condition, and observed their responses in the context of their potential usage in anti-cancer treatment. Following the 20 mins of exposure to APP, the treated HAECs were incubated for 24 - 72 hours to allow response time for cellular changes. Dramatic reduction of the cell proliferation along with alterations in cell cycle related gene expressions were observed. In addition to the decrease in the 2D migration speed, tube assay for the APP treated HAECs showed the decrease in the total tube length of the complete loops when compared to that of the untreated cells. With the treated HDFs were incubated for 24 hours, we observed a change in cellular morphology featuring rounded polygonal shapes whereas the untreated control cells were in elongated spindle shapes. This morphological change is considered the landmark feature of a MET (mesenchymal-epithelial transition)-like process. The changes in morphology were accompanied by a dramatic reduction in the level of vimentin and α-smooth muscle actin (α-SMA) expressions. In this study, our results support the potential use of APP to control the angiogenesis and the cellular transformation to invasive characteristics of cells by reversing EMT.

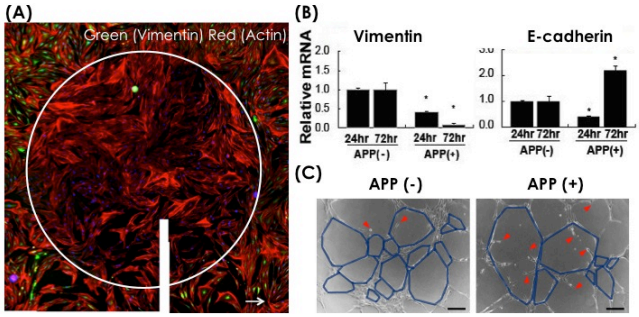


**Figure 1:** *(A) Immuno-fluorescent images of APP treated (encircled by a white line) HDFs showing distinct regions of different morphologies, (B) APP treatment suppresses mesenchymal marker vimentin while promotes epithelial marker e-cadherin, (C) APP treated HAEC show disrupted integrity (marked by red arrows) in network structures via suppression of angiogenic capability.*

**Acknowledgments**
This research was supported by the National Fusion Research Institute of Korea (NFRI) and the Korea Research Council of Fundamental Science & Technology (N04130012), and supported in part by National Research Foundation of Korea (NRF) (2011-0025465).

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# Control of time-limited activation of human primary fibroblasts through ROS generation induced by cold atmospheric plasma treatment

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Along with disinfection of living tissue and disposal of cancer cells [1,2], cold plasmas have been observed to promote tissue generation and wound healing [3].
Current knowledge emphasizes that the tuned regulation of fibroblast activity is mandatory for adequate wound-healing, paving the way for new wide-ranging strategies in the treatment of lesions.

In the present experimental study we focus on the effects of a non-thermal atmospheric pressure plasma produced by a radiofrequency low-power (1W) source using He as working gas already proven to be an effective tool in the therapy of eye infections [1,4].

It is shown that the treatment in the afterglow region of the produced plasmas controls the activation of two primary human fibroblast-like populations, hepatic stellate cells (HSCs) and intestinal subepithelial myofibroblasts (ISEMFs) through the generation of intracellular ROS with short half-life as compare to ROS generated in cells exposed to H2O2.
Plasma treatment of HSCs and ISEMFs does not significantly increase the percentage of AnnexinV- neither of AnnexinV/propidium iodide-positive cells, which means that no cell-death is induced.

Cellular proliferation and migration of plasma-exposed HSCs and ISEMFs are found to be ROS dependent. Biological effects are, indeed, prevented by pre-treatment of cells with antioxidant agents.
Morevoer, these effects are proven to be associated to plasma-induced interleukine-6 production and secretion, which is known to play an important role in the wound healing process.

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# Plasma source for fast and continuous purification of water flows

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The contamination of water by bacteria and anthropogenic origin compounds is the main causes of illnesses in developing countries and a worldwide environmental issue according to several international organizations like ONU, FAO, WHO and UN. None of common methods (filtration, sedimentation, distillation, active carbon, flocculation, clorination, UV, etc…) covers all the potential applications and their choice relies on effectiveness, fixed and operating costs. For this reason the constant development of effective methods for water purification is of great relevance in order to face the safe water scarcity issue. Several studies report on non-thermal plasma potential to decontaminate, to induce antibacterial properties and to degrade different types of contaminants in water. Plasma water purification is simple, effective and does not require the addition of expensive or harmful chemical agents.

In this work a plasma source able to treat water in a fast and continuous way without the need of recirculation or gas injection is presented. The plasma discharge is generated directly in the liquid phase to favour the introduction of reactive species and the direct decontamination and degradation of organic compounds. Furthermore the plasma source is designed to be integrated in common water supply piping system and to be easily scaled up to increase the water flow rate.

In order to assess the water purification effect of the plasma source, several tests on bacterial load and organic compound reduction were carried out.

The bacterial load reduction was evaluated for a plasma treatment of less than 1 s adding a bacterial suspension to demineralised water; two water samples collected before and after the plasma treatment were filtered and the filters incubated on plate count agar at 37°C. After 48h a bacterial load reduction was observed in plasma treated water sample in comparison with untreated water. To evaluate the plasma source potential of reducing harmful organic compound in aqueous solution we evaluated the concentration reduction of methylene blue dye by means of spectrophotometry.

Concentration of peroxide, nitrate and nitrite, pH and the temperature of the solution were measured after solution treatment in order to correlate these informations with treatment efficacy. Moreover, the discharge behaviour was investigated by means of iCCD imaging.

The results of this study may contribute to establish a new method for continuous plasma assisted water purification.

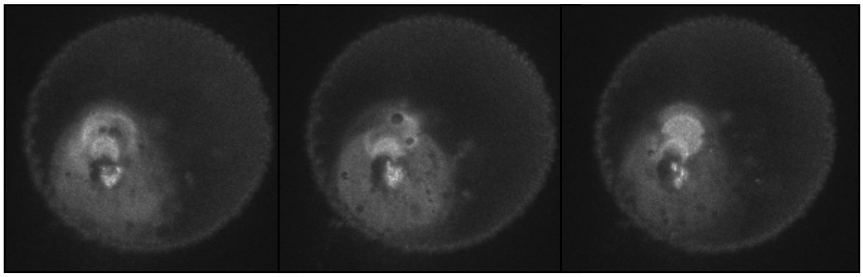

**Figure 1:** *iCCD acquisition of the plasma discharge in water*

# Bactericidal Characteristics and Material Conformity of Atmospheric-Pressure Glow Discharge

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Sterilization using non-thermal plasma at atmospheric pressure is attracting attention as an alternative method to chemical or gas sterilization.

We studied the inactivation of microorganism using plasma discharge in oxygen gas. In the plasma discharge, combinations of parameters such as frequencies, the structure of electrodes, and types of working gas,can produce a variety of solutions. We demonstrated inactivation using active species generated in the discharge space and in ultra-violet light emissions.

In the inactivation experiment, a strain of Bacillus genara: *Geobacillus stearothermophilus* was adopted as a standard microorganism and compared with other pathogenic species of microorganisms.[1 - 3] Some parts of materials such as soft silicone impression material were tested for comformity to the sterilization method.

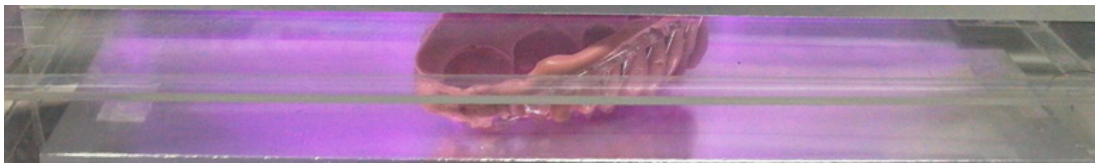

**Figure 1:** *silicon rubber impression material in plasma*

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# Inactivation of microorganism in liquid treated with neutral reactive oxygen species

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## 1. Introduction

The chemistry on gas-liquid interface has been intensively studied in order to clarify the the inactivation mechanisms of microorganisms.We have inactivated microorganisms using an atmospheric-pressure oxygen radical source in gas phase[1,2]. We measured the densities of ground-state atomic oxygen O($^3$P) and singlet oxygen molecule $O_2(^1\Delta)$ using a vacuum ultraviolet absorption spectroscopy and O($^3$P) is the effective factor for the inactivation in gas phase. On the other hand, the microorganisms in liquid are inactivated by active species (hydrogen peroxide etc) generated by the reaction with the liquid and the species in the gas phase produced by the plasma. The inactivation mechanisms of microorganisms in liquid phase has not been clarified quantitatively. In this study, we have inactivated *E. coli* cells in liquid phase using oxygen radical source in order to investigate the effects of neutral oxygen radical species.

## 2. Experimental procedure

A dish containing *E. coli* suspension (pH6.8) was placed below a neutral oxygen radical source. The radical source and the dish were enclosed with a plastic cover to avoid influences of ambient air. The total gas flow rate and the gas mixture ratio of $O_2/(Ar+O_2)$ were set at 5 L/min and 0.6%, respectively. The distance between the radical exit and the suspension surface was varied from 10 to 20 mm. The inactivation efficiencies of those cells were evaluated by colony-counting method, and the absolute densities of O($^3$P) and $O_2(^1\Delta)$ were measured with vacuum ultraviolet absorption spectroscopy.

## 3. Results and discussion

Figure 1 shows the D value as a function of number density for O($^3$P) and $O_2(^1\Delta)$. The 1/D value, which corresponds to the inactivation rate, increases from 0.14 to 0.63 min with increasing O($^3$P) density from $1.3\times10^{13}$ to $2.3\times10^{14}$ cm$^{-3}$. On the other hand, $O_2(^1\Delta)$ density is almost constant at $8.0\times10^{14}$ cm$^{-3}$ against the exposure distance. These results indicate that O($^3$P) in the gas phase contributes to the inactivation rate of *E. coli* cells in the liquid.

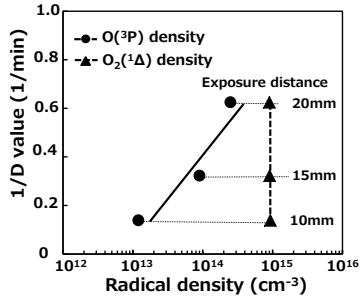


**Fig.1** *D value as a function of number density for O($^3$P) and $O_2(^1\Delta)$.*

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# Multi-torch type microwave air plasma designed for medical sterilization

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The multi-torch microwave air plasma is used for sterilization of medical equipment. The feature of this plasma production method is utilization of the ambient air. The multi-torch system has eight torches in order for filling the inside of a container with plasma uniformly. The opening edge of the torch is located in a vacuum vessel with the capacity of 17.5 L. The pressure in a vacuum vessel is kept at 100 Pa or less introducing ambient air in the vessel via multi-torch. Air plasma is generated in a glass tube by absorption of the microwave to the electrode and is blown off into the vessel.

The yellow luminescence spread uniformly in the whole vessel using the multi-torch with eight torches. When the microwave power increases, the peak intensity of a broad peak in the OES is proportional to the microwave power and the wavelength of the peak is constant, as shown in Fig. 1. Figure 2 shows the intensity at 583 nm in the OES varying the mixture ratio of nitrogen and oxygen in material gas. The intensity has maximum value at the oxygen concentration around 50 %. Above results imply that origin of the observed broad peak is the NO radical.

In order to confirm the sterilization characteristics of the multi-torch plasma system, thermophile spores with the population of $10^6$ is used as the biological indicator. Approximately 120 min is required for the inactivation of the thermophile spores. Since the discharge power is distributed to eight torches, the temperature of the discharged gas in the vacuum vessel can be reduced to below 60℃, which realizes the low-temperature medical sterilization.

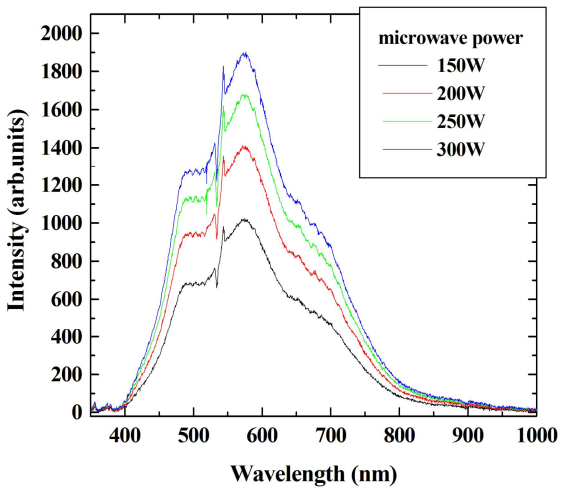


Fig.1 Typical light emission spectra of the multi-torch microwave plasma in the vessel.

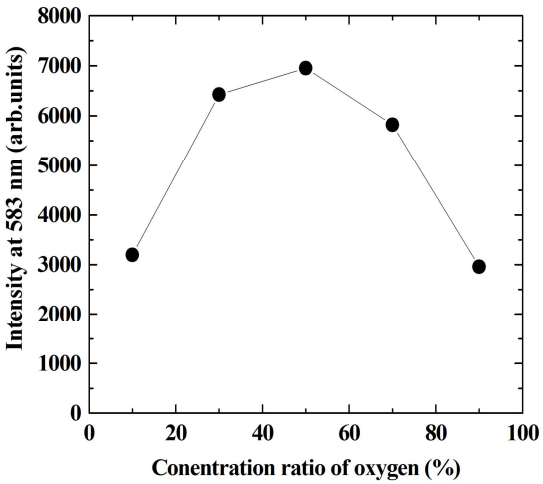


Fig.2 Light emission intensity at 583 nm varying the mixture ratio of oxygen with nitrogen.

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# Inactivation effect of marine microorganisms on hydrogen mixed gas plasma generated by dielectric barrier discharges

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Treatment methods of marine-microorganisms are required to conserve an ocean environment due to undesirable propagations caused from ballast water. We have reported that the DBD irradiation effects of the physical disruption, the bleaching effect, and the motionless state for microorganism were observed [1]. Moreover, the results indicated that the chemical reactions due to the OH radicals affect the inactivation of microorganisms. To improve the inactivation irradiated by the atmospheric pressure plasma, we investigated the inactivation ratio of microorganisms as a function of the flow ratio of hydrogen to helium.

Experimental apparatus for inactivation microorganism using DBD plasma consists of a reactor with 400 ml filled with water, aluminum electrodes covered by a dielectric as an alumina, a inverter power supply, and mass flow controller for controlling flow rate of hydrogen and helium. The high-voltage power supply (IDX Co. ltd.) is an inverter system, in which was set to be 18 $kV_{p-p}$ having 45 kHz in frequency. To avoid the damage of dielectric material, the duration of irradiating DBD plasma was set to be 20 seconds and the total irradiation time is multiplied repetition number by duration of irradiating DBD plasma. Number of inactivated samples divided by number of treated samples is defined by the inactivation ratio. To measure a generation of hydroxyl radicals, the fluorescence from 2-hydroxy terephthalic acid generated by the terephthalic acid reacted on the hydroxyl radicals is used.

The inactivation rate as a function of plasma irradiation time indecates that the introduced gas as 3% of hydrogen mixed helium gas is high inactivation rate compared to the other gases. It indicates that the generation of hydroxyl radicals qualitatively increases with the plasma irradiation time. The results from the generation of hydroxyl radicals observed by 2-hydroxy terephthalic acid reveals that the hydroxyl radicals efficiently generates in the case of 3% of hydrogen mixed helium gas. It indicates that the flow ratio $H_2$/He affects the inactivation ratio of marine microorganisms. The inactivation ratio at the $H_2$ flow ratios between 0.5 % and 2 % decreases compared to that at the $H_2$ flow ratio of 3%. It means that the balance of the chemical reactions for the generated active species in water may depend on the flow ratio $H_2$/He. The marine microorganisms are inactivated by the OH radicals. Thus, the flow ratio $H_2$/He controls the generation of the OH radicals.

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# Sterilization treatment of bacterial spores contaminated spices by Atmospheric Pressure Plasma Jet

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Spices are widely contained in various food products for their flavor and aroma. The contamination by microorganisms of imported spices has been causing problems for the food industry. It has been reported that microbial spores of Bacillus species, such as B. *subtilis* and B. *pumilus*, are the common contaminants in many spices ($10^5$ - $10^8$ spores per gram) [1]. The most popular methods for spice decontamination are superheated-steam treatment, thermal inactivation. However, these decontamination methods may cause color loss, flavor changes by thermal influence. To avoid these adverse effects, we performed the sterilization processing of the spices by using atmospheric pressure plasma jet to achieve efficient inactivation of microorganisms for thermosensitive materials like spices.

A schematic of our plasma experimental system is shown in Fig. 1. The plasma experimental system is an atmospheric pressure plasma jet source, which consists of a conical inner electrode and a grounded outer electrode with a nozzle of 5mm diameter. The inner electrode is coupled to a stepped high-frequency pulse current power supply (about 280 V, 8A, and 16.20 kHz), through a high-voltage transformer [2]. The working gas is divided into two lines, and the main line is connected through the two electrodes and the sub line including water vapor with an ultrasonic nebulizer device is connected with nozzle.

Plasma-irradiated spices (black pepper seeds) were soaked in the nutrient-rich medium (LB Broth) and incubated for 50 hours at 37°C. Figure 2 shows the results of the incubation of untreated spices (a) and $N_2$ plasma-irradiated spices (b). Although the medium containing untreated spices became crowded, the one with plasma-irradiated spices was still transparent. Therefore, the microorganisms attached with the spice could be sterilized by atmospheric plasma jet. In the presentation, we will report in detail.

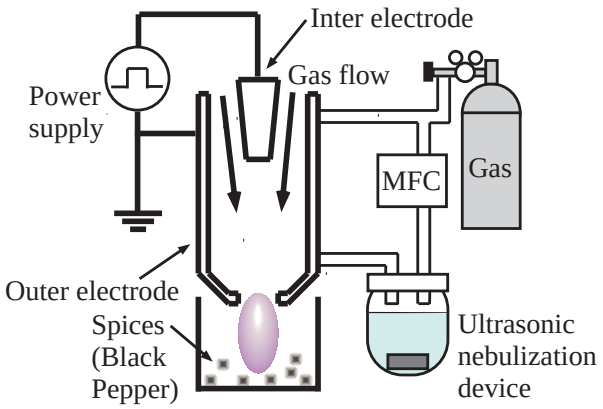


**Figure 1:** *Schematic of the germicidal treatment system*

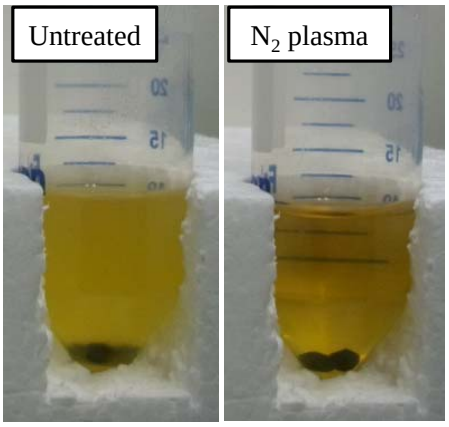


**Figure 2:** *Photograph of sterility tests of black pepper seeds in LB broth medium*

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# Difference of Cell Death Ratio between using Atmospheric-pressure Dry- and Mist- Plasma Jets

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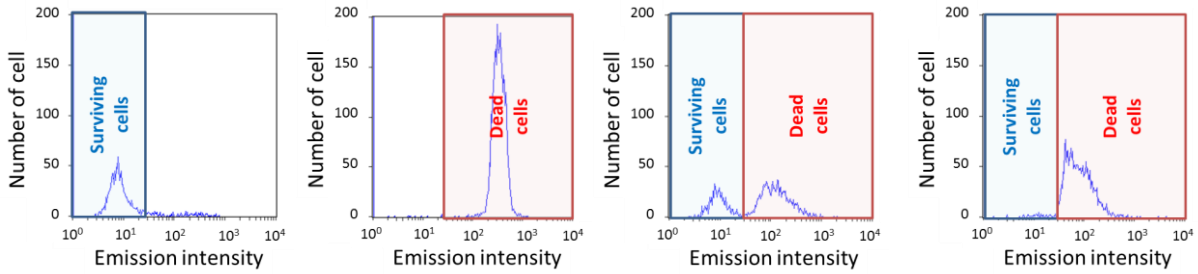
Recently, atmospheric-pressure plasmas (APPs) have attracted increasing attention in the fields of biological and medical applications, such as sterilization and surface modification [1], since they have the ability to produce UV light emission, electrons, ions, and radicals at low temperatures. Especially, it was repoted that various kinds of chemical species, e.g. $NO_2$, $HNO_3$, $O_3$ and OH, generated in air by plasma are dissolved and transported in water quickly [2], and those chemical stimuli can inactivate bacteria [3]. Furthermore, it was also reported that $H_2O_2$ produced by plasma is one of the main factors responsible for inactivation of HeLa (human cancer) cell viability [4]. In our previous study, therefore, we focused on OH or $H_2O_2$ production amount and developed an "mist plasma jet (MPJ)", which is generated using dry helium gas mixed with water mist, as alternatives to the traditional method using only dry helium gas, known as the "dry plasma jet (DPJ)" [5]. In this study, we focused on the effect of MPJ and DPJ on HeLa cells surrounded by cell culture medium, and compared those effects. The results show that there is difference of cell viability between MPJ and DPJ at the same irradiation time to the cells (see Fig. 1). The details will be shown at the conference.




*(a) Control* *(b) 35% $H_2O_2$* *(c) DPJ (5 min.)* *(d) MPJ (5 min.)*

**Figure 1:** *Comparative efficacy of DPJ and MPJ on HeLa cells using flow cytometry. (a) control, (b) 35% $H_2O_2$ added, (c) 5 min. of DPJ and (d) 5 min. of MPJ irradiated.*

**Acknowledgment:** This work was supported by JSPS KAKENHI Grant Number 25115.

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# terilizing ffect of *Xanthomonas Campestris* pv ampestris *Xcc* y orona- ischarge onthermal lasma posure at tmospheric ressure

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The Black rot disease of Chinese cabbage is caused by infection of *Xanthomonas campestris pv.Campestris* (*Xcc*). It is also kind of worldwide familiar disease of crucifers. Lots of researches have been done and the effect of nonthermal plasma exposure in sterilizing bacteria has been proved high-efficiency. In this experiment, *Xcc* is purified from the cabbages infected with black rot disease, then disposed in AC corona-discharge nonthermal plasmas at atmospheric pressure to investigate the sterilizing effect. From the result, the sterilizing effect is obvious and it has a direct ratio with exposure time and corona current. Morphological observation shows lysis of *Xcc* after the exposure and reasons are discussed following. Some cabbage seeds soaked with *Xcc* are planted and exposed to make a further study of the idea of seed-sterilization by nonthermal plasma.

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# Internal Sterilization of a Narrow Tube by ECR Plasma

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Plasma sterilization techniques have superior characteristics such as a short treatment times, non-toxicity and low thermal damages on the sterilized materials. Thus, plasma sterilization techniques have been extensively investigated as alternative sterilization method. On the other hand, it is thought that the plasma sterilization techniques is unsuitable for internal sterilization of narrow tubes such as a catheter and breathing circuit, because the production of a plasma is diffcult in a small space. In order to solve this problem, we produced ECR plasma in a narrow tube and applied it to the internal sterilization of the narrow tube.

A schematic diagram of the experimental apparatus is shown in Fig.1. The experiments was performed in the vacuum chamber which contains five cylindrical Nd permanent magnets ( $\phi$ 30mm×10mm. The magnets have a flux density of approximately 3.8kG at its surface, so that the region of the magnetic field for ECR (875G) exits near the magnet surface. A narrow tube with a inner diameter of 5mm was set at the ECR magnetic field region as shown in Figure 1. The microwave is irradiated through the tube wall into the inside. The ECR plasma is then generated in the ECR region, i.e. only in the internal tube area.

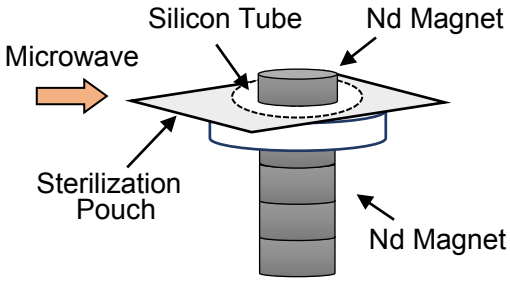


**Figure 1:** *Schematic diagram of the experimental apparatus*.

Ability of sterilization was investigated using biological indicators. *Geobacillus Stearothermophilus*, of a population of $8\times10^4$ CFU/carrier adhered on a small strip (2mm ×4mm). The biological indicator was placed in the narrow tube and it was sealed in a sterilization pouch, consisting of a Tyvek sheet. The ECR discharge in the narrow tube was kept for 0.5sec and repeated at intervals of 5sec. Discharge gas was air and gas pressure was 0.1Pa. After plasma treatment, incubation was performed in tryptic soy Broth. Subsequently, the spore survival was determined by observing the color change of the tryptic soy broth solution. Table 1 shows the effect on the sterilization by different treatment time. We confirmed that biological indicator having spore was sterilized in total treatment times of 10min at the temperature of 80℃.

**Table 1**

| Time [min] | ○ sterilized × failed |
|---|---|
| 5 | ○ × ○ ○ × |
| 7.5 | × × ○ ○ × |
| 10 | ○ ○ ○ ○ ○ |

# Cellular Responses in *E. coli* upon Exposure to Non-Thermal DBD Plasma Treated N-Acetylcysteine (NAC) Solution

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Plasma medicine is a rapidly growing field that provides novel solutions for the problems in biology and medicine. Most of the plasma medicine research studies utilize direct plasma treatement. Previously our group have introduced "fluid mediated plasma treatment" method, which can be considered as combination of direct and indirect plasma treatment [1,2]. Previously we have reported that N-Acetylcysteine solution gains antimicrobial effect when treated with non-thermal dielectric barrier discharge (DBD) plasma. Our results have revealed that 3-minute plasma-treated NAC solution can effectively inactivate up to 8 log bacteria including fungus Gram-positive, Gram-negative and multi drug resistant (MDR) strains in their planktonic forms and also can completely eradicate biofilm forms of fungus and bacteria strains [2]. However cellular mechanisms leading to antimicrobial effect are not well understood.

In the present study possible cellular responses and intracellular mechanism causing bacterial inactivation in *E. coli* were investigated. Our results revealed that during plasma treatment NAC molecule was first converted to S-NAC (S-nitroso N-Acetylcysteine), which serves as nitric oxide (NO) donor, and leads to formation of reactive nitrogen species (RNS)[3]. We have carried out various experiments including membrane damage assay, DNA damage assay and intracellular oxidative and nitrosative stress assays in order to understand possible mechanisms leading to cellular death. Our results have shown that plasma-treated NAC solution causes damages on the different components of bacterial cells. We also carried out microarray and RT-PCR studies to understand possible genes that are up or downregulated following exposure to non-lethal dose of plasma-treated NAC solution. Our microarray and RT-PCR results have suggested that plasma-treated NAC solution induces nitrosative stress in bacterial cells that leads to inactivation.

In conclusion plasma-treated NAC solution is a strong, novel, broad-spectrum antimicrobial agent that generates dominantly nitrosative stress in the cell that causes bacterial inactivation.

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# EVALUATION OF THE BACTERICIDAL EFFECT OF A HELIUM BASED ATMOSPHERIC PRESSURE NON THERMAL PLASMA JET ON THE 'ESKAPE' PATHOGENS

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Overuse and poor stewardship of antibiotics have led to the rapid dissemination of antibiotic resistant bacterial strains worldwide, resulting in a dramatic decrease in the arsenal of medicines available to treat these infections. Of particular concern are a group of bacteria termed the "ESKAPE" pathogens: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp. [1]. In 2009 the ECDC/EMEA Joint Working Group reported that infections caused by multi-drug resistant (MDR) bacteria are responsible for the death of approximately 25,000 people and cause about EUR 1.5 billion in healthcare costs and productivity losses in Europe each year [2]. Despite the desperate need for new antibiotics, in particular those from truly new compound classes, pharmaceutical companies have dramatically reduced their funding and, as a result, their output in this area, and the drug development pipeline has begun to stagnate [3].

Recently atmospheric pressure non-thermal plasmas (APNTP) have received much attention as an innovative new approach to infection and contamination control. A combination of reactive oxygen species (ROS), reactive nitrogen species (RNS), ultraviolet (UV) radiation and charged particles together contribute to a non-selective, multi-target mechanism of action that suggests a greatly reduced likelihood of the emergence of microbial resistance towards it. Our group has previously described an in-house helium based plasma jet operating with 0.5 % oxygen at 2 slm with a 6kV pulse applied at 20 kHz, and described its efficacy in the eradication of Gram-positive and Gram-negative bacterial biofilms [3][4][5]. Here we report our findings from a broader investigation of the effect of our APNTP on all six of the ESKAPE pathogens, comparing the effect of the plasma on planktonic vs. biofilm growth and on clinical strains vs. type strains. Our comprehensive study provides a platform from which to build further research to help shed light on the mechanisms of action and potential practical applications of this intriguing technology.

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# Comparison of the growth inhibition potential of different dielectric barrier discharge operating regimes

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Dielectric Barrier Discharges (DBD) are atmospheric pressure plasma sources already adopted in a wide range of biomedical and industrial applications. Depending on working voltage, gas composition and inter-electrode gap, DBD sources can be operated either in diffuse or filamentary regimes [1, 2]; each of these different modes of operation can be more or less beneficial, depending on the process to be supported.

In this work, the growth inhibition potential is compared for three different DBD regimes of operation: diffuse, filamentary with several microdischarges uniformly distributed in the inter-electrode gap (dense filaments) and filamentary with a small number of localized microdischarges (sparse filaments).

The adopted DBD plasma source consists of a cylindrical copper electrode covered by a quartz disk as a dielectric; the DBD is driven by a commercial high voltage power supply providing microsecond pulses. The contaminated agar plates used for the biological tests were placed on grounded metallic plates and used as the counter-electrode. The three operating regimes have been achieved modifying the inter-electrode gap (0,5 mm for diffuse, 1 mm for dense filaments and 2 mm for sparse filaments), while keeping constant frequency (500Hz) and impulse energy (between 1.3 and 1.7 mJ).

In order to investigate the antimicrobial potential of the different discharge regimes, the antibacterial activity against Staphylococcus aureus, a Gram positive bacteria, was evaluated. In particular, a S. aureus suspension in 0.9% NaCl was swabbed uniformly across nutrient agar plates, achieving different suspension's bacterial density (up to about $10^7$ colony forming units (CFU) $ml^{-1}$ quantified by dilution plating). The samples have been exposed to the DBD plasma for different times (10s, 30s, 60s and 120s). Both qualitative and quantitative data have been collected: after 24 hours of incubation at 37°C, different inhibition areas were observed depending on exposure time and discharge regimes; moreover, a bacterial load reduction was detected spreading different bacterial concentration in a circular limited area (diameter 30 mm) directly exposed to plasma

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# Bacterial surface decontamination of different types of materials using an UV-C dielectric barrier discharge flat lamp

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The air-conditioning, medical and food industry are continuously searching for novel technologies to improve the microbial surface decontamination. One of the directions that gained attention in the last decade is the exposure to UV-C light, a powerful non-thermal technology able to inactivate microorganisms [1]. In this study, the biocidal efficacy of a UV-C dielectric barrier discharge (DBD) lamp was assessed for different types of contaminated materials.

A DBD prototype lamp (80mm x 120mm x 6mm) was used to produce UV-C radiations in the wavelength range of 200 nm – 300 nm. This emission is generated by an internal phosphor coating of calcium doped with $Pr^{3+}$(α- $Ca_2P_2O_7$:Pr2%Na2%) excited by plasma VUV photons [2]. The typical plasma conditions were Ne-Xe (50/50) mixture at 500 mbar and square wave (1500V peak) at 20 kHz. The UV-C and visible emissions were monitored by optical spectrometry (USB 4000, Ocean Optics Inc., Dunedin, FL) with a 600µm fiber. A calibrated radiometer (System RM-22, Dr. Groebel UV-Elektronik GmbH, Germany), with a specific wide band sensor 210nm-310nm, allowed us to perform absolute measurements of the energetic fluence ($mJ/cm^2$).

The following materials were used as surfaces for decontamination: stainless steel, glass and polycarbonate carriers. A commercial *Bacillus atrophaeus* spore suspension (NRRL B4418, Steris Corporation, Mentor, IL) containing $2.1x10^8$ CFU/ml was used to inoculate the carriers. The carriers were inoculated with 10 µl of spore suspension. A part of the samples were exposed immediately to the radiation produced by the DBD lamp, and a second set of samples was dried overnight before the exposure. The treatment duration ranged from 10 to 600 seconds for the dry samples and for 10 to 60 seconds for the wet samples. Each experiment was made in triplicate. After the treatment, the spores were recovered in phosphate buffered saline (PBS). Serial dilutions 1:10 (x4) were made for each sample and each dilution was plated in duplicate on trypticase soy agar (TSA, Difco Laboratories, Detroit, MI). The plates were incubated at 35°C for 48 hours and then assessed for growth.

The preliminary results showed that the exposure to the DBD lamp of the spores inoculated on metallic carriers (wet samples) produced a 1 log reduction in 14 seconds and a complete inactivation (6 log reduction) in less than 90 seconds. The spores dried on metallic carriers proved to be more resistant. A comparison between the survival curves of *B. atrophaeus* inoculated on different types of carriers will be presented and discussed.

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# Morphologic changes observed on *E. coli* bacteria submitted to nitrogen and air plasma jets and afterglows

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Electrical discharges are able to produce large amounts of charged (electrons, ions), reactive (atoms, metastable states) and radiative species in an extended range of pressure and temperature, conferring them interesting germicidal properties. For this reason, room temperature discharges were extensively studied during the last 15 years as potential means for the sterilization of reusable medical instrumentation or for high level decontamination of wounds.

In this paper, an attempt is made to correlate the time variation of the inactivation efficiency (quantified by the log reduction of the initial bacteria population) with morphologic changes observed on the *Escherichia coli* bacteria by scanning electron microscopy (SEM) for increasing plasma exposure times.

To this end, two different discharges were used :

- a microwave reduced pressure (5 Torr) post-discharge operating in pure $N_2$ in full late afterglow conditions;
- a corona jet operating either in ambient air or in pure $N_2$ at one atmosphere.

For similar exposure times (10 min.) corresponding to approximately a 2 log reduction of the initial bacteria population, the membrane integrity appears to be preserved with the $N_2$ afterglow exposure (only strong invaginations can be seen, suggesting a cytoplasm release) while the air corona exposure conduces to a complete membrane disruption (Fig. 1).

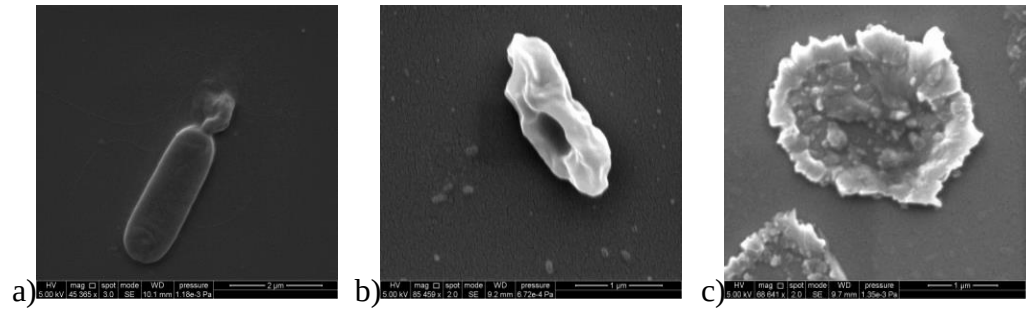


**Figure 1:** *SEM pictures of E. coli. a) Control, b) After a 10 min. exposure to a pure $N_2$ reduced pressure afterglow, c) After a 10 min. exposure to a corona plasma jet operating in ambient air.*

**Aknowledgments**

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# Inactivation Mechanism of Atmospheric Cold Plasma against *Escherichia coli* and *Staphylococcus aureus* in Liquid

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Atmospheric cold plasma (ACP) is effective against a wide range of pathogenic microorganisms, by generating groups of reactive species. Reactive oxygen species (ROS) play a crucial role when air or other oxygen containing gases are used [1]. With strong oxidative stress, cells are damaged by lipid peroxidation, enzyme inactivation and DNA cleavage [2]. In the current study, the inactivation efficacy of in-package, high voltage (80kV) ACP and the role of intracellular ROS were investigated with respect to system and process parameters.

*Escherichia coli* ME9062 and *Staphylococcus aureus* ATCC 25923 suspended in phosphate buffered saline (PBS) were used as target microorganisms. Samples were placed inside rigid polypropylene containers prior to ACP treatment, sealed within high barrier polypropylene and exposed to a high voltage plasma discharge (80 kVRMS). Intracellular ROS were measured using fluorescence spectrophotometer (Biotek synergy, 480/530nm) with the probe 2', 7'-dichlorofluorescein diacetate. Absorbance at 260 and 280 nm were used to monitor cell envelope damage. Ozone measurements were taken for each in-package treatment.

Inactivation efficacy and intracellular ROS increased with longer treatment times as well as post-treatment storage times for both direct and indirect modes of plasma exposure. A significant increase of A260/A280 was observed in *E. coli* after ACP treatment, indicating severe membrane damage. However, there was little envelope damage detected in *S. aureus*. Based on the structure difference, there are more vulnerable sites on Gram negative membrane than Gram positive. Thus, it is easier for ROS to damage the cell envelope of Gram negative bacteria. Interestingly, the intracellular ROS levels in *S. aureus* were much higher than levels in *E. coli*, while the ozone generated in-package was similar for each treatment time. Without post-treatment storage, bacteria were reduced by 0.6 to 1.5 log cycles respectively. However, introducing a post-treatment storage time led to severe cell damage and greater inactivation.

Overall, there were two mechanisms of inactivation observed in our study. Reactive species were either reacting with cell envelope or damaging intracellular components. *E. coli* was subject to both inactivation patterns , while *S. aureus* was mainly eliminated by intracellular damage.

Acknowledgement: This research is funded by the European Community's Seventh Framework Program (FP7/2207-2013) under grant agreement number 285820.

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# The application of $O_3$ and plasma generated by arc discharge in control of rice Bakanae disease caused by *Fusarium fujikuroi*

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Selective sterilization by plasma is one of the current issues in plasma bioscience. Inactivation of microbes associated with foods and host organisms requires non-toxicity to foods and hosts. The objective of our study is to examine the potential of plasma and $O_3$ for selectively inactivating fungal pathogens infecting seed rice. For this, we first investigated the feasibility of using O3 and plasma generated by arc discharge in water to inactivate *Fusarium fujikuroi*, a fungus causing rice bakanae disease which now becomes a problem in Korea. When fungal spores were exposed to gaseous $O_3$, germination was dramatically reduced within an hour. Ozone has been recognized as the most powerful oxidant next to hydroxyl and so may quickly kill insect, bacteria, and fungi. Inactivation of fungal spores was also observed in water after the arc discharge. The inactivation effect may be attributed to the presence of active plasma species, i.e., OH, O, $O_3$, $H_2O_2$, UV, electric fields and shock wave generated by the arc discharge in water. Each of these plasma species may play a role in the inactivation of microorganisms.

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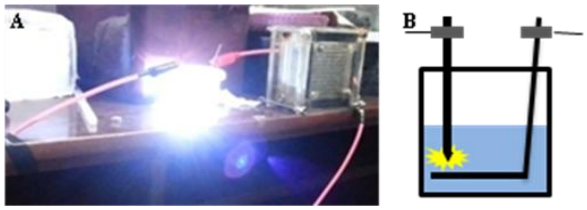


**Figure 1:** *A. Photograph showing arc discharge, B. Schematic diagram showing arc discharge electrode.*

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# Treatment of Microorganisms in Vegetables and Fruits by Gliding Arc

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A gliding arc is constructed in order to investigate the treatment of microorganisms in vegetables and fruits at atmospheric pressure, [1] [2]. Results of treatments of two microorganisms are presented namely E-Coli and Erwinia Cartovora. The plasma discharge occurs between two knife-shaped Stainless Steel electrodes of 96 mm long, and 26 mm wide at electrode's bases. A dc voltage of 2 kV is applied between the inter-electrodes' bases of 3mm separation where argon gas is injected from a Teflon nozzle. When the discharge occurs, a current up to 2 A is drawn between the two electrodes creating an expanding plasma plume. The treated specimens are located 13 cm from electrodes' tips.

E-coli is treated in a bacterial suspension prepared by fresh cultures which were grown overnight in tryptic soy broth (TSB) at 37 °C until it reached the logarithmic growth phase. The bacteria concentration is approximately $10^9$ CFU/mL, by serial dilution the concentration was diluted to $10^6$ CFU/mL. It is found that after 4 min of treatment the bacterial growth was reduced to 99.6% of the initial population

Erwinia Carotovora strain is grown on nutrient agar and potato tubers. By peeling 5 mm thick slices of area 1 $cm^2$ from the tubers skin. Erwinia Carotovora suspension of 100 µl (contains roughly $10^5$ CFUs) was deposited on the slices uniformly. These slices were allowed to dry for half an hour at room temperature. Then the samples were exposed to the gliding arc plasma for different time intervals from 15 to 60 sec. After that, the slices were put on sterile Butterfield's phosphate buffer solution PBS (90 ml and pH 7.0) and introduced into a centrifugal device to separate the bacteria from the treated slices. 100 $\mu$l of the homogenate (cell suspension) of the PBS were spread uniformly over nutrient agar plates, which are then incubated at 37 ◦C for 24 h for CFU counting. It's found that complete sterility is reached after 60 sec of treatment.

The treatment of vegetable and fruits by this method is found to be effective against common pathogens. The disinfection is rather fast with no overheating or organic changes.

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# Effects of electrical stimulation by high voltage pulse on yield in sawdust-bed cultivation *Lentinula edodes*

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Effects of electrical stimulation on sawdust-bed in Shiitake mushroom (*Lentinula edodes*) cultivation using high voltage pulse were investigated experimentally. In the experiment, *Lentinula edodes* was stimulated by applying pulse voltage on the mushroom hypha at various amplitudes and numbers of applying pulse voltage.

The pulse high-voltage was produced by high-voltage generator (*Raizo*; GM100), which was developed by Green-Techno Corp. as shown in Figure 1. The maximum output voltage of the generator *Raizo* was 100 kV with several microseconds in pulse width. The voltage applied to the sawdust-bed was changed to obtain the optimum input energy for the stimulation. The applied voltage was controlled by gap length of the closing gap switch which was connected in series to the coaxial-cable. The energy was stored in the coaxial-cable as electrostatic energy. The stored energy was transmitted to the sawdust-bed through the closing gap switch.

Figure 2 shows the number of harvested fruit body of *Lentinula edodes* at various input energies to the sawdust-bed. The number of fruit body increased gradually with increasing input energy. The number of fruit body increased to be 2.5 times larger by applying pulse voltage as electrical stimulation at 13.6 J (1200 times applying voltage) input energy.

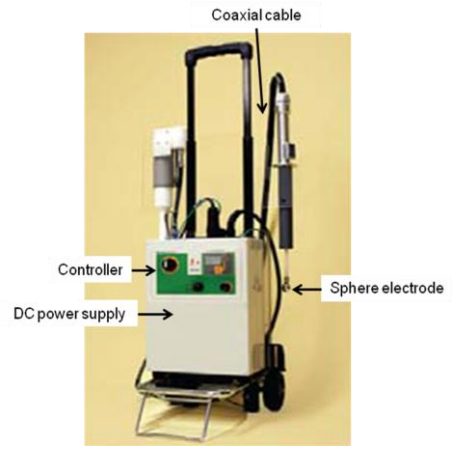


**Figure 1:** *High-voltage generator to apply the pulse stimulation on the mushroom bed developed by Green-Techno Corp.*

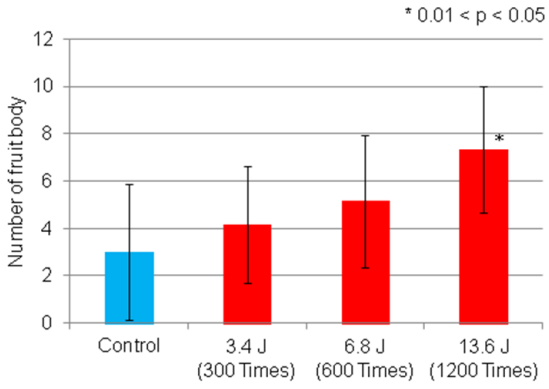


**Figure 2:** *Number of Lentinula edodes fruit body for various stimulation condition by Raizo.*

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# Histological comparison of the wound healing process between non-thermal plasma hemostasis and thermal coagulation hemostasis

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## Introduction

Plasma is a mixture of ionized molecules, radicals, and electrons that is generated by intense electromagnetic fields. Recently, techniques to generate plasma non-thermally have been developed. Because non-thermal plasma (NTP) has several effects such as blood coagulation and cancer treatment, its medical application is strongly desired. In clinical practice, bleeding from vessels is controlled by cauterization with a thermal coagulator (TC). However, heat produced from the TC injures tissues and causes long-lasting inflammation and postoperative disorders. Hemostasis by NTP may overcome this problem because the gas temperature of NTP is close to ambient temperature. However, the detailed profile of inflammation after NTP hemostasis has not elucidated yet. In this study, we histologically evaluated and compared the healing process after hemostasis between a TC and NTP.

## Methods

Helium (He) was used as the plasma source. While the He gas was flowing, plasma was excited with 14.5 V. The flow rate of the He gas was set at 2 standard liters per minute, and the distance between the plasma source and the samples was fixed at 10 mm. Male ICR mice at 10 weeks of age were used. The liver was exposed and cut (length, 20 mm; depth, 3 mm) under pentobarbital anesthesia. Blood from the wound was coagulated using either a TC or NTP. After surgery, the mice were killed on days 1, 5, 10, 15, and 20, respectively. The liver was immediately removed, and frozen sections were prepared. After fixation with 4% paraformaldehyde solution, the serial sections were subjected to hematoxylin-eosin staining and immunohistochemical staining for macrophages using anti-F4/80 antibodies. Histological analysis was performed using a microscope (Olympus IX70).

## Results and Discussion

Necrotic areas were observed in all the samples. However, the necrotic areas in the TC group were remarkably larger than those in the NTP group. In the NTP group, macrophages migrated around the hemostasis area on day 5, and the number of macrophages decreased from day 5 to day 10. The necrotic areas and macrophages disappeared on day 15. These findings indicate that in the NTP, inflammation was resolved by day 15. In contrast, macrophages were observed on day 10 in the TC group. The necrotic areas and macrophages remained even on day 20. These findings indicate that the inflammation continued in the TC group. Therefore, this study revealed that the NTP had a shorter inflammatory term than TC hemostasis.

# Generation of locally deposited Bioactive Thin Films using Atmospheric Pressure Plasma Jets

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The optimization of surface properties is an important aspect to address specific cell response suitable for biomaterials and tissue engineering. By modifying chemical and morphological surface properties the material-cell interaction and thus, adhesion and proliferation of cells, can be controlled. Tailored surface properties can be easily realized by plasma enhanced chemical vapor deposition. Especially non-thermal atmospheric pressure plasma jets represent a suitable tool for local surface coating. Furthermore, the local deposition of plasma polymerized films enables the generation of chemical and morphological patterned surfaces applicable for micro-structured cell growth, for instance. The deposition process depends on type of precursor, plasma source, and main operating parameters (e.g. precursor inlet position, gas flow, frequency).

In the present contribution, results of an experimental study on plasma enhanced chemical vapor deposition using non-thermal atmospheric pressure plasma jets are shown. Emphasis is given on thin film deposition which exhibits either hydrophilic (e.g. nitrogen-rich coatings) or hydrophobic surface properties (e.g. Teflon-like coatings). The chemical structure of these films, measured by X-ray photoelectron spectroscopy, their wettability, as well as the morphological/topological properties will be shown and discussed. The thickness of the coatings have been determined by XPS Coronene sputtering and surface profiling, whereas mass deposition rates have been examined by weighing. The influence of plasma species on the deposition processes was investigated using optical plasma diagnostics. Hence, by controlling the deposition conditions hydrophobic, cell repellent films with mass deposition rates of ~ 1 µg/s and hydrophilic, cell adhesive films with mass deposition rates of ~ 0.6 µg/s can be obtained.

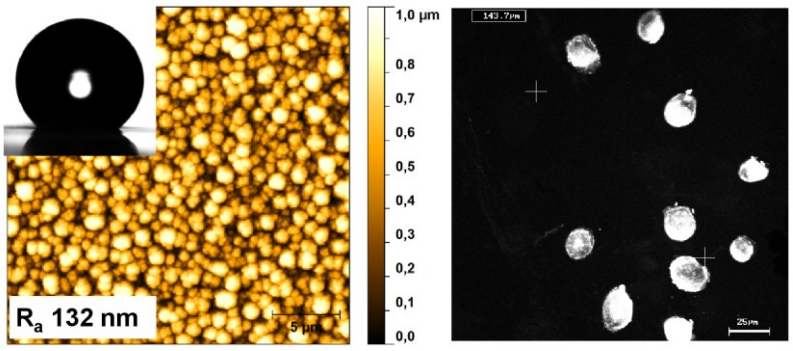


**Figure 1:** *The water droplet and AFM image show the hydrophobicity and roughness of the plasma polymerized CxFy-coating (left). The deposited fluorine-rich film inhibits the adhesion and growth of osteoblasts (right).*

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# Preparation of Bacterial Cellulose Composites with the aid of Dielectric Barrier Discharge (DBD) Plasma Treatment

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Bacterial cellulose (BC) pellicle is a polysaccharide produced by *Acetobacter xylinum*. BC pellicle has many advantages such as hydrophilicity, ultrafine 3D network structure, high purity, high water absorption capacity and never dry state of hydrogel [1]. Accordingly, BC pellicle is a good candidate for using as a wound dressing material because it can provide moist wound environment, promote wound healing process and excellent molding to all facial contour body [2]. However, in a large scale production of BC pellicle, damage from tearing of BC pellicle may occur during cultivation, sterilization and packing into a packaging. In order to reinforce BC pellicle, bacterial cellulose composites consisting of fabric embedded in BC pellicles were fabricated. Cellulose, nylon, and polyester fabrics were used to investigate the effect of the fabrics on mechanical properties, water absorption capacity and water vapor transmission rate of the composites. In addition, the surface of the fabrics was modified by dielectric barrier discharge (DBD) plasma treatment before cultivation in culture medium containing *Acetobacter xylinum*. By applying DBD plasma treatment, hydrophilicity, and surface roughness of the fabrics could be enhanced [3]. The effect of DBD plasma treatment time on production yield, change in chemical structure of the plasma-treated fabrics, morphology, mechanical properties, water absorption, and water vapor transmission rate of the BC composites was examined.

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# Plasma polymer coatings for biomedical applications: effect of aqueous media

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Nitrogen-rich plasma polymerized coatings present important advantages to enhance the biocompatibility of biomaterials. Yet, little is so far known about the interaction of these hydrophilic coatings with aqueous media, which could lead to dissolution, swelling or modification of their properties such as adhesion. The present work aimed to characterize and optimize primary amine-rich plasma-polymerized coatings (called L-PPE:N) [Truica PPP2008, Ruiz 2010] intended for use in contact with body fluids, particularly in the context of cardiovascular or endovascular applications. Coatings of different compositions were prepared in a low-pressure radiofrequency (r.f.) glow discharge reactor by varying the flow ratio, R, of anhydrous ammonia ($NH_3$) and ethylene ($C_2H_4$). The flow rate of $C_2H_4$ was kept constant at 20 sccm, while $NH_3$ flow was varied between 10 and 30 sccm, yielding respective R ($\equiv$ $F_{NH3}/F_{C2H4}$) values between 0.5 and 1.5. Plasma polymerization being performed under mild conditions, with applied power of 10 W. In addition to conventional characterization methods (contact angle, XPS, ellipsometry), we report here measurements based on quartz crystal microbalance with dissipation monitoring (QCM-D), nano-indentation and cross-hatch peel-testing, designed to investigate coatings' stability, mechanical properties and adhesion in aqueous media and under dry conditions, for comparison. The composition, aging, stability and swelling behavior were found to strongly depend on the flow ratio, R. Coatings with R d 0.85 displayed mass gains (swelling) in solution, while those corresponding to higher R values revealed mass loss, indicative of partial dissolution. In particular, R = 1.25 and 1.5 coatings dissolved very rapidly, losing about 80% of their mass within a few seconds. Coatings's Young modulus and hardness, as well as adhesion on substrates decreased substantially in water compared to dry conditions. While crosshatch tests showed good adhesion on polyethylene terephthalate (PET), surface pre-treatment of PTFE was necessary to improve film adhesion on PTFE both in dry and wet conditions.

**Acknowledgments.** Funding NSERC and CIHR. Thanks to Hysitron Inc for nanoindentation tests, as well as Annie Levasseur, Yvan Petit and JC Ruiz.

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# Patterning of Endothelial Cells and Hepatic Stellate Cells with Two Step Plasma-polymerized Processes

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Endothelial cells (ECs) and hepatic stellate cells (HSCs) adhesion and alignment were investigated on a micro-patterned surface fabricated by two step plasma polymerization processes. The plasma process is rapid, sterile, more cost effective, less labor intensive, and relatively easy to perform reproducibly on a large number samples simultaneously. A first functionalization step was the deposition of the nitrogen-rich plasma-polymerized film for rendering the entire surface the cell-adherent region. Following this, the hydrophobic plasma-polymerized film was formed through grid metal mask with hundred micrometer-sized openings, that was, rendering them the cell-repellent region (Fig. 1). Imaging ellipsometry showed that the 100 μm-width-groove and -ridge with tens nanometer steps was obtained. EC and HSC culture experiments were conducted on the patterned surfaces (Fig. 2). EC rapidly adhered and aligned along the cell-adherent groove of the patterned surface, and did not do on the cell-repellent ridge. HSC patterning succeeded only when the height of cell-repellent ridge was 20 nm, whereas the patterning failed when the height of ridge was 10 nm. This indicated that EC patterning was possible only with chemical effect, to the contrast, HSC patterning required with both chemical effect and "topological" constraints of the patterned surface.

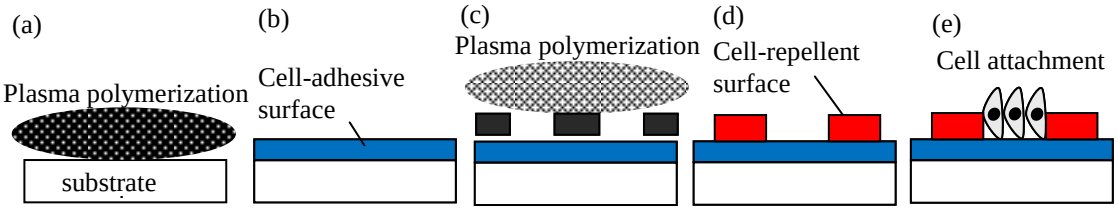


**Figure 1:** Schematic illustration of cell patterning process based on plasma polymerization. (a) Plasma polymerization. (b) Formation of cell-adhesive surface. (c) Partial plasma polymerization with shadow mask. (d) Formation of patterning surface. (f) Cell attachment.

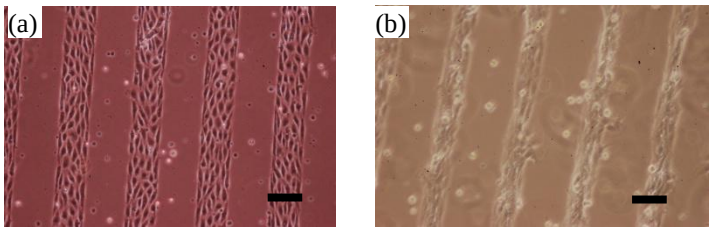


**Figure 2:** Optical microscopic images of (a) ECs and (b) HSCs seeded on PPF patterned surfaces. Scale bar represents 100 μm.

# Treatment of Polymer Surface in APPJ

Joanna Pawłat[1], Tomasz Cieplak[2], Jarosław Diatczyk[1], Piotr Krupski[1], Michał Kwiatkowski[1], Radosław Samoń[1], Piotr Terebun[1], Henryka Stryczewska[1]

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Polymers are widely used in biotechnology, medicine and chemistry; in food and automotive industries as common and cheap materials offering a broad range of beneficial optical, thermal, electrical, mechanical, and chemical properties. In surface processing, plasma is mostly used for surface cleaning, deposition, ablation or etching, surface activation, crosslinking and functionalization.
In the case of plasma treatment of bulk materials the modification depth depends on several factors including exposure time, plasma power, gas temperature and content (including oxygen, water vapor, noble gases, carbon dioxide, nitrogen), and also on geometrical factors.

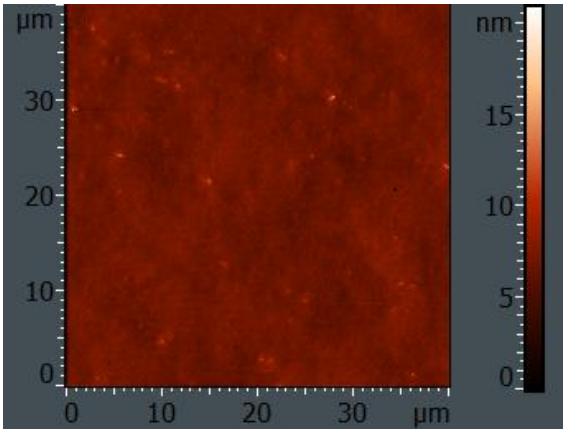


**Figure 1:** *AFM analyzed structure of plasma treated HIPS' surface (Gas flow rate- 0.71 $m^3$/h: 0.43 $m^3$/h He + 0.28 $m^3$/h $O_2$, 50 W, 14.23 MHz, 60 s.*

Atmospheric pressure plasma jet (APPJ) for treatment and decontamination of polymer surface was developed. In plasma physics, temperature is an extremely important parameter, which determinates the type and energy of plasma particles, and thus their chemical and electrical properties. This is particularly important in the case of non-heat resistant materials used biotechnology and medicine, where use of plasma jet reactor is limited by maximum permitted temperature of exhaust gas.
Properly selected composition and flow rate of substrate gas, reactors' geometry and power supply parameters allow to achieve broad range of outlet gas temperatures, with the lowest even below 40 $^{o}$C, what can be useful for treatment of non-heat resistant materials. AFM analysis of high-impact polystyrene (HIPS) surface after plasma treatment showed change in roughness, which is an important parameter correlated with hydrophilic character of the surface. The example of surface structure after 60 s plasma treatment is depicted in Fig.1.

# Cell adhesion enhancement of electrospun microtube array membrane (MTAM) by acetic acid (AA) plasma treatment for hollow fiber assay

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An effective in vivo assay to determine the cytotoxic effect of drugs against cancer cells, hollow fiber assay (HFA) relies cell-HF interaction critically. To improve hydrophilicity of HF substrate and enhance its adhesion with cells, biocompatible PLLA (poly-L-lactic acid) MTAM were treated with acetic acid plasma. PLLA MTAM with better cells attachment in conjunction with original porosity which facilitate both nutrient and drug perfusion will potentially outperform the commercial HFs. Three different tumor cell-lines were cultured both in vitro and in vivo with the MTAM, which were tuned with dimension, surface treatment and porosity for better cell viability and proliferation. The surface properties of plasma-treated, porous PLLA MTAMs were characterized by FT-IR and WCA. Results indicated MATMs were rendered more hydrophilic by plasma treatment. Compared with commercial HFs, tumor cells numbers were significantly increased one-two order in in vitro experiment. In in vivo test, tumor cells growth rates were also higher than that of commercial one. Based on these finding, it is anticipated that PLLA MTAM could be an efficient drug delivery platform for HFA. In summary, plasma-treated, porous MTAMs may serve as an advanced substrate for hollow fiber assay.

This work was partially supported by grant from National Sciences Council of Taiwan ( NSC102-2221-E-038-015)

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# Mechanical and biocompatibility of tunable TaOxNy thin films

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Oxynitrides of transition metals show tunable optical, mechanical, electrical, and bio-related properties when the ratio of oxygen to nitrogen is varied. In this study, a plasma assisted co-sputtering was used to produce TaOxNy thin films with the variation of O/N. After deposition, the films' mechanical properties were first examined. Following the biocompatibility of these films were studied using 3-T-3 cells. The results show that films' hardness as well as the toughness could reach the maximum when the ratio of O/N was at 1/5. However, from the biocompatibility testing, it was found that the O/N ratio should be enlarged. The viability of the cells was also tested using MTTassay. The applications of these films for medical devices was discussed.

# Laser-assisted biomimetic process for calcium phosphate deposition on a titanium metal

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Calcium phosphate is a major mineral component of human hard tissues, and shows good biocompatibility and bone-bonding ability. Recently, we developed a laser-assisted biomimetic (LAB) process for calcium phosphate deposition on an ethylene-vinyl alcohol copolymer substrate utilizing weak pulsed laser light [1]. The LAB process is simple (single-step), quick (within 30 min), and effective in area-specific calcium phosphate deposition. Hence, the LAB process has potential as a calcium phosphate deposition method on biomaterials.

In this study, the LAB process was applied to a substrate of titanium (Ti) metal that has long been used as an implant material in orthopedic and dental applications. The LAB process was carried out by irradiating pulsed laser light (Nd:YAG, 355 nm, 30 Hz, 4 W/cm$^2$) without focusing onto the Ti substrate that was immersed in a supersaturated calcium phosphate solution (Figure 1, left). Within 30 min of irradiation, calcium phosphate deposited on the laser-irradiated region of the Ti surface (Figure 1, right). The calcium phosphate deposition is attributed to the laser light absorption by the Ti substrate, and the resulting Ti surface oxidation and increase in the solution temperature near the Ti surface.

In summary, the present calcium phosphate deposition method by the LAB process was applicable to the Ti substrate. The LAB process would be useful for the production and surface functionalization of Ti biomaterials.

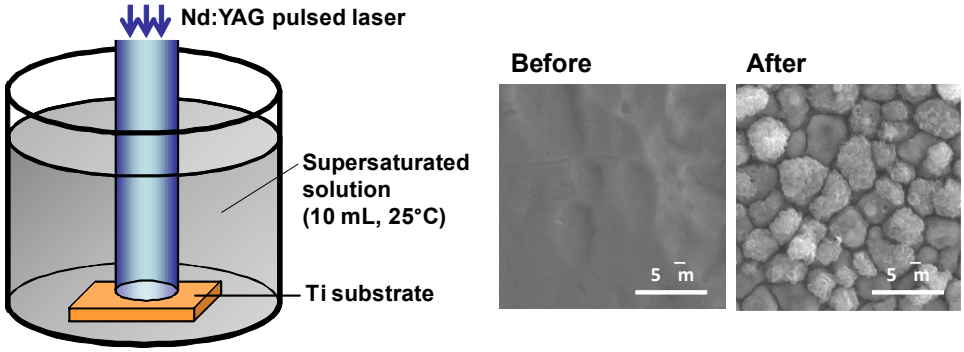


**Figure 1:** *Schematic of the LAB process (left) and scanning electron microscopic images of the Ti surfaces before and after the LAB process (right).*

Acknowledgments : This work was supported by KAKENHI (25108517) from The Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

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# Surfactant-Free Green Approach to Obtain Water-Dispersible Carbon Nanotubes by RF Plasma Treatment

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Water-dispersible carbon nanotubes (CNTs) possess a great importance in biotechnology and medical engineering, microelectronics, and composite materials owing to their unique chemical and physical properties [1-3]. Usually CNTs are dispersed in water using surfactants or through strong acidic treatments [4]. The surfactants are difficult to separate at the final stage, and the acidic treatment clearly deviates from the principles of green chemistry, which also alters the basic properties of CNTs [4]. To solve these problems, an easy and environment-friendly approach has been developed to obtain water-dispersible single-walled and multiwalled CNTs (SWNTs and MWNTs) by citric-acid-assisted oxygen plasma. CNTs are at first sonicated in pure ethanol to decoagulate them temporarily for the easy access of citric acid molecules, and to increase the sites for functionalization. In the second step, CNTs are kept in the citric acid solution where they are effectively wetted by the citrate ions. Finally, RF (13.56 MHz) oxygen plasma reaction is operated on the wet CNTs under optimum condition. The CNTs are effectively functionalized by the attachment of a large number of carboxyl (COOH) groups, which are created during the plasma process. The highly energetic plasma electrons and UV light attack the citric acid, water and oxygen molecules to produce many oxygen containing radicals and COOH groups, which eventually are attached as COOH on the surfaces of CNTs. The resultant CNTs can be easily dispersed in pure water by sonication without using any surfactant, and the suspension remains stable for more than one month. The structural qualities of the functionalized CNTs are also observed to be preserved.

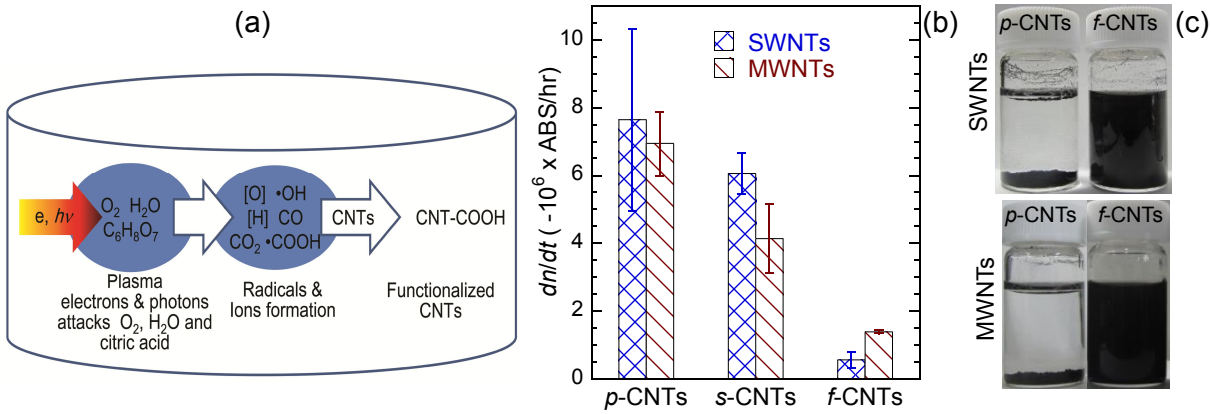


**Figure 1:** *(a) The plasma reaction process to produce CNT-COOH. (b) Settling speed, dn/dt, calculated from the Abs (250 nm) versus time graphs for the pristine CNTs (p-CNTs), sonicated CNTs (s-CNTs), and functionalized CNTs (f-CNTs). (c) Photographs of the suspension of p-CNTs and f-CNTs in pure water taken after 20 days.*

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# Influence of polymer surface on cell proliferation and cell oxidative homeostasis

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The formation of endothelial cell monolayer on prosthetic implants is not sufficiently explored. The main reasons leading to the development of thromboses and/or intimal hyperplasia is the lack of endothelialization of prosthetic implants. In the present work we have studied the influence of oxygen and fluorine plasma treatment of polyethylene terephthalate (PET) polymer on human microvascular endothelial cell adhesion and proliferation. We have checked the influence of plasma treatment on induction of cellular oxidative stress and cell proliferation. The obtained results showed that treatment with oxygen plasma decreased the oxidation potential of the PET surface and revealed the highest affinity for binding of serum and media components. Accordingly, the cells reflected the best adhesion and morphological properties on oxygen treated PET polymers. Moreover, treatment with oxygen plasma did not induce intracellular reactive oxygen species production while it stimulated endothelial cell proliferation by 25% suggesting the possible use of oxygen plasma treatment to enhance endothelialization of synthetic vascular grafts.

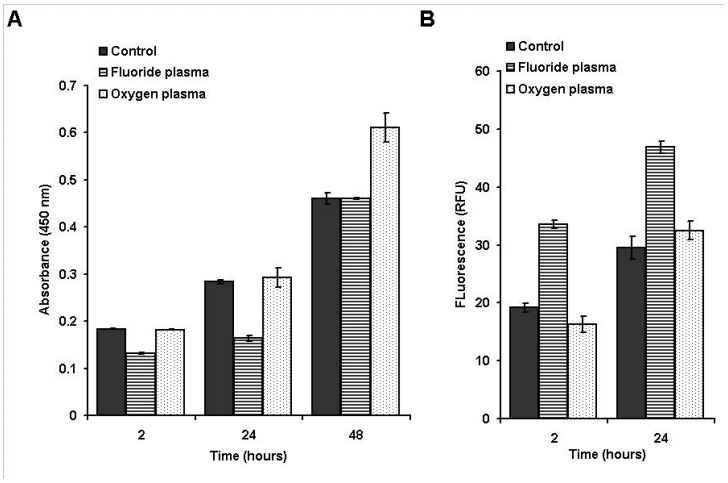


**Figure 1:** *Effect of chemical surface composition on cell proliferation and oxidative homeostasis. A. Proliferation of HMEC-1 cells on plasma untreated and treated PET polymers determined by MTT assay. Mean values (± SE) for the respective triplicates are given. B. Intracellular HMEC-1 ROS production for plasma untreated and plasma treated PET polymers. Mean values (± SE) for the respective triplicates are given.*

The authors acknowledges the financial support from Slovenian Research Agency through the project L7-4035 (Toward ecologically benign alternative for cleaning of delicate biomedical instruments).

# Plasma-patterned PDMS Coated with Vitronectin and γ-globulin Enables Patterning of Human iPS Cells

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The goal of this article is to make a pattern of human induced pluripotent stem cells (hiPSCs) on Polydimethylsiloxane (PDMS) surface. Because hiPSCs contain the donor's genetic information, medical applications of autologous hiPSCs offer the hope of rejection-free transplantation of tissues and patient-specific drug screening. Recently, the development of PDMS microdevices to control the microenvironment of hiPSCs has been extensively studied. Low pressure plasma-treatment is easy to use and have variety of usage including PDMS–PDMS and PDMS–glass bonding, to clean the surfaces, and to facilitate coating of the surfaces with cell-adhesive extracellular matrix (ECM) proteins [1]. Therefore, we frequently use plasma treatment to fabricate PDMS micro devices for cell culture [2, 3].

Here, we studied the effects of vitronectin and γ-globulin on hiPSC adhesion to plasma-treated and untreated PDMS surfaces under defined culture conditions. We chose these proteins because they have opposite properties: vitronectin mediates hiPSC attachment to hydrophilic siliceous surfaces, whereas γ-globulin is adsorbed by hydrophobic surfaces and does not mediate cell adhesion. To elucidate protein adsorption and hiPSC adhesion on a PDMS surface with and without plasma treatment, we applied our defined culture conditions, including purified protein coating and the culture medium for culturing hiPSCs.

Immunostaining showed that vitronectin and γ-globulin adsorped to both plasma-treated and -untreated PDMS surfaces when these surfaces were coated with a single protein; however, vitronectin and γ-globulin adsorped more onto plasma-treated and -untreated PDMS surfaces, respectively, when these surfaces were coated with a mixture of both proteins. Human iPS cells adhered to the vitronectin-rich surface but not to the γ-globulin-rich surface. Based on the results, we succeeded to make pattering of hiPSCs by patterned plasma treatment followed by coating with a mixture of vitronectin and γ-globulin (Figure 1). The result is useful for future applications of plasma-treatment for controlling human iPSCs adhesion.

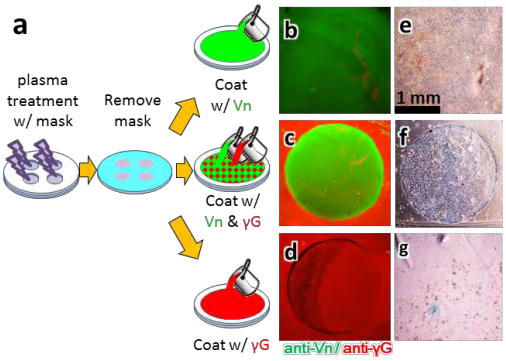


**Figure 1:** *Pattering of hiPSCs by patterned plasma treatment.* ***a:*** *Schematics of experiments.* ***bcd:*** *Immunostaining of vitronectin (green) and γ-globulin (red).* ***efg:*** *Micrographs of hiPSCs.*

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# Research for Tissue Regeneration Using Micro-Spot Atmospheric Pressure Plasma Source

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Atmospheric pressure plasma is reported in recent years that direct plasma irradiation promotes wound healing of living-tissues. However, these mechanisms have not been clarified. Against this background, we performed experiments involving direct irradiation to the culture cells using an atmospheric-pressure plasma source for various biomedical engineering applications.

The plasma source using helium (He) gas has a coaxial structure having a tungsten wire installed inside a glass capillary, and a grounded tubular electrode. The experiment was conducted by preparing a culture medium containing mouse embryonic fibroblast (NIH3T3 cell) line on a culture dish. The culture dish was irradiated by the plasma and was then placed in a $CO_2$ incubator (temperature: 37℃, $CO_2$ concentration: 5 %). After culturing for 24 hr in the $CO_2$ incubator, the number of cells counted using a hemocytometer. 24 hr cell culture and count number of cells repeat 7 days, growth curve was drew on the number of cells in 7 days.

According to the typical microscope images of NIH 3T3 cells that had been cultured in a serum-free medium for 24 h, cell growth was inhibited when only He gas was flowed through the medium because gas flow promotes cell floatation by agitating the gas in the culture. In contrast, cell floatation did not occur and healthy growth was observed for plasma irradiation.

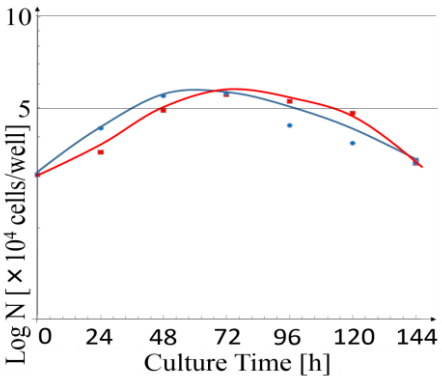


**Figure 1:** *Cell growth comparison 1*

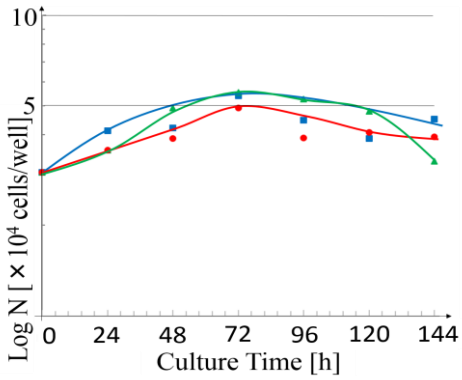


**Figure 2:** *Cell growth comparison 2*

First, we created cell growth curves by performing He gas flow and plasma irradiation processing on NIH3T3 cells for 30 s/day and plotting the increase in number of cells in each group for 1 week. Plasma irradiation group proliferate speedier than He gas flow group for Figure 1. Second, they transiently irradiated plasma for NIH3T3 cell line at each condition on logarithmic growth phase, stationary phase, and death phase. When irradiated plasma at the death phase, had no effect on cell proliferation. These results suggest that plasma exposure significantly accelerates cell doubling, and effective irradiation timing for cell activation is logarithmic growth phase and stationary phase.

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# Deacetylation and Depolymerization of Chitin Hydrogel via Solution Plasma Process

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Solution Plasma Process (SPP) is a liquid-phase plasma system which has been applied in several applications such as synthesis of metal nano-particles and decomposition of organic compounds. In this research, SPP was introduced to deacetylation and depolymerization reactions of chitin hydrogel in order to produce low-molecular-weight chitosan (LMWC) with inherent biological properties such as antimicrobial and antitumor activities.Chitin hydrogel, a highly reactive form of chitin with low crystalline structure, was prepared by dissolving chitin in calcium chloride dihydrate-saturated methanol, followed by precipitation in distilled water. Deacetylation reaction of chitin hydrogel was performed by suspending chitin hydrogel in alkaline/alcohol solutions before being subjected to SPP. The alkaline concentration and plasma treatment time were varied in order to obtain chitosan with high values of degree of deacetylation (%DD). It was found that the %DD of plasma-treated chitin hydrogel was increased from 35.79 % to 74.44% after repeatedly deacetylating chitin hydrogel in 10 % KOH/MeOH solution for 5 cycles using the operating condition as follows: the frequency of 15.0 Hz, primary voltage of 2.24 kV, pulse width of 2μs, and electrode gap of 1 mm. The reaction time for each cycle was 1 hour and the KOH/MeOH solution was changed every cycle in order to achieve high value of %DD. The solubility of the deacetylated product having %DD of 74.44% in 1% acetic acid solution was 76.19%. The effect of plasma treatment on depolymerization of chitin hydrogel was investigated under acidic condition. The reduction in molecular weight was measured by gel permeation chromatography (GPC). Acid hydrolysis without plasma treatment was performed as a control.

**Acknowledgement**

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# Gene Transfection to Human Skin Cells by Microplasma Irradiation Using Microcapillary Electrode

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On a unique gene-transfection technique using plasma irradiation, we have developed spatio-temporally stabilized microplasma irradiation system by employing small capillary tube having combined functions of the gas nozzle and the HV (high voltage) electrode [1]. Using this technique, 60% transfection rate of pCX-EGFP plasmid into COS 7 cells, which were taken from African green monkey kidney, have been realized without necrosis. In this study, as a next step to the regenerative medicine, we have tried realizing gene transfection to human skin cells.

A 96 well plate was placed on the GND (grounded) electrode plate. The capillary HV electrode with outside diameter of 70 μm was located above them and was put into a target well. Sinusoidal voltage of 20 kHz was applied on the capillary electrode, which was pulse-modulated at a repetitive frequency of 25–30 pps with a duty ratio of 1%. Typical conditions of the voltage and the current were 10–20 kV (pp: peak to peak) and 5–100 mA (pp) respectively. The discharge gap was set at 1 mm. Plasma irradiation duration was varied from 0.1–0.5 s. Two kinds of human skin cells were employed. One was HaCaT cell, which is located in epidermis and the other was fibroblast cell, which is located in dermis. One of them was cultured on the 96 well plate and 4.0 μg / 4.0 μl of pCX-EGFP solution was dropped into each well. After 24 h culture following plasma irradiation, the number of living cells was counted and the transfection rate was estimated by fluorescence observation.

Figure 1 shows maximum GFP transfection and cell survival rates for COS 7, HaCaT and fibroblast cells. It is shown that the present method shows an ability to transfect plasmids into human skin cells under the similar condition for COS 7. Optimization of plasma irradiation conditions is going on in order to achieve high transfection and survival rates for human skin cells as for COS 7.

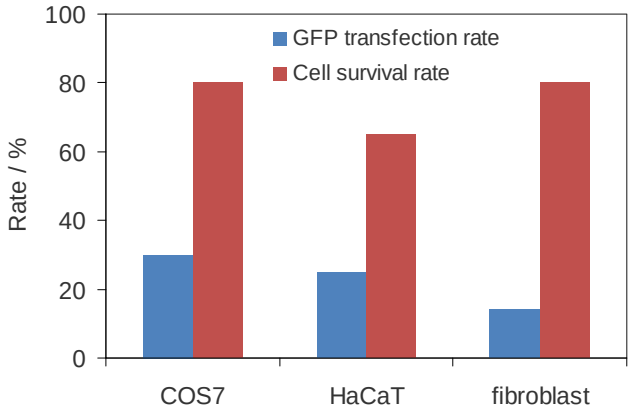


**Figure 1:** *Maximum transfection and survival rates for each cell*

This work was partly supported by the Grant-in-Aid (25108509) from JSPS.

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# Analysis of Plasma Irradiation Effect on Cell Membrane Using Artificial Cells

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On a unique gene-transfection technique using plasma irradiation developed by some of us (Sato *et al.*), in order to analyze the plasma irradiation effect on the cell membrane, artificial cells are synthesized, which simply consist of phospholipids bilayer and deionized water (see Fig. 1.a). The effect of plasma irradiation on the artificial cells was investigated by varying the phospholipids concentration.

Figure 1.b shows the outline of the experimental setup. A copper capillary of 70 µm of external diameter and 20 µm of internal diameter was placed 1 mm above the sample in a Petri dish containing artificial-cell solution, with different concentration of 2 and 5 mM for 1.2-Dioleoyl-sn-glycero-3-Phosphatidylcholine (DOPC) in 200 µl of deionized water. The capillary worked as a high voltage electrode, while the grounded electrode was a metal plate placed under the Petri dish. One-shot voltage of 3 kV was applied on the capillary with the duration of 1 µs. The fluorescence observation was performed before and after the plasma irradiation.

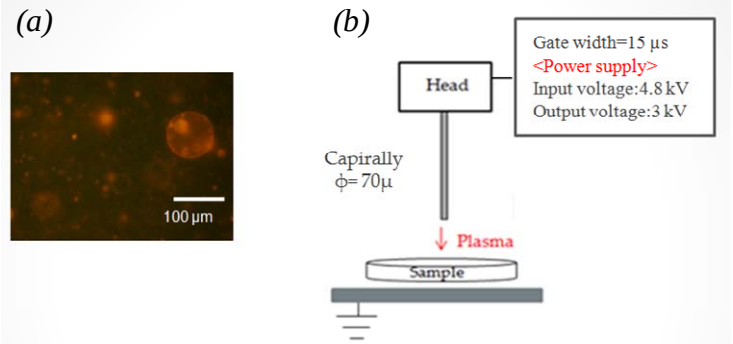


**Figure 1:** *(a) Fluorescence image of synthesized artificial cell and (b) experimental setup.*

Figure 2 shows the fluorescence observation obtained for two different phospholipid concentrations of 2 and 5 mM. The results showed coagulation for the higher concentration of 5 mM after plasma irradiation. This phenomenon was not observed for lower concentration of 2 mM. The results imply that the charge amount on the surface of artificial cells was changed by plasma irradiation. Analysis of the effect of the surface charge on the gene transfection is now under progress.

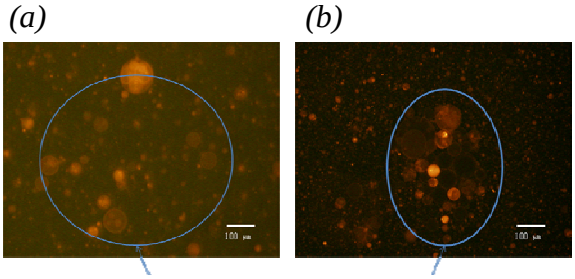


**Figure 2:** *Fluorescence images of the cells after plasma irradiation for (a) 2 mM and (b) 5 mM phospholipids concentrations*

This work was partly supported by the Grant-in-Aid (25108509) from JSPS.

# How to Improve the Reproducibility of Treatment Using Helium Atmospheric Pressure Plasma Jet (He-APPJ)

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Atmospheric Pressure Plasma Jets (APPJs) are used in various bio-medical applications such as living tissue sterilization and surface modification because profound radicals and charged particles can be transported remotely in air [1]. Plasma Jets are consist of discrete 'Bullet' and it propagates in ambient air with several km/sec velocity which is much faster than the speed of carrier gas, approximately a few m/sec. In detail, plasma is generated and maintained in the bullet so the propagation is closely related with the plasma generation mechanisms based on the penning ionization along the plasma jet plume. Bullet velocity along the plasma plume does not have constant value but distribution which was known as [2]. It corresponds to the ill-reproduced treatment using APPJs due to the absence of understanding the bullet velocity distribution. Therefore the formation of velocity distribution in space according to the substrate position should be reconsidered. Since the bullet velocity in ambient air is closely related to the mobility of local charges and electric field in the bullet region, the relation between the gas mixture condition of He(carrier gas) with $N_2/O_2$(air) and the induced E-field by the discharge in the source region and the accumulated charges on the target substrate should be understood.

Observation reveals that the bullet velocity distribution consists of three phases regardless of the ambient oxygen molar fraction: Phase I is the acceleration, phase II is relatively constant velocity and phase III is the deceleration. In phase I, bullet velocity increases exponentially due to penning ionization of $N_2$ and $O_2$ by $He^m$. In phase II charges accumulated near the electrode induces the opposite E-field on the bullet and it may reduce the bullet speed. In phase III, electron attachment by $O_2$ results in linear decrease of bullet velocity. When the metal grounded substrate located farther than plume length, the bullet velocity becomes faster and the phase II is shorter due to the electric field induced between bullet head and the image charges on the metal substrate. When the grounded metal substrate is in the plume, the bullet speed is increased by E-field induced by the image charges on the metal plate so the region II and III is disappeared. When the ground metal substrate is in the region of phase I where it is too close to the discharge source, the discharge in reactor is not stable and changed the discharge mode in plume. Consequently, the bullet delivers the radicals, charges, and the electric field on the target so its velocity distribution provides how to choose the location of treated substrate. Then we can expect what happens when the insulated substrate is located in APPJ plume and the results will be presented in conference.

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# Effect of voltage polarity and surface condition on active species production by an atmospheric-pressure helium plasma jet

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Atmospheric pressure plasma jets are getting much attention for biomedical applications such as cancer treatment, sterilization and wound healing. In those applications, active species generated by the plasma plays important roles. Especially, reactive oxygen species (ROS) are recognized to be main factors. Although it is widely know that OH, O and NO are important for plasma biomedical applications, their production mechanisms are still unclear [1] [2].

When the plasma jet extends toward a surface, discharge and ROS production vary from when the plasma jet extends into atmosphere. In plasma biomedical applications, it is estimated that the plasma jet extends toward surfaces: skin, organs and cell. However, many studies have focused on when the plasma jet extends into atmosphere. In this study, OH, O and NO were measured nearby surfaces using laser-induced fluorescence (LIF). The helium plasma jet consists of intermittent propagation of ionized front called “plasma bullet” [3]. When the plasma jet extends toward a surface, plasma bullet propagation is affected by surface condition, polarity of applied voltage and helium flow velocity. Since ROS production depends on the plasma bullet propagation, ROS amount is estimate to change depending on those parameters.

Figure 1 shows the plasma bullet propagation onto and along the glass surface. It shows that the plasma bullet propagates as surface discharge after it arrives at the surface. When the surface condition was changed, surface discharge propagation changed depending on wetness, roughness and conductivity of the surface. Meanwhile, ROS amount and provided region also changed. This result indicates that ROS production is affected by many parameters and condition in plasma biomedical applications should be deeply considered.

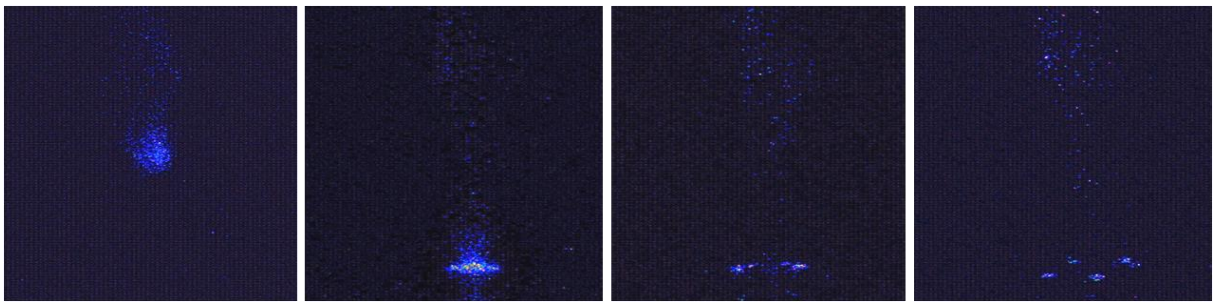

**Figure 1:** *Plasma bullet propagation onto and along the surface.*

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# Physical processes in the low-frequency nonequilibrium atmospheric plasma jets

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The field of plasma medicine has been significantly grown in recent years. This development is triggered by intensive study of non-equilibrium atmospheric plasma sources and their applications for biomedicine. Non-equilibrium atmospheric plasmas are traditionally utilized for sterilization and disinfection [1] [2]. More exotic applications include cancer treatment, skin dentistry, drug delivery, dermatology, cosmetics, wound healing, cellular modifications, etc. [1]-[5]. The non-equilibrium atmospheric plasma jet (NEAPJ) facility utilized at the George Washington University (GWU) demonstrated efficacy for treatment of various cancer types (lung, bladder, breast, head, neck, brain and skin). This work presents some insights on the physics of low-frequency nonequilibrium atmospheric plasma jets obtained using unique diagnostic facilities available at GWU.

Experimental facility created at GWU utilizes NEAPJ powered by about 20-30 kHz AC high-voltage source (up to 4-5 kV) [6] [7]. The NEAPJ uses central electrode with non-insulated metal tip on the axis of the discharge tube (4 mm outlet diameter) and grounded ring electrode. The device operates on helium (typically around 11-12 L/min) and produces 4-5 cm length jet. Diagnostic tools include Rayleigh Microwave Scattering facility, DC potential scattering setup, Rogowski coils, ICCD camera and optical emission spectroscopy [6] [7].

Transient dynamics of plasma and discharge parameters will be presented and physical processes involved in the discharge including streamer breakdown, electrical coupling of the streamer tip with discharge electrodes, discharge current spreading, factors determining NEAPJ length, streamer characteristics such as length, diameter and propagation path etc. will be analyzed [6] [7].

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# Effects of humidity on gas heating in atmospheric-pressure streamer discharge

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Gas heating in an atmospheric pressure streamer discharge was analyzed by a two-dimensional streamer discharge simulation model describing internal molecular energy transfer. Our two-dimensional streamer simulation model incorporates concepts from the fast gas heating mechanism proposed by Popov [1] and our self-developed state-to-state vibrational kinetics. To compute the streamer propagation in air, we use the first-order electro-hydrodynamic model for electrons and positive and negative ions in the framework of the drift-diffusion approximation. Detailed numerical techniques, including methods for solving transport and poisson, are given in our previous paper [2].

Figure 1(a) shows a diagram of energy relaxation processes between discharge products. The vertical axis corresponds to the enthalpy of formation of each molecule or atom. The energy stored in electronically-excited $N_2$ can be used to produce $O(^3P)$ through reactions R2, R3, and R6. In the same manner, the production of $O(^3P)$ can occur by the oxygen dissociation reaction, R4. $O(^1D)$, which is also produced by R4, is quenched to $O(^3P)$ through R1 in dry air. The energy stored in $O(^3P)$ is gradually released to form $O_3$ after the discharge. Since the $O_3$ enthalpy of formation is lower than that of $O(^3P)$, the energy difference is released through R5. On the other hand, in humid air, about half of the energy stored in $O(^1D)$ and a part of the energy stored in $O(^3P)$ are used to produce OH with accompanying exothermic energy release. As a result, humid air is more easily heated than dry air by an atmospheric-pressure streamer discharge.

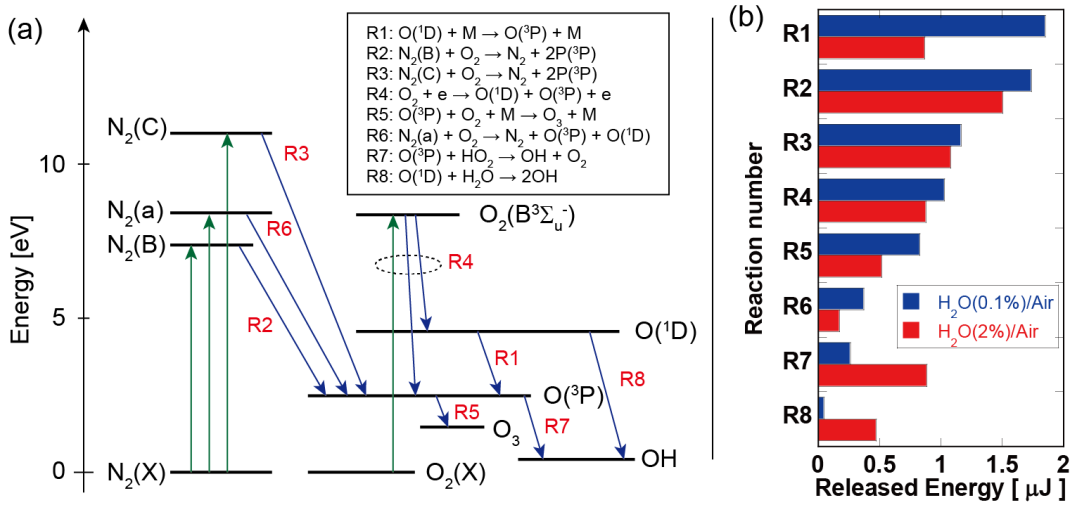


**Figure 1:** *(a) Diagram of energy relaxation processes of discharge products. (b) Contribution of each reactions to gas heating up to 3 μs for V = 24 kV.*

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# On the microwave plasma jet characteristics by impedance analysis

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Recently, atmospheric pressure plasmas are continuously expanding their distinct field in industries and medical applications. They are considered as fine alternative ways with efficiency and effectiveness. Among them, atmospheric pressure microwave plasma jets are one of the strongest tools for plasma bio-medical applications with its handiness and shock-free nature.

Diagnosis of plasmas has great importance for effective and safe operation of them. However it is quite difficult to diagnose the atmospheric pressure plasmas because of their high pressures which involve complex collisions of atmospheric molecules. Usually we use several optical methods to analyze the plasma discharges, but the scope of measurement is considerably limited.

The relation between some significant plasma parameters and plasma impedance was suggested by Spitzer resistivity model. De-embedding method, a new way of the plasma impedance measurement was implemented, which has its own advantages in simplicity and usability. It can provide us the indirect diagnosis of the plasma parameters via Spitzer resistivity.

In this study, we measured the impedance of the microwave plasma jet using de-embedding method. The characteristics of the microwave plasma jets in different discharge conditions were analyzed by adopting Spitzer resistivity. The experimental data was also compared with the computational simulation results.

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# Free Radical Generation by Cold Atmospheric Argon Plasma in Aqueous Solutions. An ESR Spin Trapping Study.

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The progress in understanding cold atmospheric plasma (CAP), together with the development of medical applications has been expected. Although CAP has been known to produce many reactive species in aqueous solutions, the mechanism in detail has been still unclear.

Here, electron spin resonance (ESR) and spin trapping were used to identify free radicals in aqueous solutions exposed to argon cold atmospheric plasma (Ar-CAP) generated by 20 kHz RF at 18 kV with Ar flow at 2 L/min. Characteristics of Ar-CAP were estimated by measurements of vacuum UV absorption and emission spectra. Hydroxyl ($OH^{\cdot}$) radicals and hydrogen ($H^{\cdot}$) atoms were detected and identified with 5,5'-dimethyl-1-pyrroline-N-oxide (DMPO) and α-4-pyridyl-1-oxide-N-tert-butylnitrone (POBN), respectively. The observed ESR spectra were compared with those obtained from radiolysis of air-saturated water. Relatively large quantity of DMPO-OH adducts was found in aqueous solutions exposed to Ar-CAP. When the formation of $OH^{\cdot}$ radicals was examined in the presence of rare gases, the yield was in the order of Kr > Ar =Ne > He, in almost an inverse accord with the ionization energy of rare gases.

The effects of Ar-CAP on aqueous solutions of DNA constituents, sodium acetate, an amino acid (L-alanine), and dimethylsulfoxide were also investigated by ESR and spin trapping using 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS) as a spin trap, to examine the possibility of detecting new radicals (e.g. typically methyl radicals in sodium acetate) which were reported to form in the high temperature interfacial regions induced by ultrasonic cavitation [1, 2]. However, at lower concentrations as well as at a higher concentration of these solutes, no evidence for specific new radicals was obtained, and only radicals formed by $OH^{\cdot}$ radical and $H^{\cdot}$ atom abstraction reactions could be detected. These results indicate that no pyrolysis radicals were formed in aqueous solutions exposed to Ar-CAP. The evidence for free radical formation in aqueous solutions by Ar-CAP will be discussed with respect to the biological effects and future therapeutic applications.

Acknowledgment: This study was in part supported by the grants from the Ministry of Education, Culture, Sports, Science and Technology, and Toyama Prefecture, Japan.

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# Influence of Spore Deposition onto the Dielectric Surface on the Mode of Dielectric Barrier Discharge

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Dielectric barrier discharge (DBD) is an easy and robust solution to generate low temperature discharge at atmospheric pressure and its biomedical applications have been intensively studied. However, the mechanism controlling the homogeneity of DBD is still controversial, especially in $N_2$-$O_2$ system. The homogeneous DBD has been achieved with pure $N_2$ and the transition from diffuse to filamentary mode at oxygen concentration about 500ppm has been reported by several groups [1]. However, homogeneous DBD at oxygen concentrations higher than 500ppm, even in air, has been also reported with organic plate [2] or porous alumina [3, 4] as barrier material. Therefore, it is reasonable to consider that characteristics of DBD are influenced by the microorganism on the dielectric surface and is investigated in this study.

The DBD device in this study consists of two gold electrodes (40mm x 40mm x 0.2um) deposited on dielectric plates. The barrier materials are quartz and alumina plates (50m x 50mm x 1mm) with or without spore deposition. Spores of Geobacillus Stearothermophilus ATCC7953 (5-20 x $10^6$ spores) are deposited on the quartz plates. The distance between two dielectric plates was 1mm. The DBD was driven by a sinusoidal voltage (frequency 1-5 kHz; peak-to-peak amplitude 10–15 kV; Trek Model 20/20C) and monitored by digital oscilloscope (Tektronix DPO3012) with current and voltage probe (Tektronix TCP0030 and P6015A).

Figure 1 shows current and voltage oscillograms of DBD. The diffuse and filamentary modes are clearly seen in pure nitrogen (a) and dry air (b), respectively, without spore deposition. The current oscillogram of spore-deposited DBD in air system is shown in Fig. 1(c). The number of microdischarges is slightly reduced and homogeneity of DBD is improved with spore deposition onto the dielectric materials. The morphological change of treated spores has been investigated by means of optical microscope and SEM.

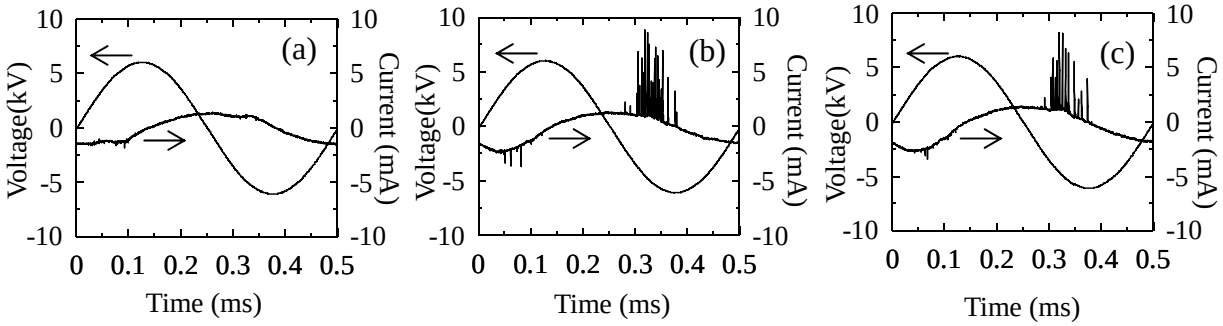


Fig. 1 Current and voltage oscillograms of DBD in (a) pure nitrogen and (b) dry air, respectively, without spore deposition and (c) dry air with spore deposition.

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# Relation between Plasma Plume Density and Helium Gas Flow in Atmospheric Pressure Plasma

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Recently, plasma techniques under atmospheric pressure have been adopted for industrial, biological, and medical applications. Atmospheric pressure plasma of dielectric barrier discharge is intermittently generated using a dielectric, rare gas, and metal electrode by applying RF high voltage [1]. A quartz tube is used as a dielectric and plasma is released into the atmosphere. Plasma irradiation is applied for material processing without thermal damage under atmospheric pressure. A plasma plume is small bullet-like volume of plasma traveling at unusually high velocities. The plasma density is an important factor to examine the mechanism of plasma generation in such atmospheric pressure plasmas. In the development of industrial, biological, and medical applications, it is necessary to express the values of the plasma current and plasma density for an effective plasma supplement for materials. The plasma density is estimated from the plasma current, the plasma drift velocity, and the current cross-section. The plasma plume current is estimated from the difference in currents on the circuit and the plume drift velocity is measured using a photodetector [2]. In addition, the release of the plasma plume into the atmosphere is influenced by the gas flow state [3] and the behavior of plasma plume is related to the hydrodynamic instability [4]. The gas flow velocity is related with dynamic pressure and the gas pressure is related with density. It is necessary to examine experimentally the relations among the plasma plume density, the gas flow state, and the gas flow velocity.

In this study, we will describe the experimental results on atmospheric pressure plasma using a quartz tube, helium gas, and copper foil electrodes by applying RF high voltage. To study the properties of the plasma plume, the relation between the plasma plume density $n_{plu}$ and the gas flow velocity $v_{gas}$ is examined with various tube inner diameters. It is found that the dependence of the density on the gas flow velocity has relations of $n_{plu} \propto \log(v_{gas})$ [5]. The plasma plume density in the atmosphere increases with increasing the dynamic pressure. However, the plasma plume density in the laminar flow is higher than that in the turbulent flow because the drift velocity in the laminar flow is slower than that in the turbulent flow. The energy loss from electric charge to frictional force in the turbulent flow is larger than that in the laminar flow, because the propagation of the plasma plume in the turbulent flow is complicated.

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# Generation of reactive species in water exposed to low-temperature atmospheric-pressure plasma jets

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Nonequilibrium atmospheric-pressure plasma jets typicallyh can generate highly chemirally reactive speceis in air at low-gas temperature and therefore are widely used for low-temperature treatment of material surfaces and modification of biological systems such as sterailization [1], blood coagulation, and wound healing. In many biological applications, plasmas are not directly in contact with living bodies and reactve species generated by plasmas reach them through a liquid such as cell culture medium or a body fluid. Recent numerical simulations for reactive oxygen species (ROS)/reactive nitrogen species (RNS) in puter water have indicated that highly reactive species such as OH radicals are typically convereted to less reactive species (such as $H_2O_2$) before diffusing through the liquid [2]. In this study we have measured concentrations of reactive species using colorimetric methods for ceratin chemical reactions and compared the results with those of numerical simulations. For example, Figure 1 shows $H_2O_2$ and $NO_2$ concentrations in 2 ml pure water as functions of He plasma jet irradiation time.

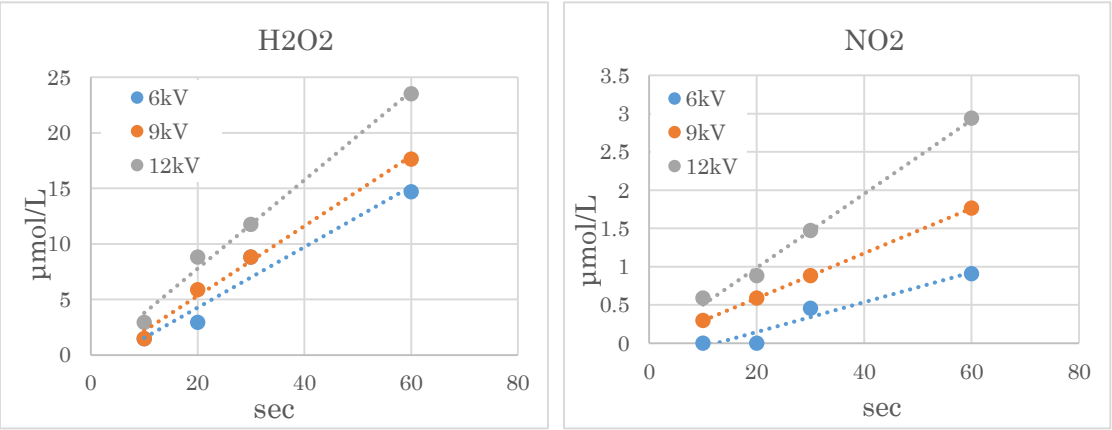


**Figure 1:** *$H_2O_2$ and $NO_2$ concentrations in 1 ml pure water as functions of He plasma jet irradiation time at various applied voltages.*

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# Effect of Plasma Jet on Carbohydrate Derivatives

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Recent studies demonstrate that the atmospheric-pressure plasma jet promotes the wound healing after operations, the mechanism of which is not yet known. It is known that the surface of human cells are coated with dense cluster of carbohydrates called glycocalyx, which is mainly composed of proteoglycans and glycoproteins. Thus, it is probable that the initial event in the wound healing is the interaction of plasma itself or radicals produced in the humor with the cell surface glycocalyx.

As the carbohydrates on glycocalyx are highly heterogeneous, it is very difficult to analyze their structural changes caused by the plasma jet experimentally. A simpler model would be required to clarify the effect of plasma jet on the carbohydrates. In this paper, we prepared several carbohydrates and their mimics, such as D-glucose and methyl-D-galactoside, and plasma jet was irradiated to these samples. We will report on the structural changes of these samples, which were analyzed by NMR and mass analyses.

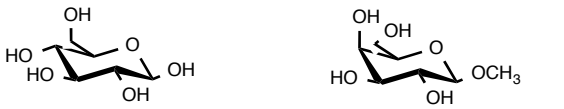


**Figure 1:** *Carbohydrate samples used for plasma irradiation*

# Evaluation of Cell Growth on Nanostructured and Functionalized Polystyrene

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In recent years there is a focus to the influence of surface structuring in the nano- and micro scale to cell growth characteristics. Because of transparent properties polystyrene is a well-established material for cell culture and tissue engineering applications. Especially for light microscopy and fluorescence analysis the transparency is of prime importance. However, the basic material of polystyrene owns of a high hydrophobic feature, whereby a surface treatment before cell cultivation becomes essential. Therefore, for conventional cell culture consumables plasma treatment and collagen coating are current methods for permission of hydrophilic properties and cell adhesion. Because of the loss of transparency by the use of nano-structuring of polystyrene, it has not been established for commercial cell culture products at all.
In this project, methods for nano-structuring and functionalization of the growth surface of cell culture products under prevention of the optical properties of polystyrene are presented. Plasma surface modification is performed by low pressure plasmas using different processing gases. The surface nano-structures are generated by an ion assisted deposition process [1]. Additionally a combination of the nano-structuring and subsequent functionalization was investigated regarding cell adhesion. The effectiveness has been pointed out by cell culture experiments with fibroblast cell line 3T3 as well as embryonic lung cell line MRC5. The cell growth parameters are investigated and evaluated in 6 well plates over a culture period of 12 days under standard culture conditions.
Surface modifications with these methods were successfully performed. UV-Vis and FTIR spectroscopy shows the transparency before and after treatment. Further surface analysis experiments as XPS, AFM and SEM are evaluated regarding cell adhesive properties.
Analysis of the growth curves of both cell lines shows that the surface modifications by both, nano-structuring and functionalization are performed successful regarding cell adhesion and growth velocity.
Nano-structuring by plasma etching and plasma functionalization of polystyrene surfaces causes improved surface properties regarding cell adhesion, while keeping material transparency. With the conducted methods cell growth surfaces were generated out of untreated polystyrene raw material which offers good cell adhesion for both standard cell line and less adhesive cells like MRC5. This may constitutes to a novel surface modification able to implement into industrial production of cell culture products.

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# Local Injection using Reagent-laden Micro-bubbles

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We have successfully carried out local reagent injection using electrically induced bubble knife. The air-liquid interfaces of the dispensed bubbles have novel characteristics which can carry the various kinds of the materials in different phase and viscosity of solution. This technique has advantages in high efficiency of injection of limited amount of valuable samples.

Conventionally, limited method was available for the high efficient injection. For example, the cell wall of the plant cell was too hard to enable to make a hole for injection [1]. The authors were invented an electrically induced bubble knife [2], which can produce directional high-speed mono-dispersed micro-gas bubbles (Fig 1(b)) whose diameter was less than around 10 μm. For the present study, we utilized the air-liquid interface of bubbles to carry various kinds of reagent or particles for the versatile gene transfer technique. The concept and experimental setup were shown in the Figure 1(a). First of all, a single bubble was produced by local heat and electrolysis due to by the electric discharge. Then mono-dispersed directional bubbles are created. Finally, simultaneous cell ablation and injection can be achieved by a crush of high-speed bubble with cavitation and by the diffusion of reagent simultaneously. Figure 1(c) shows the methylene blue-laden bubble transportation in medium.

The current technique contributes to the various gene transformations for a wide range of cells. Due to the large stiction force at the interface of bubble, a wide range of materials can be transported without damage.

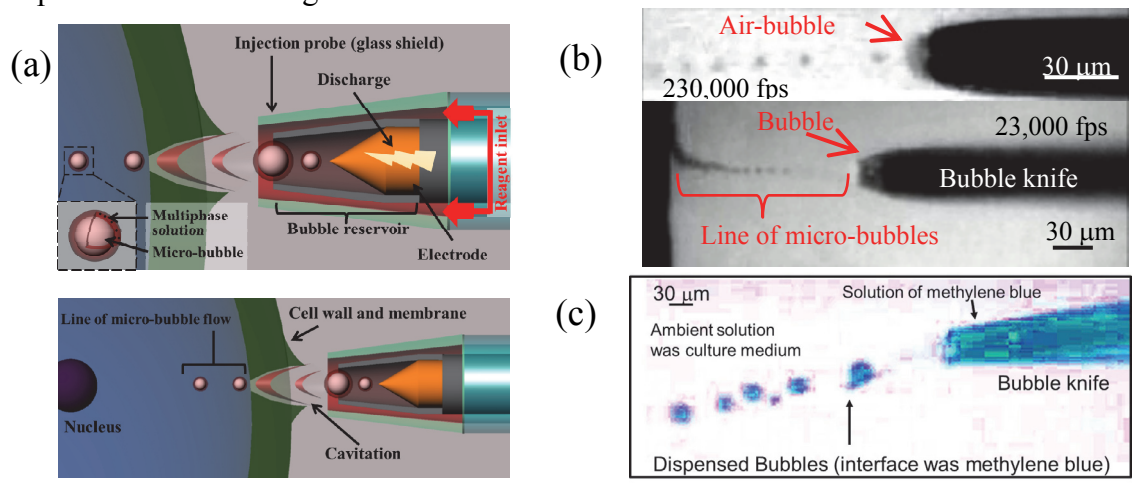


**Figure 1:** *(a) Concept of the reagent injection by electrically driven bubble knife, (b) dispensing of directional micro-bubbles, (c) dispensing of reagent-laden microbubbles*

## References

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# Plasma Surface Functionalization of Graphite-Encapsulated Gold Nanoparticles for Bio-medical Application

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Gold nanoparticle (AuNP) is used widely in biological and medical fields such as bio-sensing, bio-imaging, drug delivery, photothermal cancer therapy and so on. Usually the AuNP is prepared in solutions by chemical reactions. Surface modification is necessary for AuNP to improve its properties in biological and biomedical use. In this paper, we report a different way to fabricate the graphite-encapsulated gold nanoparticle (Au@C NP) and modify its surface by low pressure plasma processings. One of our research goals is establishing a selective virus detection system using the specific antibody-immobilized nanoparticles. Experiments devices and methods have been described in details in our previous papers. [1, 2] Firstly, Au@C NP was fabricated by the DC arc discharge method. Secondly, the nanoparticles were collected and placed into the RF plasma device for surface modification. After surface modification, the Au@C NPs can be immobilized by designed specific biomolecules, through which nanoparticles were examined for the practical biological and biomedical use.

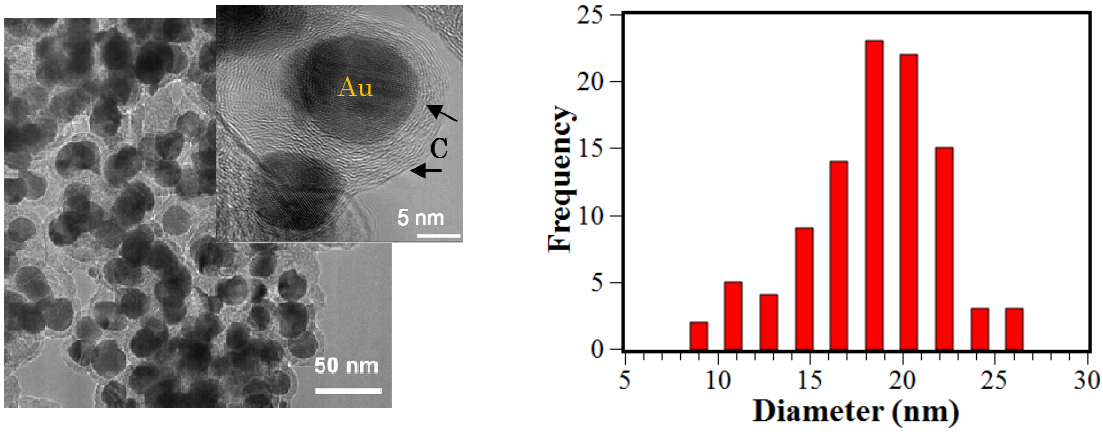


**Figure 1 a)** *TEM image and* **b)** *Size distribution of as-fabricated Au@C NP.*

So far we have successfully demonstrated the fabrication of Au@NP by DC arc discharge method and the surface modification of them by RF ammonia plasma processing in which the amino groups have been grafted onto the surface of the nanoparticles linking with the carbon atoms. [1] Characterization including the SEM, TEM, XRD, and XPS of the as-fabricated and functionalized Au@C NPs has been done to analysis the morphology, size distribution, crystallite and surface component information respectively. Figure 1 shows part of our experiment result for the as-fabricated Au@C NPs. We are now investigating the feasibility of immobilizing some biomolecules onto the aminated nanoparticles, which has been already performed on the graphite-encapsulated magnetic nanoparticles in our previous research.[2] More detailed experiment results will be presented at the conference.

**References**

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[2] T. E. Saraswati, A. Ogino, M. Nagatsu, Carbon 50 (2012) 1253.

# Surface Functionalization of Graphite-encapsulated Magnetic Nanoparticles with Amino Groups Using RF Excited Ar/$NH_3$ Plasma

Han Chou[1], Enbo Yang[2], Anchu Viswan[2], Kazunori Koga[3], Masaharu Shiratani[3], Masaaki Nagatsu[1,2]

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## 1 . Introduction

The graphite-encapsulated magnetic nanoparticles (GEMNPs) have a great advantage to control them by external magnetic force because of their excellent superparamagnetic property. Recently, various bio-medical applications with MNPs, such as drug delivery system and MRI contrast agent, have been extensively investigated. To use the MNPs for bio-application, it is generally required to modify the surface of nanoparticles by grafting relevant functional groups such as carboxyl group or amino group, etc. In this study, we present the characteristics of surface functionalization of GEMNPs with amino groups by using RF excited Ar/$NH_3$ plasma under various plasma treatment conditions. We also present some preliminary results of plasma diagnostics during the plasma-particle interaction and aging effect of surface-aminated GEMNPs.

## 2. Experimental setup

We used an inductively coupled RF plasma device to study the surface modification of GEMNPs. The plasma discharges are operated at a gas pressure of 50 Pa and RF power of 80 W, where the reflected power was adjusted to almost zero by matching box. The amino groups surface modification has been made via two steps; the first step is Ar plasma pre-treatment to activate the surface of graphite layers and second one is Ar/$NH_3$ plasma post-treatment to introduce the amino groups.

## 3 . Result and conclusion

To study Ar plasma pre-treatment effect, we changed treatment time at a constant RF power of 80 W. Figure 1 shows the result of $I_D/I_G$ intensity ratio measured by Raman spectroscopy and the number of amino groups per nanoparticle. Figure 2 shows the RF power dependency of amino group population. According to these results, it is found that the numbers of amino groups have a strong correlation with the treatment time of Ar plasma pre-treatment and the RF power in the $NH_3$ plasma post-treatment. At the conference, we will present the experimental results of the optical emission measurement during the plasma-particle interactions and the aging effect of aminated GEMNPs.

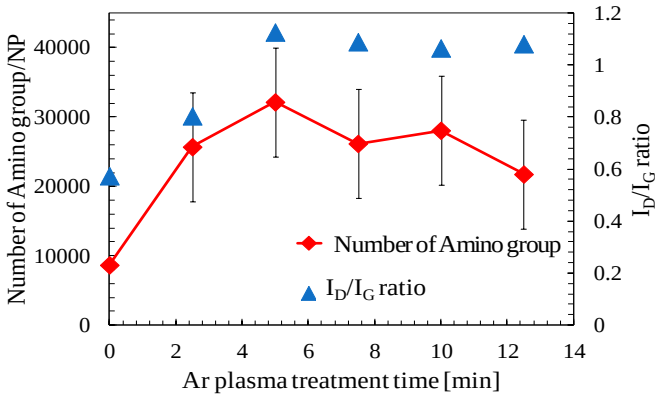


**Figure 1** *Effect of Ar plasma pre-treatment time on surface defect and amino group population per particle.*

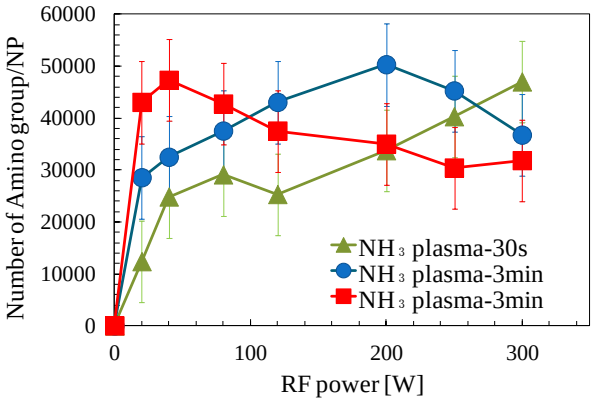


**Figure 2** *RF power dependency of amino group populations for different treatment times.*

# Investigation of the effectiveness of a *Gatling machine gun*-like plasma source for biomedical and materials treatment applications

S. Bianconi[1], F. Cavrini[2], V. Colombo[1,2], M. Gherardi[1], R. Laurita[1], A. Liguori[1], P. Sanibondi[1] and A. Stancampiano[1]

[1] *Department of Industrial Engineering (D.I.N.), Bologna, 40131, Italy*
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A particular type of plasma source, reminding the shape of a Gatling machine gun, has been recently developed to generate high intensity, high ionization rate plasmas at atmospheric pressure [1]. Gatling sources are an array of plasma jets, adjacent to one another, that rely on jet-to-jet coupling to merge plasma plumes in a single combined intense jet [2] [3].

The Gatling plasma source adopted in this work is an array of seven PTFE tubes, one in the center and six surrounding it, having inner-outer diameters of 1-1.6 mm. The tubes are arranged in an axisymmetric structure. The source is driven by a generator producing pulses with a rise time of 9 ns and peak voltages in the range 7-20 kV into a 100-200 Ω load impedance. As working gas, 99.999% pure helium is used.

Gatling sources are known to exhibit both an uncoupled mode and a coupled one. As shown in Fig. 1, a transition from uncoupled (Fig. 1a) to coupled mode (Fig. 1b) occurs changing GFR from 8 slpm to 2 slpm, with a constant peak voltage (PV) of 28 kV and a 476 Hz pulse repetition frequency (PRF).

In the present work, effects of Gatling plasma source for biomedical and materials treatment applications, focusing on bacterial growth inhibition and material properties modification, are investigated. From the achieved results, a 60 s treatment (PV: 27.2 kV, PRF: 476 Hz) of B. atrophaeus, enables a growth inhibition area with diameter 4 mm and 8 mm for uncoupled (GFR: 5.5 slpm) and coupled mode (GFR: 2 slpm), respectively. Moreover a reduction of WCA is evaluated with respect to pristine (107°) when treating (PV: 28 kV, PRF: 476 Hz) for 1 s silicone films in uncoupled (GFR: 5.5 slpm; WCA: 77°) or coupled (GFR: 2 slpm; WCA: 50°) mode.

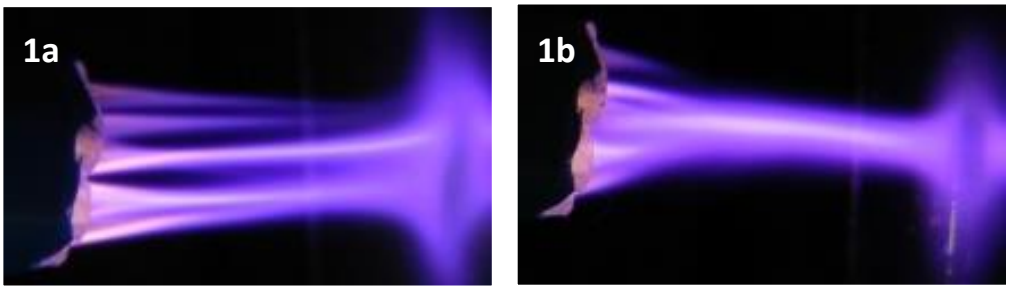


**Figure 1:** *Low-speed images of the Gatling source in uncoupled (1a) and coupled (1b) modes*

**References**

[1] Kim, J. Y. Kim, D. Y. Kim, J. Ballato, Appl. Phys. Lett., 101, 173503 (2012)
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# Experimental characterization of a coaxial microwave plasma source and efficiency on microbial surface decontamination

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For surface treatment, uniform high density plasmas are necessary in order to obtain homogeneous effects. In this context, Sairem SAS designed a new Electron Cyclotron Resonance coaxial 2.45 GHz microwave plasma source named *Aura-Wave* with very high performances [1] in terms of gas pressure range, plasma sustaining minimum power and operating frequency range. In this study, an experimental characterization of *Aura-Wave* was performed and a preliminary evaluation for bacterial decontamination efficiency was done.

The *Aura-Wave* ECR coaxial microwave plasma source was designed to sustain microwave plasma over several decades of pressure (from $10^{-4}$ mbar to a few $10^{-1}$ mbar), to avoid inside power-losses and to be matched with no additional impedance tuning system [1]. This source (fig. 1) was implemented in our laboratory setup allowing pumping, gas flow monitoring and plasma diagnostics. Plasma characterization was performed using optical spectrometer and linearly driven double Langmuir probe. These measurements provided basic plasma characteristics - i.e. plasma optical emission, plasma homogeneity, electron temperature, charged species densities and plasma potential - in the chamber. The experiments were done for different gas types - argon, helium, air -, pressures and injected powers (up to 200 W). The experimental results will be presented according to the variation of the above parameters.

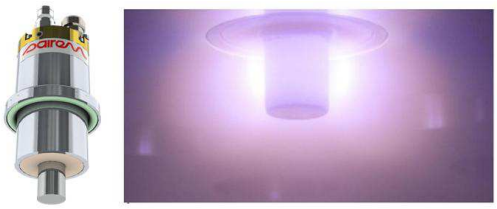

Figure 1: *Aura-Wave ECR coaxial microwave plasma source in argon at low pressure.*

For the evaluation of bactericidal efficiency, glass samples were used as carriers. Each carrier was inoculated with approximately $10^6$ spores of *Bacillus atrophaeus* (NRRL B4418, Steris Corporation) and was left to dry overnight before the plasma exposure. After the treatment, the spores were resuspended in phosphate buffered saline. Serial dilutions 1:10 (x4) of each sample were plated in duplicate on trypticase soy agar. The plates were incubated at 35°C for 48 h and then assessed for growth [2]. A comparison between the survival rates of *B. atrophaeus* spores obtained for different plasma parameters will be presented and discussed.

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# Sterilization of *Escherichia coli* by atmospheric pressure plasma irradiation using superimposed waveform pulsed-power generator

Satoru Hida [1], Yuki Kondo[1], Kiyoshi Ohnuma[1], Kazumasa Takahashi[1], Toru Sasaki[1], Tsukasa Aso[1], Takashi Kikuchi[1], Nob. Harada[1]

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Methods to sterilize bacteria are heat, ultraviolet irradiation, and chemical processes, conventionally. Atmospheric pressure plasma generates dense active species without vacuum system. The plasmas are applied to various fields such as environmental, surface, and medical treatments [1]. In comparisons with the conventional sterilization methods, the plasma irradiation is expected to be rapid and continuous processing without heat damage. However, the sterilization mechanism of bacteria using the plasma irradiation is unclear due to the complex processes. We consider the processes using a controllable pulsed-power supply based on high power semiconductor switches. The purpose of this study is to investigate the sterilizing effect on *Escherichia coli* (*E. coli*) by the superimposed waveform produced from the pulsed-power supply.

Schematic diagram of the experimental apparatus is shown in Fig. 1. The experimental apparatus consists of the controllable pulsed-power supply or an inverter power supply, a dielectric made by alumina, cultivated *E. coli* on a dish (ϕ60×25 mm), and supply system for helium gas to generate atmospheric pressure plasma. The voltage waveform of controllable pulsed-power supply consists of an ignition pulse of 14 kV with 200 ns and a sustained pulse of 1 kV with 100 μs. Water depth of the culture medium is about 10 mm (15 ml). The concentration and sterilization of *E. coli* were evaluated with the spectrophotometer and its growth rate, respectively.

The inactivating effect of *E. coli* due to the high voltage power supplies is shown in Fig. 2. The results indicate that *E. coli* is sterilized by the plasma irradiation. From the Fig.2, the inverter power supply well sterilized *E. coli*. To consider the sterilizing effect for *E. coli*, we will demonstrate the dependence on the pulse shape from pulsed-power generator.

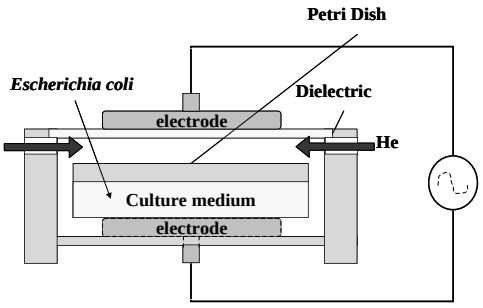


**Figure 1:** *Experimental apparatus*

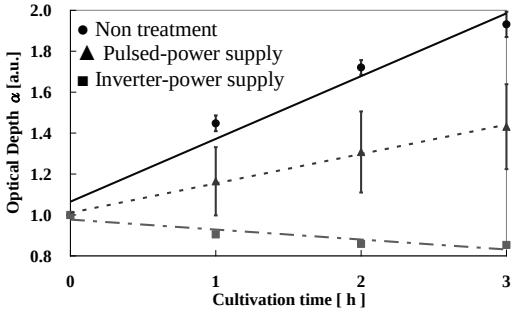


**Figure 2:** *Inactivation characteristics of E. coli*

## References

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# Generation of Multiple Plasma Plumes and Biomedical Applications in an Atmospheric Pressure Plasma Jet Array

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An atmospheric pressure plasma jet array source driven by a pulsed bipolar wave of several tens of kilohertz was designed and characterized. Plasma plumes were generated and propagated to grounded-surface relatively uniformly in entire single jets without an auxiliary circuit. The optical emission intensity and discharge current of the plasma jets were observed to be stable along the array axis. The plasma jet array consists of 16 tungsten pin wire electrodes, Teflon tubes, quartz tubes (3 mm inner diameter and 5 mm outer diameter) with pencil-shaped nozzle (1 mm inner diameter at the exit). This source was assembled with each individual module. Thus, it is possible to adjust the area of treatment by arranging the number of jets. In plasma plume, the temperature was less than 32 ℃.With an increase of the applied voltage, it was increased from 31.3 ℃ to 36.9 ℃. This structure has the potential to greatly enhance the scale of surface treatment over that of a single plasma jet and the gas temperature is low enough to treat heat-sensitive materials or biomedical samples. To examine the involvement of plasma in mitochondria-dependent cellular response, we evaluated whether plasma-induced ROS production also occurs in human rho 0 cells. Rho 0 cells depleted of mtDNA were developed by long term culture with ethidium bromide. To verify loss of mtDNA encoded protein, the expression of mtDNA-encoded cytochrome c oxidase (COX II) was assessed by western blotting.

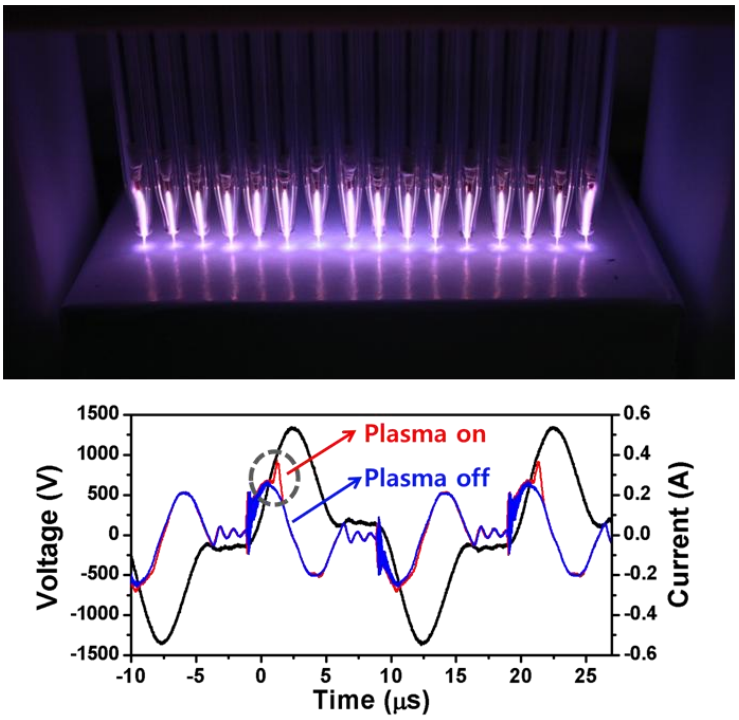


**Figure 1:** *The photograph of a plasma plume and waveforms of the voltage and the total current.*

# Study on Low Temperature Plasma by using Pulse Mode SSPA

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For the bio-plasma applications such as sterilization of medical instruments, high-precision surgery, cells treatment and deactivation of bacteria and viruses, the microwave source has been developed in recent years [1] [2] [3]. High efficiency solid-state power amplifiers(SSPA) using GaN HEMT and MOSFET has been designed for the generation of high quality bio-plasma. Typically, the temperature of the plasma using microwave is very high. For the medical application, the temperature must be no larger than 40 degrees Celsius.

The overall diagram of plasma experimental setup is shown in Fig. 1. We measured temperature of plasma by heat paper in various conditions and confirmed condition of low temperature plasma generation. When temperature of plasma is over 40 degrees Celsius, the color of the heat paper begins to change. As a result, when the peak power is 40 W and the base power is 15.8 W, the temperature of the plasma is below 40 degrees Celsius in Ar gas flow rate 3.5~5.0 lpm. Otherwise, when the peak power is 200 W and the base power is 14.5 W, the temperature of the plasma is below 40 degrees Celsius in Ar gas flow rate 3.0~3.5 lpm.

This work was funded by the National Foundation of Korea (NRF).

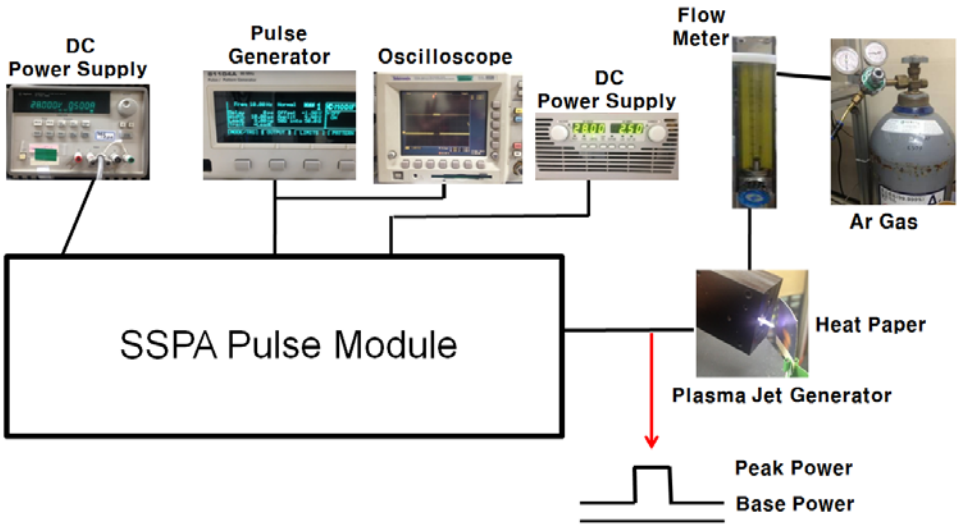


**Figure 1:** *Overall Experimental Setup*

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# Characteristics of Nonthermal Plasma source in Various Liquids

Seung-Ju Lim [1], Boo-ki Min [2], Jong-Yong Lee [1], Nathan Taylor [3], Seung-Oun Kang [2], and Eun Ha Choi [1,2,3]*

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Recently non-thermal plasma has been frequently applied to various research fields. The liquid plasma have received much attention lately because of interests in surgical [1] and nanomaterial synthesis applications [2]. Especially, intensive researches have been carried out for non-thermal plasma in liquid by using various electrode configurations and power supplies [3,4].

We have developed a bioplasma source which could be used in a liquid, in which outer insulator has been covered onto the outer electrode. Also we have also put an insulator between the inner and outer electrode. Based on the surface discharge mode, the nonthermal bioplasma has been generated inside a liquid by using an alternating current voltage generator with peak voltage of 12 kV under driving frequency of 22 KHz. Here the discharge voltage and current have been measured for electrical characteristics. Especially, We have measured discharge and optical characteristics under various liquids of deionized (DI) water, tap water, and saline by using monochromator. We have also observed nitric oxide (NO), hydrogen peroxide ($H_2O_2$), and hydroxyl (OH) radical species by optical emission spectroscopy during the operation of bioplasma discharge inside various kinds of DI water, tap water, and saline. Here the temperature has been kept to be 40 ℃ or less when discharge in liquid has been operated in this experiment. Also we have measured plasma temperature by high speed camera image and density by using H-alpha Stark broadening method.

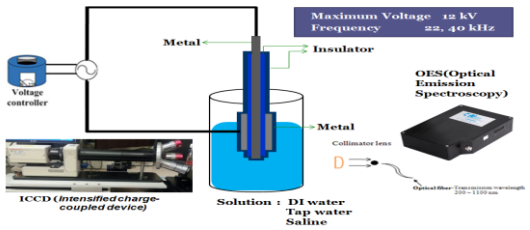


**Figure 1:** *Experimental setup for bioplasma source.*

**Acknowledgments**

This work was supported by the National Research Foundation of Korea (NRF) Grant funded by the Korean Government (MEST) (No. 2010-0029421).

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# Characteristics of Bovine Teeth Whitening in Accordance with Gas Environments of Atmospheric Pressure Nonthermal Plasma Jet

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**Abstract**

If hydrogen peroxide concentration is too high for treatment of maximized teeth whitening effect [1], it is harmful to the human body [2]. To the maximum effective and no harmful teeth whitening effect in a short period of time at home, we have observed the whitening effect using carbamide peroxide (15%) and a low-temperature atmospheric pressure plasma jet which is regulated by the Food and Drug Administration.

The gas supplied conditions of the non-thermal atmospheric pressure plasma jet was with the humidified (0.2~1 %) gas in nitrogen or in air at gas flow rate of 1000 sccm. Also, the measurement of chemical species from the jet was carried out using the optical emission spectroscopy (OES).

Evidence of increased reactive oxygen species was compared with that of non-humidified plasma jet. We have found that the whitening effect of the plasma is very excellent through this experiment, when bovine teeth are treated in carbamide peroxide (15%) and water vapor (0.2 to 1%). The brightness of whitening teeth was increased up to 2 times longer in the CIE chromaticity coordinates. The colorimetric spectrometer (CM-3500d) can measure color degree of whitening effect.

Non-thermal atmospheric pressure plasma jet with humidified gas resulted in effective tooth whitening on bovine tooth for the case of either with low concentration of carbamide peroxide or without cabamide peroxide. It is found in this study that the plasma jet is more effective than conventional LED method.

The white bleaching could be optimized when the plasma jet with inclusion of humid air has been employed along with the cabamide peroxide rather than the conventional LED along with the carmaide peroxide.

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# Investigation of Sterilization Effect with Low Pressure RF Oxygen Plasma

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Authors have researched metal-oxide material coating with low pressure oxygen plasmas. However because biomedical plasma applications have attracted much attention these days and it is required to establish novel applications of our RF plasma, it is attempted to investigate a feasibility of sterilization with our device in this study [1] [2].

The used device consists of a quartz discharge tube and a stainless-steel chamber. The quartz discharge tube is 50 mm in outer diameter and 400 mm in length, and dimensions of the stainless-steel chamber are 216 mm in outer diamter and 400 mm in length. After the device is evacuated by a turbo molecular pump with an oil-free scroll vacuum pump, operating gas, which is pure argon, nitrogen, oxygen and their mixtures, is fed and goes through the tube into the stainless-steel chamber. Total gas flow rates are less than 100 sccm, and the pressure of inside the device is about $10^{-1}$ Pa. A three-turn coil antenna is set around the tube and 13.56 MHz electric power (CW) is supplied to it via a matching system from a power supply. Typical value of the input electric power is 100 W. Plasma is generated under the coil anntena region and flows into the chamber.

Sterilization effect of the generated plasma is investigated with culture of *Geobacillus Stearothermophilus* (ATCC7953). A bacterial spore test strip (Mesa Laboratories, Inc.), whose spore population is about $10^4$, is placed in the quartz discharge tube or the stainless-steel chamber and exposed to the generated plasma. The exposured duration is changed from 5 min to 30 min. After the treatment, the test strip is transferred into a prepared culture media (Triptic Soy Broth with Bromocresol Purple manufactured by Mesa Laboratories, Inc.) and incubated at 50 degrees centigrade. If the sterilization efficacy is sufficient, little spores survive and the color of the culture media remains purple because the bacteria can not multiply in the culture media enough to vary its appearance. In contrast, when the sterilization is incomplete, the culture media changes from purple to yellow. The media is typically discolored between 18 and 19 hours later with no treated spore strip.

The spores used here are still alive after they are left in the circumstance at base pressure of $10^{-3}$ Pa. It is also found that the sample, which is placed in the stainless-steel chamber, is not influenced by the plasma and thus the culture media changes its color after the same time as with no treated one. On the contrary, when the test strip is at the edge of quartz discharge tube and exposed to the plasma including oxygen, some culture results show that beginning of color variation of the media delays compared to that of no treated spore strip case. Terms of the delay distribute between several hours and several days. This result indicates that some spores certainly can be killed or inactivated by the plasma treatment, although the sterilization is not complete yet under these experimental conditions.

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# Development of a Hand Sanitizer Employing Non-thermal Plasma Activated Mist

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As the use of alcohol-based hand sanitizers has become more widespread within hospitals to control methicillin-resistant Staphylococcus (MRSA), Pseudomonas, and other multidrug-resistant bacteria, reliance on the more time-consuming and often less convenient practice of hand-washing with soap and water has declined. Unfortunately, alcohol-based hand sanitizers do not kill the spores of *Clostridium difficile*, and the use of soap and water for hand washing is therefore recommended during the care of patients with *Clostridium difficile*-associated disease (CDAD) [1].

The degree to which such recommendations are complied with and the resultant effectiveness of this practice has been found to be suboptimal [2]. In addition, since patients can be colonized with *Clostridium difficile* in the absence of recognized CDAD, they may unknowingly serve as a source for spread to other patients if hand washing with an alcohol-based product is routinely used in their care.

Non-thermal plasma (NTP) has been shown to be an effective agent in the killing of a number of vegetative bacterial species [3] as well as the spores of Bacillus species and *Clostridium difficile* [4]. Therefore, the routine use of NTP for hand hygiene in the hospital setting would be an attractive means by which to ensure control not only of the many bacterial pathogens killed by alcohol-based hand sanitizers, but also of *Clostridium difficile*.

We have developed a method to deliver NTP antimicrobial elements via an aerosol. The reactive oxygen species (ROS) synthesized in NTP can be transferred to water droplets in fractions of a second. The antimicrobial properties paired with the xerographic characteristics of the charged droplets result in uniform coverage, independent of droplet size, with a 5-log kill of microbes. Our device will be comprised of 3 stages and will reduce both the cost and treatment time of producing a NTP disinfectant device. In stage 1, an atomizer vaporizes liquid water into a fine mist. The droplets are then infused with antimicrobial ROS in stage 2 as it passes through a dielectric barrier discharge (DBD) plasma. Stage 3 will evenly dispense the antimicrobial mist onto the user's hands. Due to the xerographic nature of the charged mist, hand rubbing is not required to ensure uniformity, as it is with alcohol sanitizers and conventional hand washing.

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# Low Voltage Plasma-on-a-Chip for Infection Treatment

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We report a plasma-on-a-chip (POC) which provides stable atmospheric plasma for infection treatment. The POC was fabricated using micromachining techniques and nickel electrodes are integrated on a glass substrate with micron size gap (Fig. 1). A glow discharge was obtained across 280 um gap at a very low voltage(~400 V) by injecting initiation carriers prior to the discharge. It is found that the injection of the initiation carrier can significantly lower a gas breakdown voltage. An array of the integrated electrodes enables a well controlled initiation carrier injection and it can also provide a stable two dimensional plasma source for medical applications. Non-thermal atmospheric plasma was demonstrated using a pulsed glow discharge and the electron density of $5x10^{13}$ $cm^{-3}$ in the plasma was obtained. A photographic image of the generated atmospheric plasma is shown in Fig. 2. Configuration of the electrodes allows delivery of reactive ions to the surface to be treated without a strong gas blow, offering an alternative to a plasma jet. Inactivation of an anti-biotic resistant bacteria (Methicillin-Resistant Staphylococcus Aureus) was experimentally tested and the measured inactivation rate exhibits a strong dependence on the plasma exposure (Fig. 3).

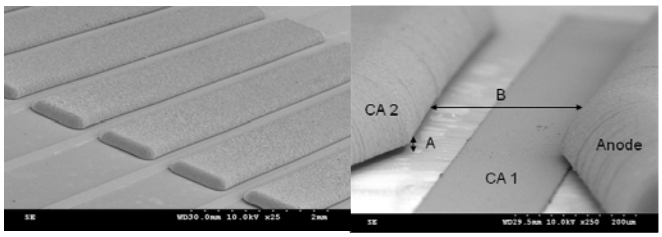


**Fig.1:** *Fabricated Plasma-on-a-chip. CA1:cathode1 and CA2:cathode2*

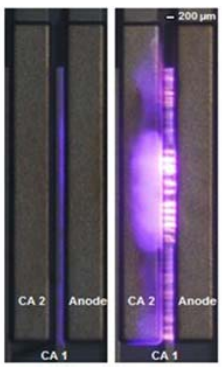


**Fig. 2:** *Generated atmospheric plasma*

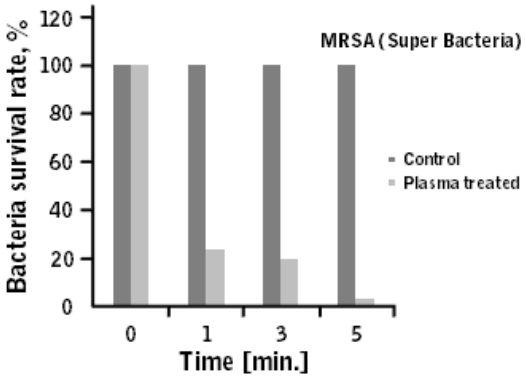


**Fig. 3:** *Inactivation of an anti-biotic resistive bacteria*

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# Bladder cancer cell lines cultured by plasma treated cell culture medium

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Atmospheric-pressure plasmas (APPs) are widely used in many biomedical applications due to their non-thermal characteristic [1]. APP jet is well kwon as a kind of APPs. It can do localized plasma process and having a direction to deliver secondary reactive species which generated in an interaction between emerging charged species and ambient air [2]. Here, we modified cell culture medium using an APPJ as shown in figure 1. Here, plasma source is consisted of a glass capillary (2.4 mm inner diameter) and a copper ring electrode, and it working with a bipolar high voltage pulse of 7 $kV_{p-p}$ at a fixed low frequency of 10 kHz. Helium gas was fed into the glass tube with a fixed flow rate of 5 L $min^{-1}$ (gas speed of around 20 m/s). The optimized cell culture medium was prepared based on Dulbecco's modified eagle medium (D-MEM) supplemented with 10% fetal bovine serum (FBS). Average pH value of around 7.6 was measured. The 3 mL medium in a-6-cm petri dish was treated by APPJ with a fixed distance for 3 min.

For the cell culture in the plasma treated medium, both bladder cancer cell lines (253JB-V and T24) were incubated at 37˚C with 5% $CO_2$ and 95% air over a day passage cycle. About $10^4$ cells of each cell line were cultured in the medium and the cultured cells were counted after 3, 6 and 24 hours, respectively. As results, the death of over 60 % 253JB-V cells in plasma treated medium were observed within 3 hours while it took 24 hours in case of T24 cells. It will be discussed that the possibility of an APPJ generated reactive oxygen and nitrogen species (ROS/RNS) in the medium and cells got damaged by the radicals [3].

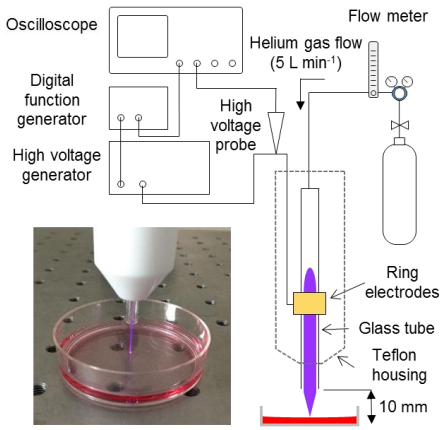


Figure 1

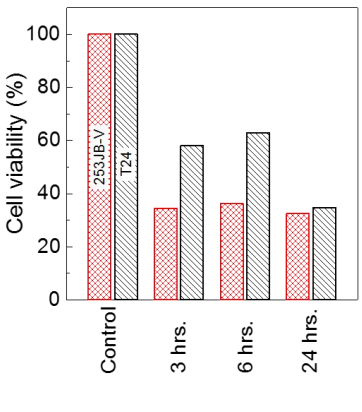


Figure 2

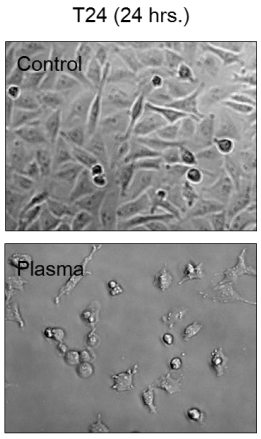


Figure 3

**Fig. 1** experimental setup for plasma treated cell culture medium for 3 min. **Fig. 2** shows cell viability. **Fig. 3** phase contrast images of T24 cell lines are cultured for 24 hours.

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# Diagnostics of a low power inductively coupled plasma source for potential biomedical applications

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Low power inductively coupled plasma (ICP) sources integrated with a quenching device for the efficient production of reactive species at atmospheric pressure have been recently developed for potential biomedical applications [1]. The aim of this work is to characterize the ICP source with and without quenching device in terms of discharge behaviour, effluent temperature and fluid-dynamics, reactive species and UV radiation production, thus providing fundamental data and insights useful for the application of the ICP source in biomedical treatments.

The adopted ICP torch is sustained by a 1 kW-13.56 MHz power generator and can be operated with argon/air mixtures. The quenching device placed at the torch outlet consists of a dielectric tube, with suitably designed air injection ports and an exit orifice for the gaseous effluent.

Ignition transients, discharge behaviour and flow fields downstream the plasma source have been investigated using high speed imaging equipment and a Z-type optical setup for Schlieren imaging (see Fig. 1), whereas the temperature of the substrate treated by the source effluent has been measured by means of fiber optic sensors. Moreover, reactive species produced by the plasma source have been investigated in the gas phase and in treated liquid samples. Finally, the emission spectrum of the ICP source in the UV-VIS range has been investigated with OES, while the spectrum integrated irradiance in the UV range has been measured by means of a calibrated sensor. Results are used to optimize the design of the ICP source for an effective production of reactive species, while keeping effluent temperature and UV radiation at values compatible with biomedical treatments.

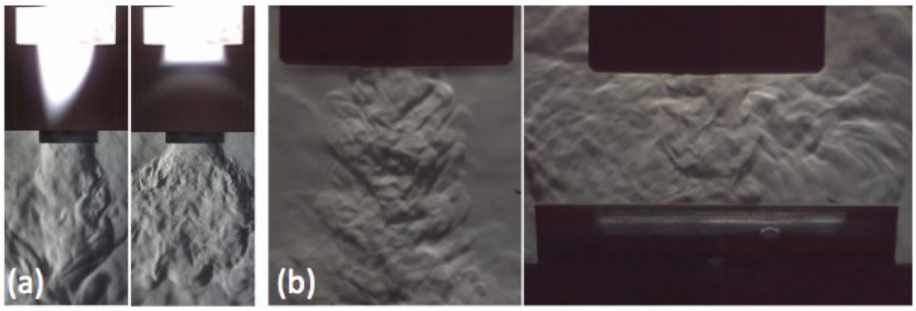


**Figure 1** *High-speed and Schlieren imaging of the ICP source without (a) and with (b) quenching device*

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# Atmospheric-pressure microplasmas for medical/biological applications

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In recent years, atmosphere-pressure cold plasmas were becoming increasingly important in the field of plasma medicine. Here, various hollow-core optical fiber based plasma generators were reported for medical/biological applications. The high-density and microns-sized plasmas were formed when the ionization energy was confined inside the hollow cores of the microns-thick fibers. The propagation of atmospheric-pressure microplasma results from the anode-driven pulse discharges with their durations of 30 ns – 15 μs. The pulse current density generated at an i.d. of 100 μm is about four magnitudes higher than the glow-like one at an i.d. of 2000 μm. These hollow-core optical fiber based plasma generators may be utilized to generate large-area and uniform atmospheric plasmas. These large area plasma generators including plasma brush and surface discharge plasma device were successfully used for the application in the plasma inactivation of *Candida Albicans* cells. The room-temperature air plasma plume consisting of well-aligned and stable microplasma jets can be formed in the vicinity of the ends of hollow optical fibers at atmospheric pressure. This plasma plume may lead to the uniform and large-area surface modification of PET polymers. The plasma plume may efficiently prevent the heat-sensitive polymers from being damaged and significantly affect the surface properties of treated polymers, such as surface chemical compositions, hydrophobicity, and biocompatibility. The microplasma in contact with water solution is highly efficient for killing various viruses in water. The hollow-core optical fiber based plasma device can be also used to form large-area and atmospheric-pressure plasmas in a sealed package for the low-temperature disinfection of *fungi*. The atmospheric microplasma is very effective in killing various funguses for plant disease management. The hollow-core optical fiber based plasma generators running without the complicated fabrication processes show their potentials for plasma inactivation, plant disease management, and surface modification.

# An atmospheric-pressure microplasma jet device with Ni-Co alloy electrode and glass insulator

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In recent years, microplasma jets for bio-medical applications have been reported [1]. However, the reported microplasma jet has a small diameter, so the area treated at once is small. To overcome this drawback, we developed a microplasma jet array device. In our former study, we used two metal electrodes and a porous alumina insulator [2]. However, the reliability of the nozzle is not satisfactory because to fabricate uniform porous alumina insulators is difficult.

We propose a microplasma jet array device composed of two metal electrodes and glass insulator in this study. The cathode and the glass insulator were fabricated by micromachining technology and the anode was fabricated with stainless steel as shown Fig. 1. To fabricated the cathode and the glass insulator, we used electroplating and sand-blast processes.

In the discharge test of the fabricated device, we applied bias voltage of 15 $kV_{p-p}$ at 15 kHz. With air and $N_2$ gas, the plasma jet was successfully generated. In order to characterize plasma jet, we obtained the electrical characteristics and optical emission spectra (OES) of plasma jet. From the electrical characteristics, we calculated the electron temperature and the electron density. The air plasma has the higher electron temperature than $N_2$ plasma while vice versa in case of the electron density. From OES results, we confirmed that air and $N_2$ plasma generate species related with oxygen and nitrogen.

We observed that microplasma jet array device having glass insulator limits the overcurrent leading to arc and generates stable and uniform glow plasma at atmospheric pressure.

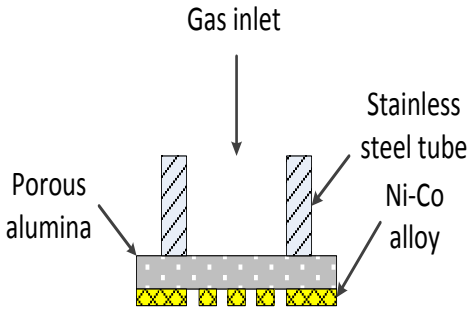


Fig. 1. The cross-sectional schematic view of microplasma jet device

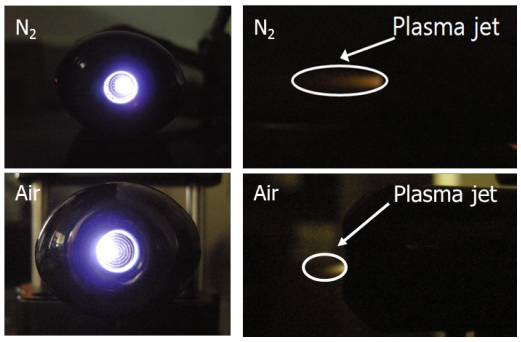


Fig. 2. Photo images of plasma jet for $N_2$ gas and air.

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# Property of OH Radicals Generated in $H_2O$ Dominant Discharge with Voltage Polarity on Power Electrode

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The non-thermal water plasmas are often adapted in the bio- or medicine applications, which plasmas are generated by the pin to water discharges or aqua-needle plasma discharges in water. In those applications, OH radical plays an important role in the bio-chemical reactions however, the thermal damages on the target material is often very serious. In this study, the radical generation and thermal property are investigated with varying the polarity of power electrode and the generation mechanism is considered. Since the water vapor are rich in the discharge region and they are polarized due to dipole and the resident electron attachment, the water particles are easily arranged by the electric field distributed in between the powered tip and the water surface. Then the discharge property is sensitive to the polarity of power electrode. Previous study reported that the discharge conditions, electrode voltage polarity and water mole fraction, change the rotational temperature of OH ($T_{rot\_OH}$) [2]. The time-varying OH generation mechanism in the ac-driven electrode is investigated with the 1-dimensional model considering $H_2O$ mole fraction profile, OH involved reations, rotiational energy state transfer in OH, particle densities, and particle temperatures. Experiments were carried out with the tip-electrolyte discharges generated by ±1 kV ac voltages on the metal tip and the 0.9 wt% of NaCl and KCl solutions are placed for the electrode. The time-resolved normalized optical intensity profiles of OH and $T_{rot\_OH}$ in plasma were measured by using the Intensified Charge Coupled Device and monochromator. The normalized OH densities with discharge conditions in the electrolytes are measured by using the Electron Spin Resonance (ESR). It is found that the mechanism of OH generation is temporally changed with ac voltage phase. The radical density and $T_{rot\_OH}$ have dependency of $H_2O$ vapor distribution above the electrolyte surface, resulting in the distributed OH density and the temperature along the discharge path. The ratio of OH density with voltage polarity ($n_{OH}$ at negative discharge / $n_{OH}$ at positive discharge) was ~6.5 from ESR and ~7.1 from optical intensity of OH. The uniform $T_{rot\_OH}$ of ~3500 K was observed in the positive discharge region and it was decreased to ~500 K at electrolyte surface. The joule heating by plasma electrons increases the temperature of OH while the quenching effect by $H_2O$ cooled the temperature of OH radical on the electrolyte surface. Two temperatures of OH radical also observed in aqua-needle-plasma in electrolyte. The thermal distribution in the tip discharge on the electrolyte will be analyzed with the observed results, which will be discussed in the presentation.

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# Spectroscopic Investigation of Nitrogen Radical Transport in Atmospheric Jet Plasma

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A 2.45 GHz microwave excited atmospheric jet plasma (AJP) has been characterized using optical emission spectroscopy and Langmuir probe diagnostics to determine the active species present and the electron temperature of the plasma, respectively. The steady state AJP implements a swirl gas system wherein argon serves as the main gas (3, 5, 7 LPM) and nitrogen acts as the swirl gas (1, 3, 5 LPM) that detaches the plasma plume from the discharge tube. The electron temperatures of the AJP for different discharge conditions were 0.38 eV at 5:1 LPM Ar:$N_2$ flow rate increasing up to 0.52 eV at 7:5 LPM Ar:$N_2$ flow rate, both at 600W microwave input power. The optical emission spectrograph revealed that the ambient air contributes to the detected $N_2$ species. Moreover, oxygen content is also evident for plasma with no swirl gas injection. The nitrogen swirl gas is observed to have an apparent effect in the N I and Ar I intensities as other discharge condition is varied.

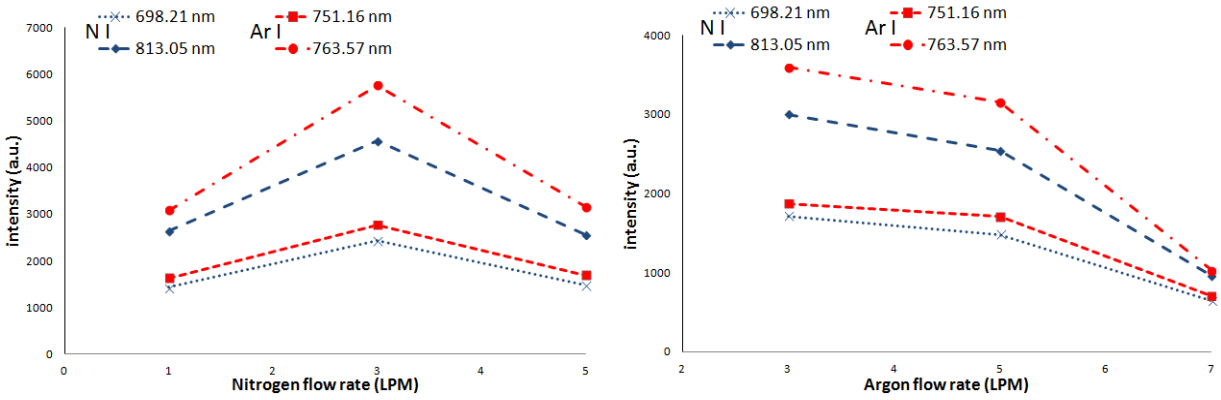


**Figure 1:** *N I and Ar I intensities plotted against a) varying nitrogen flow rate at constant 7 LPM argon and b) varying argon flow rate, constant 5 LPM nitrogen at 600 W input power*

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# Diagnostics of cold atmospheric pressure plasma generated by various plasma sources

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In this work we present methods and results of diagnostics of cold atmospheric plasma (CAP), and possible methods of a control and monitoring of its influencing factors, which allow analyzing a contribution of separate components of plasma torch in cumulative effect of CAP. In the experiments we used the argon microwave plasma sources and ferroelectric reactor. The results of probe and optical diagnostics of CAP under various regimes of plasma generation suitable for working with biological objects are presented. By optical diagnostics we obtained experimental data of spectral analysis of plasma flow, including emission and absorption spectra. As a result of spectral measurements we determined plasma components that are corresponded to different spectral line series. Also estimations of metastable argon concentrations in plasma torch, based on analysis of absorption spectra, are made. We obtained integrated absorption coefficient which is equal 1,65 x $10^{11}$ and the concentration of metastable argon, which is equal 19,4 ppm. As for probe diagnostics, we obtained distribution profile of electron concentration for different plasma source. Also a diagnostics of chemical composition of CAP, based on measurements of chemical gas analyzer, was conducted. We measured concentrations of active oxidizing agents in plasma, such as $O_3$, $NO_2$ and NO, in dependence on a distance from plasma torch. Also a power of SHF-radiation under various working regimes of generator was measured. The measurements were conducted with the use of SHF-analyzer, which allowed making a diagnostics in a wide range of wave lengths and radiation power. We studied a dependence of power flow density of SHF-radiation on power of magnetron in a range from 60 to 150 W. Obtained curve is linear with a good accuracy. A range of flux density is 0,005- 0,05 mW/cm$^2$. These values do not exceed permissible levels of radiation, which is 0,1 mW/cm$^2$, that is allowed during 2 hours under frequency 2,45 GHz. Finally, in this work we considered problems concerned with a selecting of physical factors that have the most sufficient bactericidal effect.

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# Characteristics of AC excited Non-equilibrium Atmospheric Pressure Helium Plasma Jet for Medical Application

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Non-equilibrium atmospheric pressure plasma jets (NEAPPJ) are frequently used for plasma bio and medical applications. The activated species generated by the plasma jet play important roles to react with biomedical samples. Therefore, higher density plasma jet sources are very important for realizing the higher performance. Our group has developed a 60 Hz alternative current (AC) excited Ar NEAPPJ with an electron density as high as $10^{15}$ $cm^{-3}$ [1]. Using the plasma jet, the selective killing of cancer cells [2], the inactivate spores of *Penicillium digitatum* [3], etc. have been successfully realized. In the bio and medical applications, it is required to reduce thermal effects from plasma jets and to realize a plasma jet which enables us to treat a small area of samples. Therefore, a new spot-size AC excited NEAPPJ with He gas as discharge gas have been developed in this study. Using the He plasma jet, we are investigating the effects of activated species on the interaction between plasma and biomedical sample on the basis of the results of plasma gas-phase measurements.

Gas temperature and electron density of the AC excited He NEAPPJ has been measured by optical emission spectroscopy. The discharge condition of the plasma jet was He gas flow rate of 2 slm and discharge voltage of 9 kV (20 mA). From results, the gas temperature and the electron density was estimated to be 343 K and around $10^{14}$ $cm^{-3}$ from $N_2$ emission spectra (2nd Positive System ($C^3\Pi_u$ - $B^3\Pi_g$), λ: 380.4 nm) and Stark broadening of $H_\beta$ (λ: 486.1 nm ). Moreover, activated species have been measured with absorption spectroscopy. Figure 1 shows $O_3$ density as a function of radical distance at 20 mm (the plasma edge region) from a gas nozzle of plasma head. Although the diameter of plasma jet was around 1 mm, the $O_3$ density was almost constant along the radial direction. It is considered that the $O_3$ which has a relatively long life time was generated by the engulfment of ambient air into the plasma jet, and then was transported to downstream region by the gas flow and diffused to the radial direction of the plasma jet.

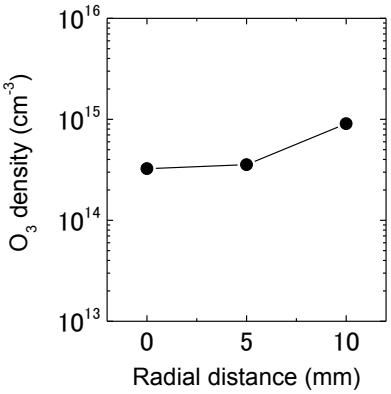


**Figure 1:** *$O_3$ density as a function of radial distance at 20 mm from the gas nozzle of plasma head.*

**Acknowledgements**
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# OH LIF for *in situ* plasma jet diagnostics

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The LIF technique and associated numerical model, including detailed vibrational energy transfer analysis, developed for years in Bari [1,2] for hydroxyl radical quantification in atmospheric pressure discharge has been applied for the challenging diagnostics of helium plasma jets expanding in ambient air.
The plasma jets were produced by the Plasma Gun (PG) [3] and special emphasis was paid for the OH LIF diagnostics in set ups close to those involved for biomedical applications. The key influence of the presence of a metallic target, mimicking to some extent the interaction of the plasma jet with living animals, but also of liquid targets having various conductivities on the OH spatial distributions and densities has been evidenced in agreement with preliminary approaches based on ICCD plasma imaging and optical emission spectroscopy.
Good agreement or at least strong correlation were found between emission and LIF diagnostics confirming the strong interplay between plasma and neutral helium flows but also the generation of upstream plasma following plasma jet impingement on conductive targets [4]. It has been evidenced that in most situations, complementary analysis appears as a unique tool to provide a comprehensive description of the spatial distribution of hydroxyl radical. Following a calibration experiment based on broad band absorption and validated for helium DBD plasmas, plasma jets LIF diagnostics results in the absolute OH density measurement, ranging from a few $10^{12}$ to $10^{14}$ $cm^{-3}$ with an accuracy of about 20 %.

The authors acknowledge supports from ANR BLAN093001 "PAMPA" and MP1101 COST STSM. DR is supported by CNRS and Région Centre.

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# Ion measurements of a cold atmospheric pressure plasma jet: The influence of ambient air humidity

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Charged particles, which are produced in an atmospheric pressure argon plasma jet interact with ambient air and generate nitrogen and oxygen reactive species (RNS & ROS) for instances the radicals $^{\bullet}OH$ (hydroxyl), $^{\bullet}NO$ (nitric monoxide), and $O_2^{\bullet-}$ (superoxide anion). These radicals are well known in biology and interact with eukaryotic cells to activate different cell signaling pathways. In order to have a close look at the reaction and signaling pathways, the plasma generated negative and positive ions were measured in the gas phase by means of molecular beam mass spectrometry (MBMS). Particularly the influence of ambient humidity was investigated, as it was depicted in the work of Winter et al. [1] that humidity has a large effect on the chemical composition in the liquid and on the biological response. The present work shows the presence of a huge variety of positive and negative ions, particularly the presence of water cluster consisting of an ion and several water molecules, e.g. $H_3O^+(H_2O)_2$ [2]. These water clusters have a long lifetime and could therefore transport the short living species to a target which is located at larger distances.

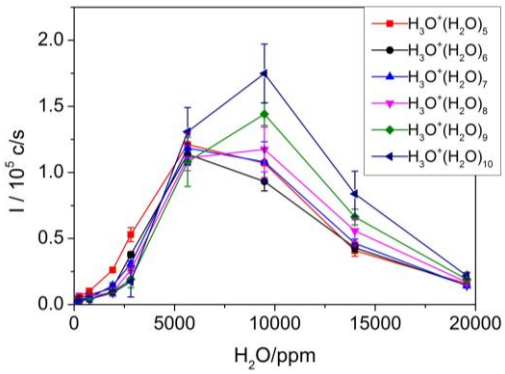


**Figure 1:** *Positive ion cluster produced by an atmospheric pressure argon plasma jet as a function of the ambient air humidity concentration. The different clusters show maxima at around 10000 ppm.*

It is shown that with an increase of ambient humidity the intensities of positive as well as negative ion signals change only slightly. However the intensity of the water cluster signal increases if the humidity level of the ambient air rises up until a maximum at a humidity level of 10000 ppm, see figure 1. To show resulting changes in liquids and cells, different liquid diagnostic (e.g. $NO_2^-$ and $NO_3^-$) as well as cell changes in the expression level of several oxidative stress and mitochondrial metabolism responsible genes (e.g. SOD3, IPCEF1, SDHB, NDUFS4) were performed.

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# Revealing the NO generation mechanism in a needle type plasma jet

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Nitrogen oxides ($NO_x$) are considered to be a key agent in plasma medicine applications, especially for wound healing. Although it remains to some extend unsure which beneficial processes are triggered by which specific type(s) of $NO_x$ in the biological samples, we see that all the different $NO_x$ molecules are initially created from NO in the gas phase.

Numerical simulations can be a very powerful tool for obtaining a detailed insight in the reaction chemistry of the plasma species. Note that, in our case, a large reaction set is required due to the complex background gas composition ($Ar/N_2/O_2/H_2O$) and more importantly, because we are interested in the biomedical species which are often not the main plasma components. As this increases the calculation time, we use a zero-dimensional (0D) fluid model, based on the GlobalKin source code [1]. The original model was adapted to mimic the operating conditions (*i.e.*, gas temperature, power deposition, humid air diffusion, gas flow velocity) of two needle type plasma jet configurations developed at the Eindhoven University of Technology by P. Bruggeman (see Figure 1). The total amount of power deposited into the plasma is equal for both plasma jet devices. Our calculations provide species densities as a function of the distance from the nozzle exit, among other information. Very good agreement was obtained between the experimental data and our numerical simulations. Furthermore, we will also show in detail which reactions are responsible for the NO production and how this chemistry is affected by the two different geometries.

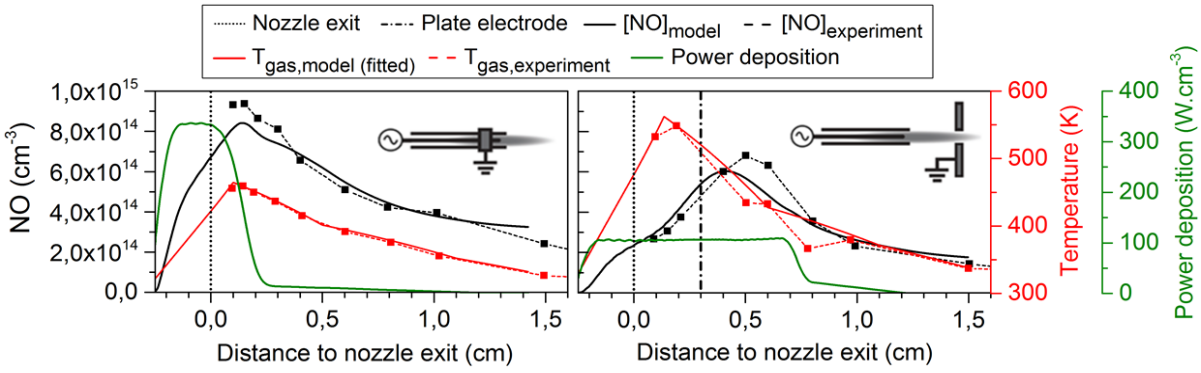


**Figure 1:** *NO density along the plasma jet, for two different geometries of the grounded electrode. The calculated densities are compared with experimentally data (taken from [2]).*

We acknowledge the Institute for the Promotion of Innovation by Science and Technology in Flanders for financial support and M. Kushner for supplying the numerical code.

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# Reactive Molecular Dynamics between Oxygen Radial and Phosphatidylcholine by Plasma Irradiation

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Biomedical applications of non-thermal discharge at atmospheric pressure have been developed on the basis of stabilization techniques of the plasma formation [1]. However, the molecular mechanisms in biological cells are not well understood. Recently, reactive molecular dynamics were reported with respect to the interaction of radical species and biological molecules [2] [3]. In the present work, the influence of the incident energy and direction of oxygen radial on the elementary process of reaction in a phospholipid was investigated with quantum mechanical molecular dynamics for the validation of plasma irradiation to cell membrane.

The bombardment of oxygen atom (O) to phosphatidylcholine (PC) was modeled as a basic interaction of plasma radical and biological surface. The structure of PC was optimized by energy minimization under general AMBER force field. The initial distance from O to PC was set to 6 Å. The incident energy of O was varied from 0 to 1 eV. The lateral and upper parts of PC were examined as to irradiation position. The analysis time was 10 ps. The force field of each time increment was derived using a semi-empirical molecular orbital method PM3. The molecular behavior was calculated with the molecular dynamics software AMBER12.

As is shown in figure 1 (a), a hydrogen atom was abstracted by O and a hydroxyl group was generated at low incident energy. Subsequently, the product was recombined with carbon chain of PC. On the other hand, a portion of PC was separated above incident energy of 0.7 eV (see figure 1 (b)). The energy threshold did not depend on irradiation direction under the present condition. These results suggest that the former reaction process is dominant in water at early stage of plasma irradiation, whereas the latter one is in air.

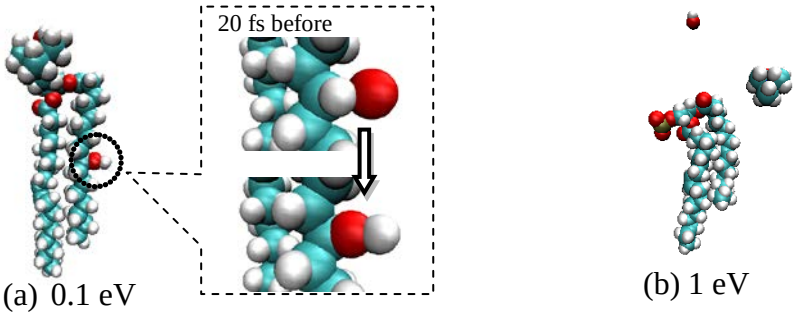


**Figure 1:** *Dependence of chemical interaction between O and PC on incident energy.*

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# The penetration process of gaseous reactive species into aqueous solution: A modeling study

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Although a great progress has been achieved for plasma medicine in the last decade, an fundamental problem, i.e. the interaction between gas plasma and living cells, tissues and organisms, is still far from understood [1,2]. Considering that these living substances are in moisture circumstance or even in aqueous solution, here we construct a model to study the penetration process of plasma-induced reactive species into aqueous solution.

For both ubiquity and simplification, a surface micro-discharge (SMD) in air and a petri dish of distilled water, 1cm depth and 1cm beneath the SMD, are modeled. A zero dimensional global model is constucted for the SMD, a 1-D diffusion model for the air gap between the plasma and the solution, and a 1-D penetration model for the aqueous solution. All these three models are integrated together for calculation, and in total 53 species and 776 chemical reactions are incorporated. For validation of the simulation results, the distribution of $O_3$ in the air gap is measured by absorption spectroscopy, and the density of $H_2O_2$ in aqueous solution after plasma treatment is measured by enzyme linked immunosorbent assay.

In the gas phase, it is found that $O_3$ dominates in both the plasma and the air gap. Due to the short life cycle, the diffusion distance of most species like O, OH, NO, $O_2(a^1\Delta_g)$, etc. is less than 1mm from the plasma. So, the main species which can diffuse longer than 1cm air gap and then penetrate into the aqueous solution are $O_3$, $H_2O_2$, $NO_2$, $HNO_3$, $HNO_2$, $N_2O_5$ and $N_2O$.

However, in the liquid phase the main reactive species are much different to its gas source. Many kinds of ionic species are generated due to 1) the self-ionization of water molecule and some reactive species like $HNO_3$ and 2) the corresponding chemical reactions. The penetration depth (d) of $O_3$ is just d<30μm, due to the fast chemical conversion to form $O_3^-$, OH, $HO_2$, etc. The density of NO is higher than 1nM and its penetration depth increases linear with time. The NO is totally generated in the liquid phase, indicating that some short-life species can be generated by in-situ reaction. This also applies to $O_2^-$. It is interesting that the diffusion process dominates the loss of $H_2O_2$ in the surface layer of d<20μm, but after that the chemical process dominates. For the first time, these results provide a spatial-temporal description of the penetration process of gaseous reactive species into aqueous solution, the key species and their dosages are quantified which may benefits biomedical applications.

This work was supported by the National Science Foundation of China (Grant No. 51307134), and the Fundamental Research Funds for the Central Universities.

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# Preservation of Fresh Food Using AC Electric Field

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Maintaining freshness for a relatively longer period is important for the preservation of foods. However, it is difficult to keep the freshness of perishables such as fish and shellfish. The drip, eluted from foods by defrosting, increases due to the tissue breakdown which is caused by cryohydrate in freezing cells. Such drips lead to the quality loss in foods.

Recently, an incubator using the AC high voltage for high-quality preservation of foods has become commercially available. Here, we report the effect of the AC high voltage on keeping freshness of foods using such an excellent incubator. In this study, purple sea urchin (*Helicoidaris crassispina*), which was one of popular foods for the Japanese people, was used as a specimen. The freshness was estimated by the amount of the proteins with the molecular weight of less than 70,000 included in the drip which eluted from samples, where the amount of proteins was evaluated using the most widely used technique for analyzing mixtures of proteins, called SDS polyacrylamide gel electrophoresis[1].

It was found that the amount of the protein with the molecular weight of less than 70,000 decreased by applying the AC high voltage as shown in **Fig. 1**. This result indicates that the degeneration in proteins is inhibited by the effect of the AC high voltage. Therefore, it is possible that the effect of the AC high voltage improves the technique keeping freshness because the degeneration in proteins is related to freshness of foods.

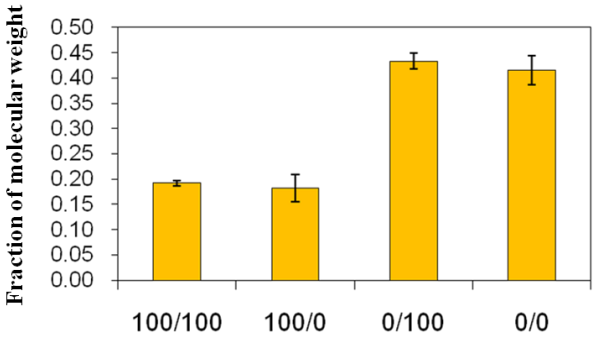


**Figure 1 :** *The amount of proteins with the molecular weight of less than 70,000 included in the drip which eluted from samples*.

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# Signaling circuits that are affected by plasma-activated medium in brain tumor cells

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We had previously developed NTP with ultrahigh electron density (approximately $2 \times 10^{16}$ $cm^{-3}$), and applied to therapy for ovarian cancers [1]. We also found that plasma-activated medium (PAM) selectively killed glioblastoma brain tumor cells and induced apoptosis [2]. Our goal is to understand the intracellular molecular mechanisms by which PAM induces apoptosis in glioblastoma.

There are many pathways that induce apoptosis in signaling circuits. Survival and proliferation signaling pathways are constitutively active in most cancer cells. These pathways inhibit apoptosis in cancer cells. We investigated several nodes in the signaling circuits, and we found that PAM down-regulated key factors in the signaling circuits such as AKT, MAPK (Erk1/2), and mTOR in glioblastoma brain tumor cells (Figure 1, [3]).

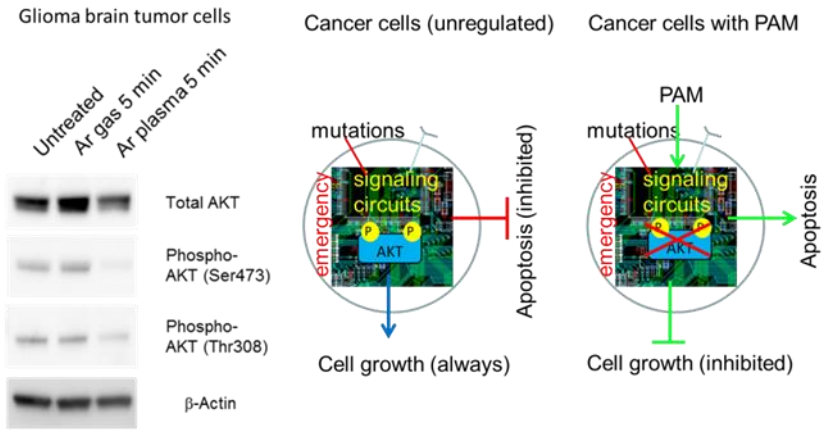


Figure 1: Survival and proliferation signaling circuits were down-regulated by plasma-activated medium in glioblastoma brain tumor cells.

PAM did not affect normal cells such as fibroblasts and astrocytes [2, 3]. These results suggest that PAM is a promising tool for cancer chemotherapy. Current trends in chemotherapy are molecularly targeted cancer therapy based on intracellular molecular mechanisms. We believe that these findings provide significant insight into the intracellular molecular mechanisms of cell death of cancer cells mediated by PAM.

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