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.

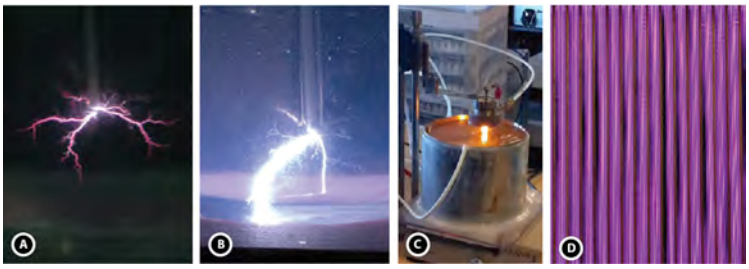

Fig.1. Photographs of four discharges used for water treatment: spark in water (A), pulsed arc in water (B), gliding arc plasmatron (C), and dielectric barrier discharge (D).

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.

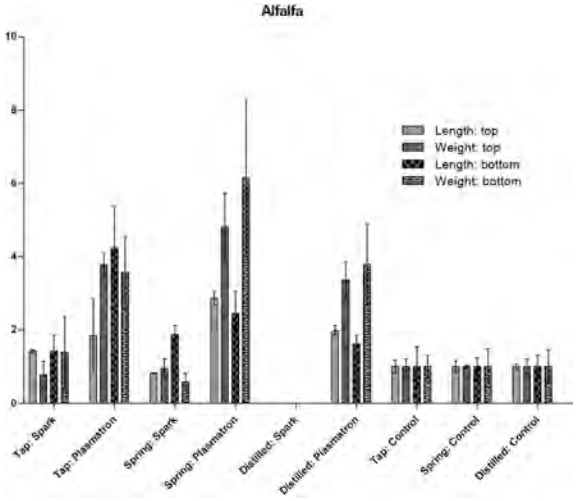

Fig.2. Results of length and weight of Alfalfa sprouts following treatment by plasma-treated water.

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.

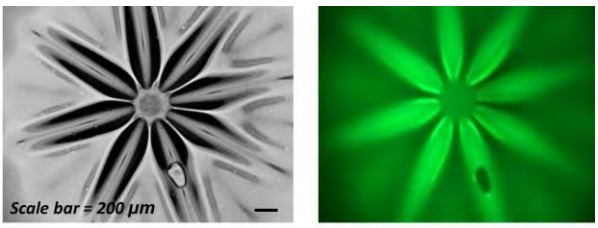

Figure 1: *Plasma jet treatment of the synthetic biological sensor resulting in a pattern of microchannels formed within the gelatin matrix (left bright-field image) enabling the direct plasma treatment of sensitive vesicles within the material (right fluorescence image).*

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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.

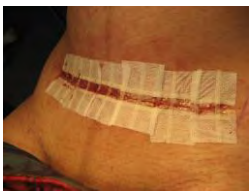

Figure 1: *Day of operation*

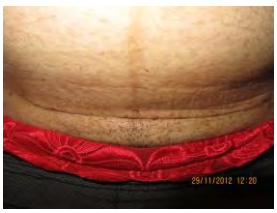

Figure 2: *Post-operative day 7*

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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.

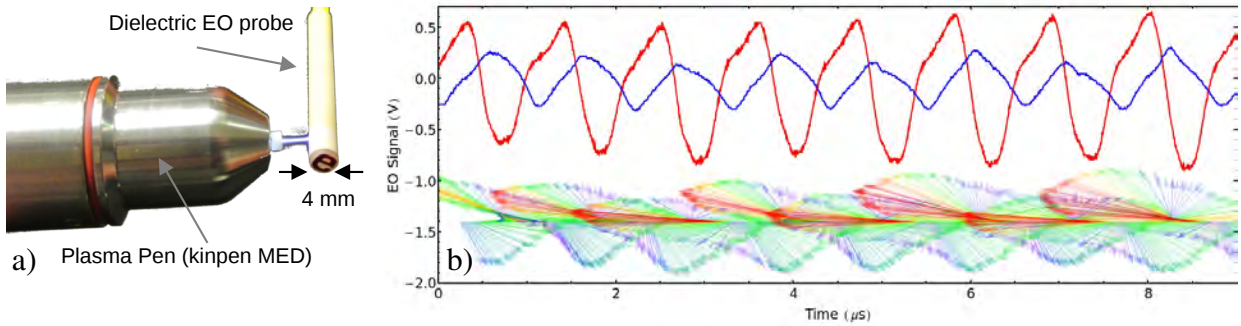

Figure 1: *Transient evolution of the E-field associated to the plasma jet. a) Photography of the experiment. b) Real time measurement of the two transverse components of the E-field vector. The reconstructed E-field vectors are given bellow the curves.*

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.

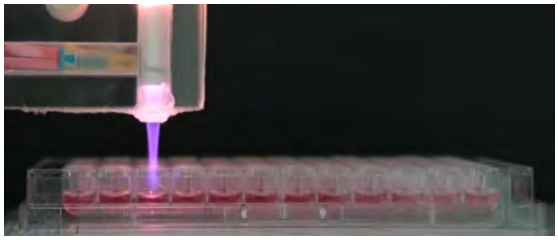

Figure 1. *Exposure to the He low temperature plasma jet of spheroid grown in 96-round bottom well plates*

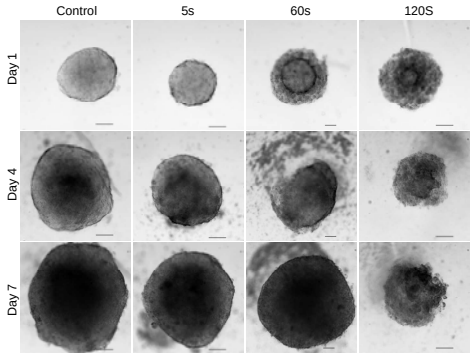

Figure 2. *Variation of HCT116 volume 1, 4 and 7 days after plasma exposure.*

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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.

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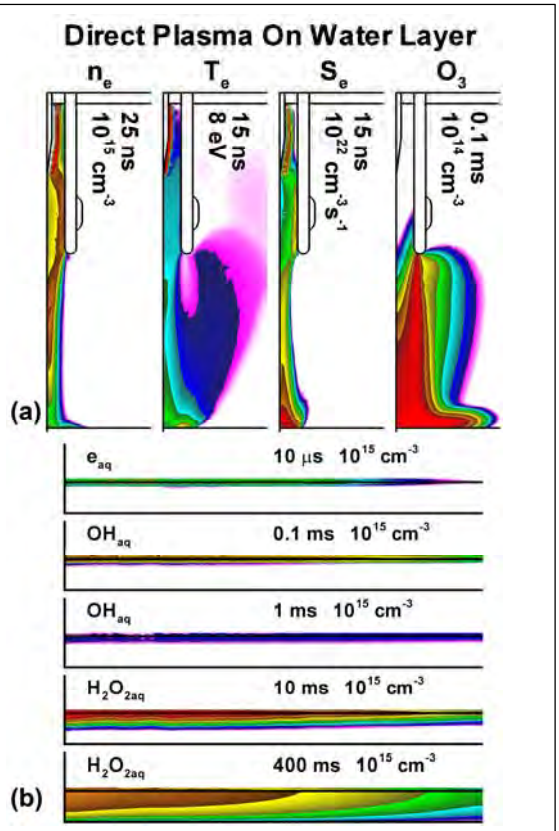

Figure 1: *He/O_2 plasma jet interacting with a water layer over tissue. (a) The effluent of the plasma jet. (b) Diffusion of species over time through the water layer.*

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.

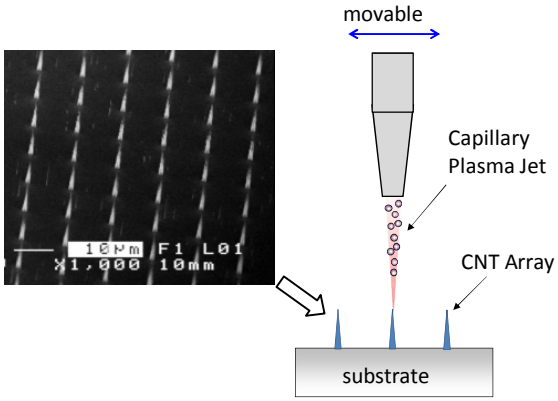

Figure 1. FE-SEM image of dot-arrayed CNTs for 1µm dot sizes and 10 µm dot intervals and schematic of surface treatment

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.

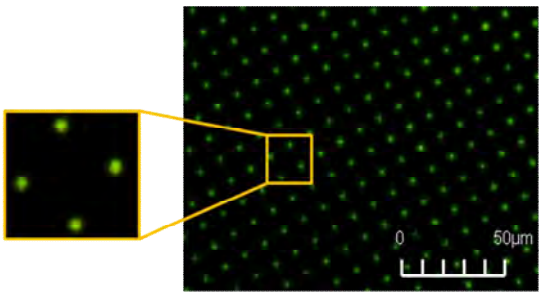

Figure 2. Fluorescent microscope image of the surface aminated dot-arrayed CNTs.

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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.

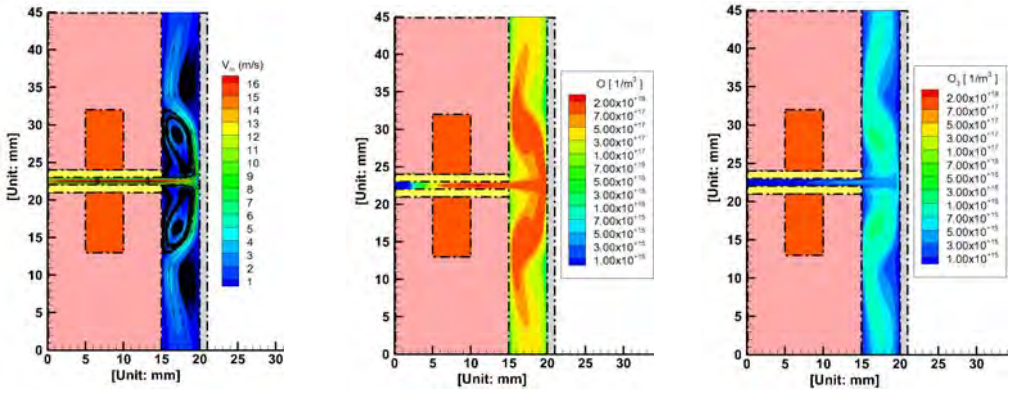

Figure 1: *Typical results of a planar DBD jet impinging on a flat surface.*

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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.

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

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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.

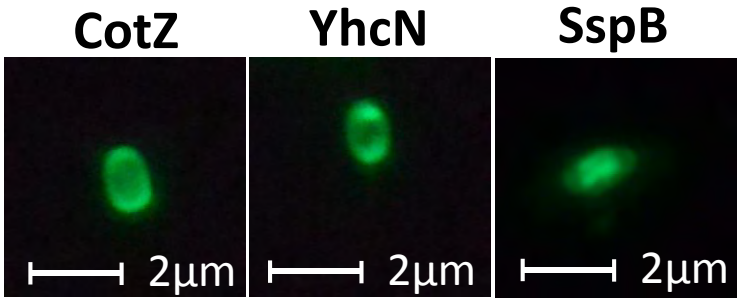

Figure 1: *Typical Bacillus subtilis spores differentially labeled with GFP*

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.

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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 2.1×10^{19} to 9.8×10^{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 2.1×10^{19}, 9.8×10^{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 2.1×10^{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 9.8×10^{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.

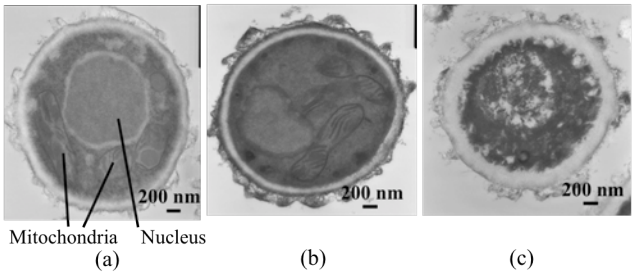

Figure 1. *TEM images of cross sections of P. digitatum spore: (a) control, and (b) 2.1×10^{19} and (c) 9.8×10^{19} cm^{-2} in $O(^3P_j)$ dose.*

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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.

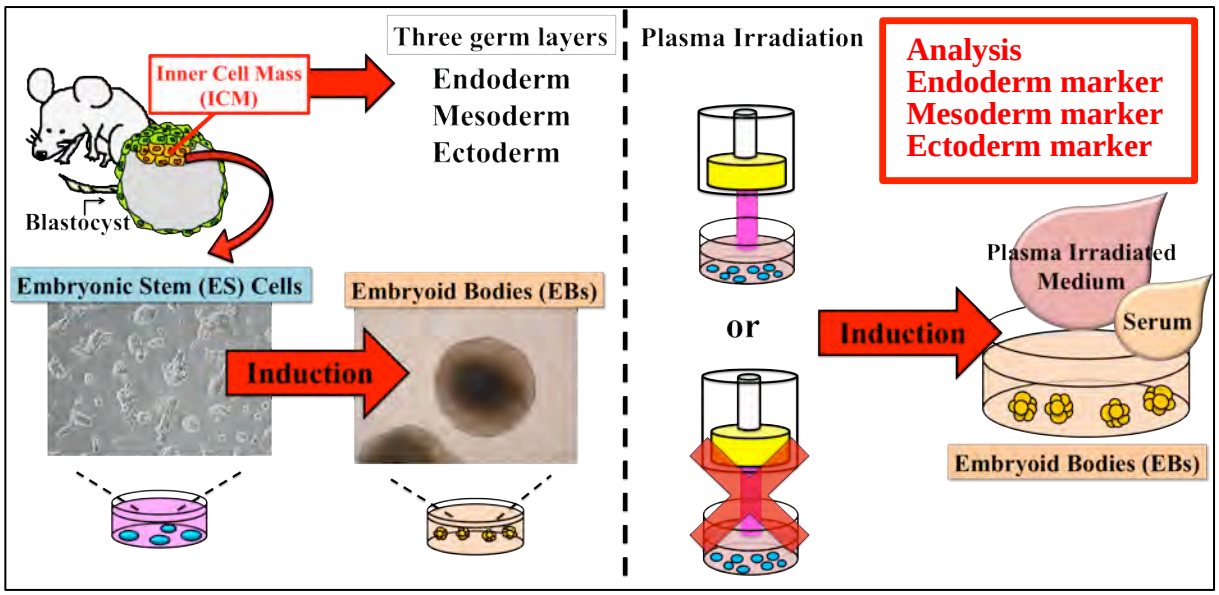

Figure 1: *The analysis of the effect of gas plasma irradiation on the differentiation of mouse ES cells*

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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

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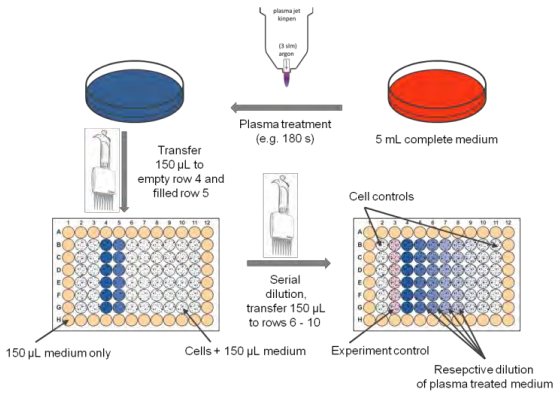
During the last decade the interests and efforts in Plasma Medicine increased with time. Since today there are several leading institutes developed their own plasma sources as well as treatment protocols for biological experiments. Due to the different sources including various feed gases there are dozens 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).

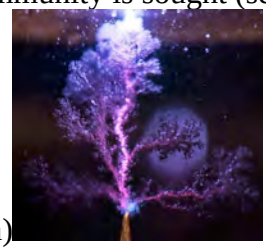
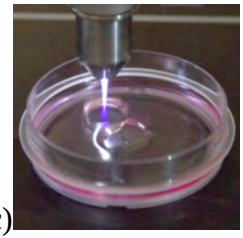
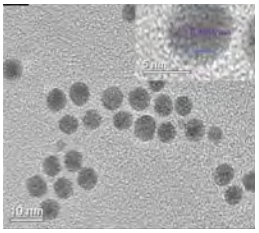
a)  b)  c)  d) 

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
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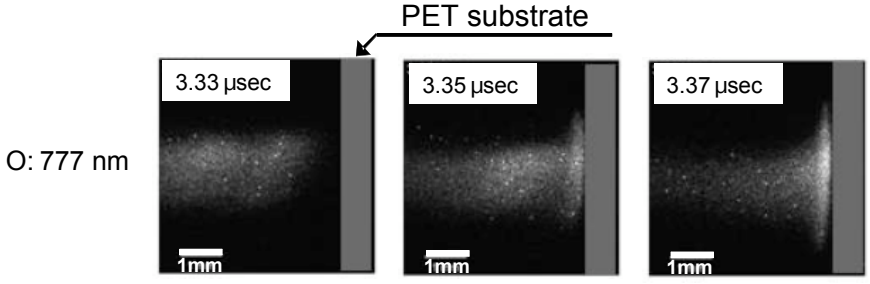

Figure 1: *ICCD images of plasma bullet impinging on PET substrate.*

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).

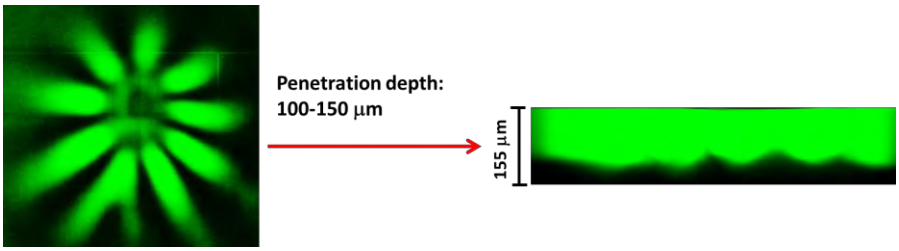

Figure 1: *Helium plasma jet delivery of ROS into the biological tissue model. ROS is detected in the material by the green fluorescence. The spatial distribution of ROS on the tissue surface resembles a star shaped pattern (left fluorescence micrograph) and ROS penetrates the material to a depth of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

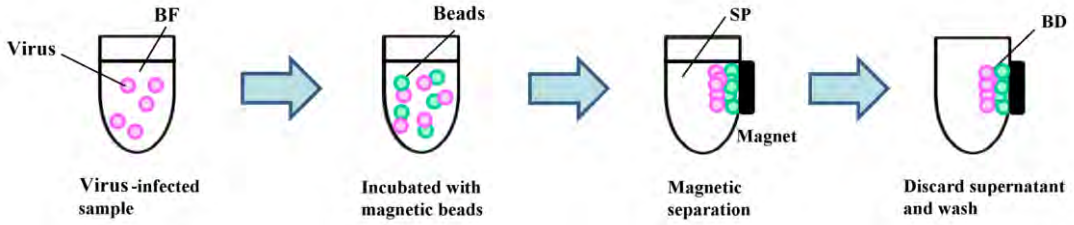

Fig. 1 Procedure of magnetic collection of influenza virus by antibody-immobilized magnetic nanoparticles(MNPs).

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.

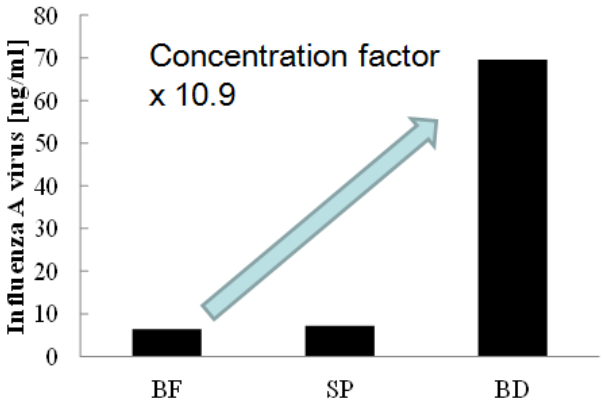

Fig. 2 Results of virus condensation for MNPs using C111 antibody.

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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.

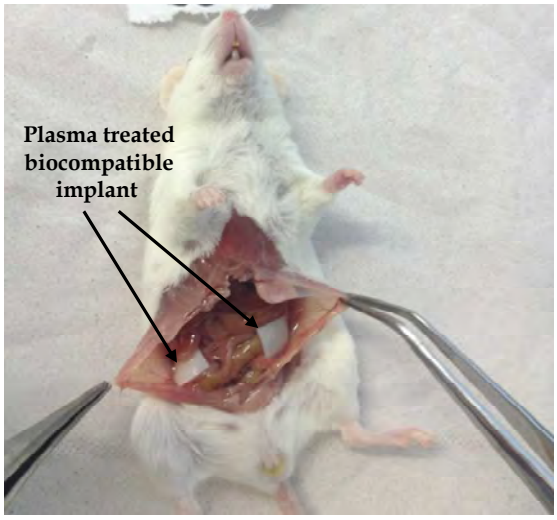

Figure 1: *Intraperitoneal biodegradable plasma treated implant in mice removed after 3 month. No inflammation was observed in the tissue exposed to the plasma coatings.*

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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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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.

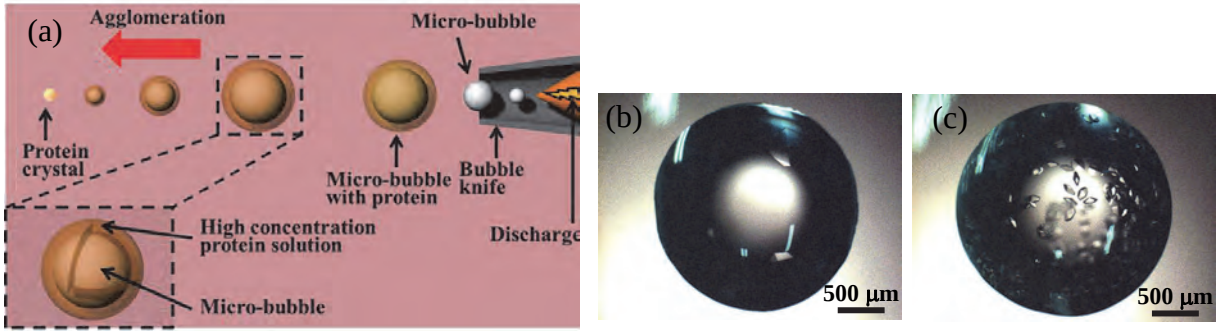

Figure 1: *(a) Concept of the protein crystallization assisted by electrically driven bubble knife, (b) protein crystallization by conventional hanging drop methods, (c) protein crystallization assisted with electrically driven micro-bubbles*

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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.

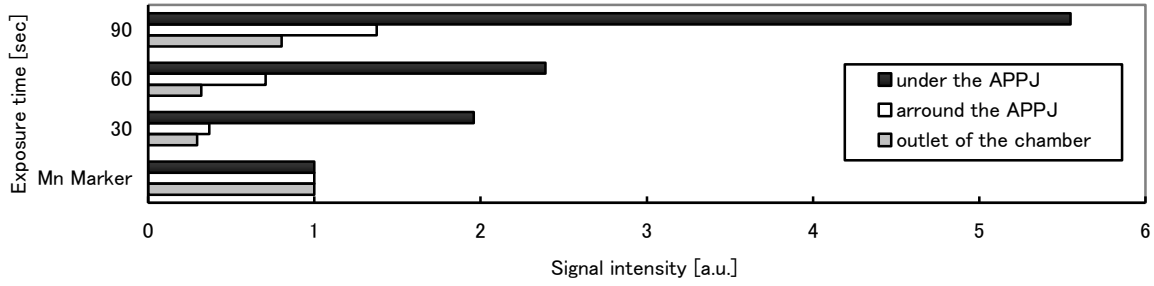

Figure 1: *BMPO-OH spin adduct signal intensity*

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.

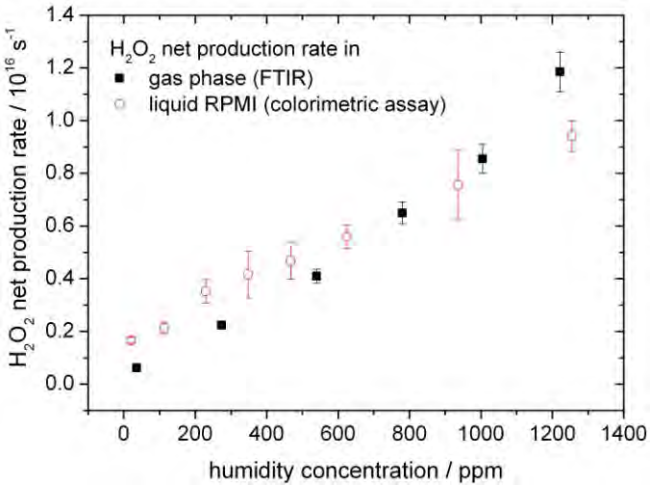

Figure 1: H_2O_2 production rate is identical in the gas and in the liquid phase

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.

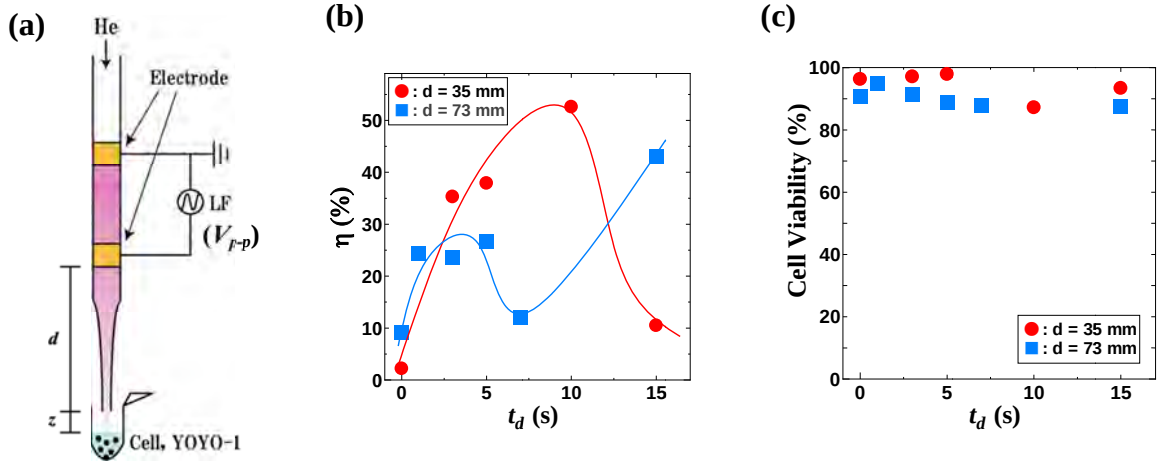

Fig. 1: (a) Schematic of an experimental setup for atmospheric pressure plasma irradiation, (b) the transfection efficiency **η** (%) and (c) cell viability (%) as a function of plasma irradiation time with the diffusion distance d as a parameter for $V_{p\text{-}p}$ = 7.8 kV, c = 5μM, z = 5mm.

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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.

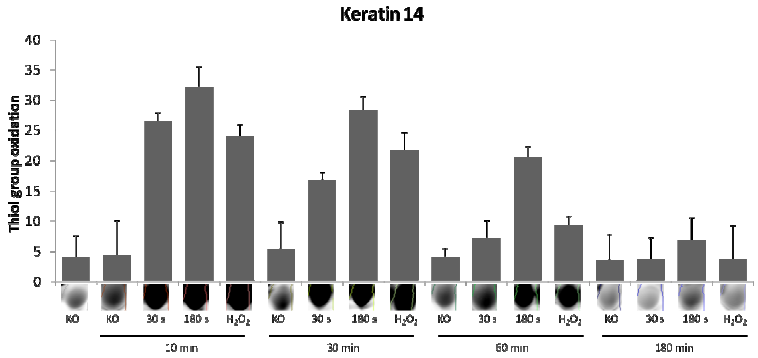

Figure 1: Thiol oxidation of intracellular keratin 14 after plasma returns to control within 3h

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 and10^3 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.

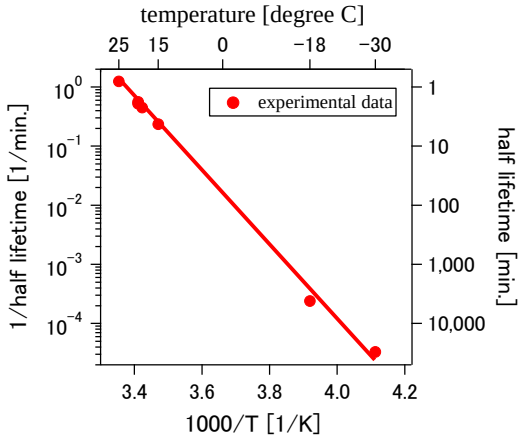

Figure 1: *Arrhenius plot for bactericidal activity of PTW.*

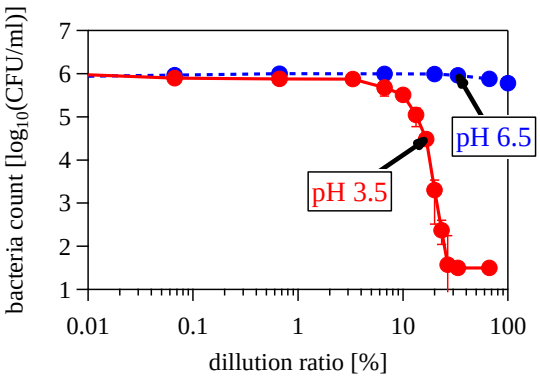

Figure 2: *Bactericidal activity (B. subtilis) of diluted PTW with the reduced pH method.*

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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.

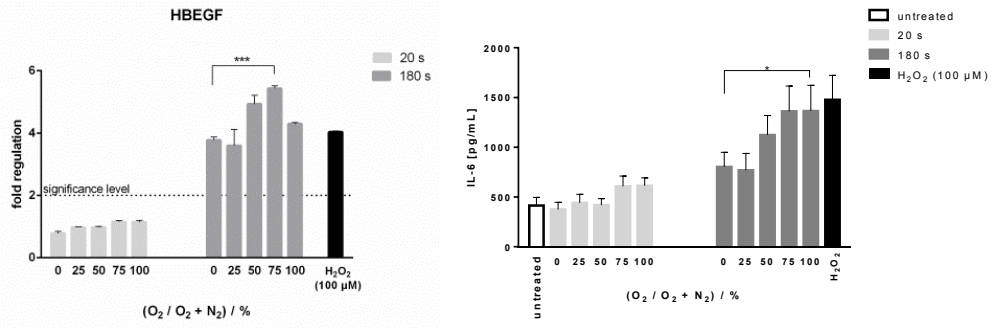

Figure 1: *Up-regulated gene expression of growth factor HBEGF (A), and increased IL-6 secretion (B) due to altered environmental conditions*

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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.

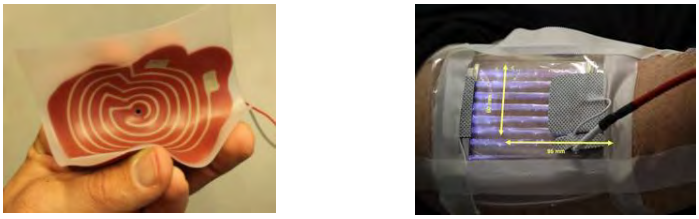

Figure 1: *Flexible dielectric barrier discharge arrangements*

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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.

This work was partially supported by the Ministry of Education and Science and by the Program of the Presidium of RAS.

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.

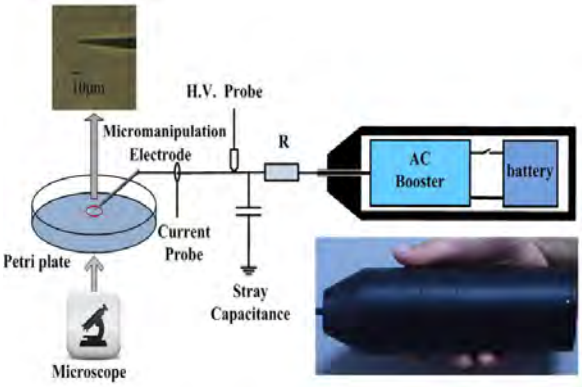

Figure 1: *Schematic of the microplasma jet setup and a sketch of the biomedical treatment*

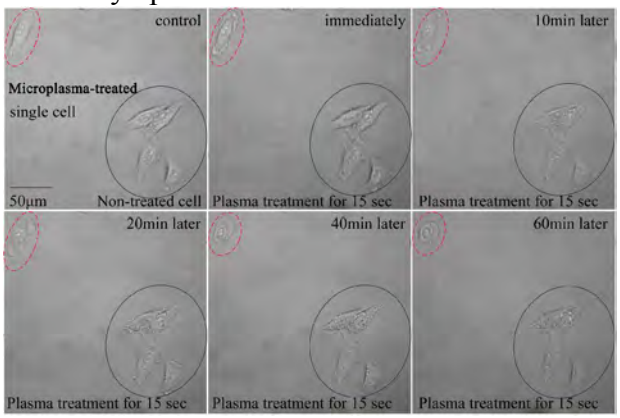

Figure 2: *Real-time monitoring of morphological changes at the single-cell level in HepG2 cell.*

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.

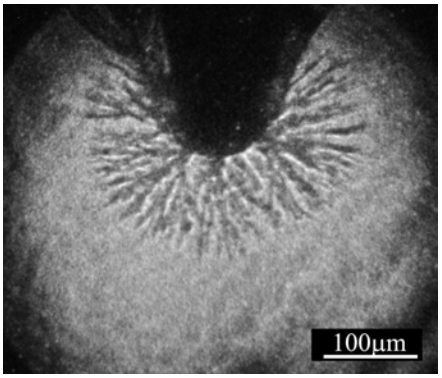

Fig. 1 Shadowgraph image of the primary streamer during propagation.

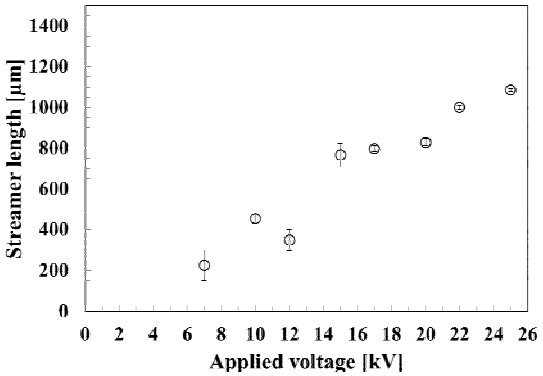

Fig. 2 Length of the developed primary streamer vs. applied voltage.

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

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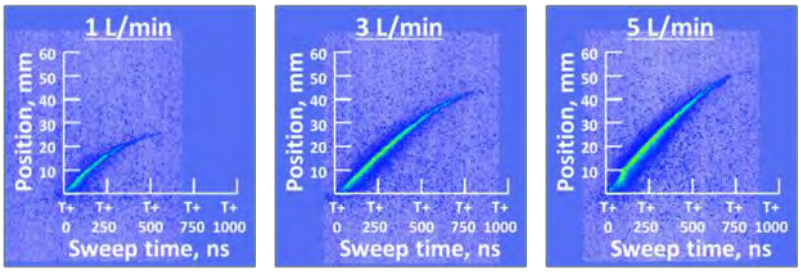
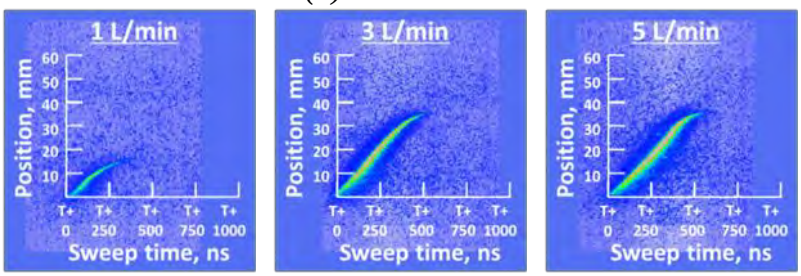
Atmospheric-pressure plasma jets (APPJs) have rapidly become widespread in the field of material modification and biomedical applications. These applications are based on various phenomena produced by the APPJs, such as UV light emission, electrons, ions, and radicals. In the past, it was reported that 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.

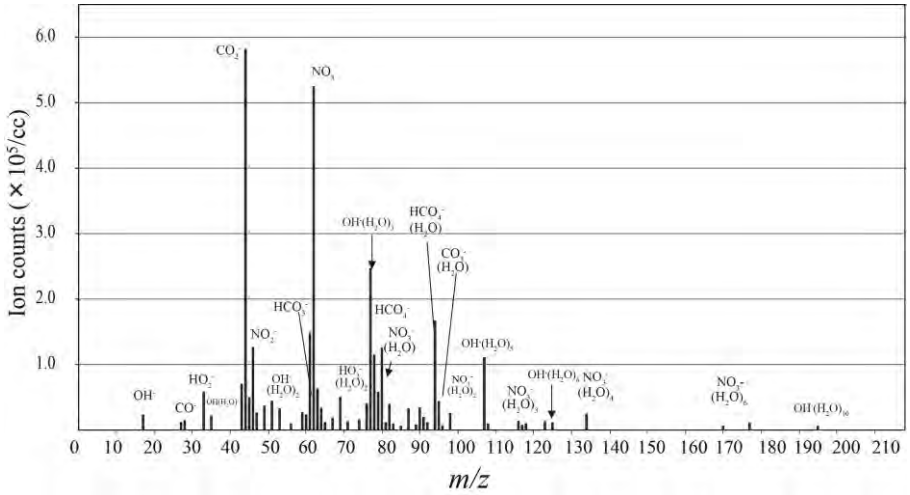

Fig. 1. *Mass spectrum of negative ions emitted from a He APP jet. The He gas flow rate was 3l/min and the peak-to-peak voltage Vpp was 9 kV.*

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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.

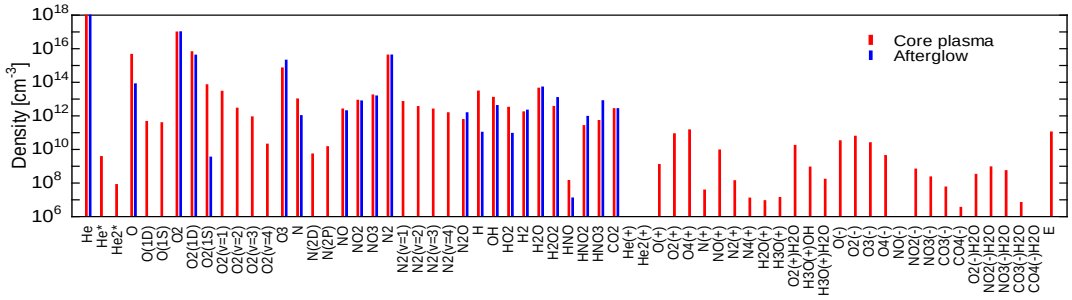

Figure 1: *The absolute densities of 65 species in He+0.5%O_2+0.025%humid air plasma*

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 μ 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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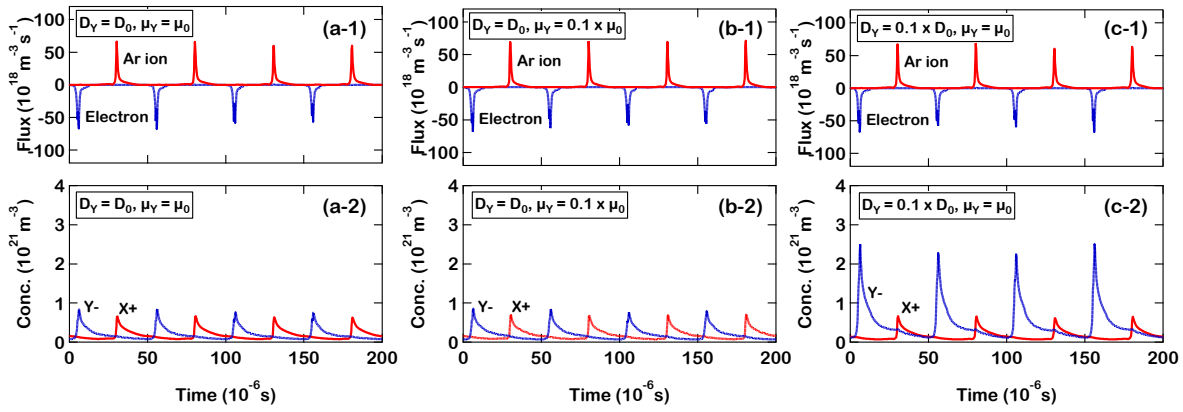

Figure 1: Effects of mobility and diffusion coefficient of liquid ions X+ and Y- on the concentration of these ions on the top of the liquid surface in contact with DBD.

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.

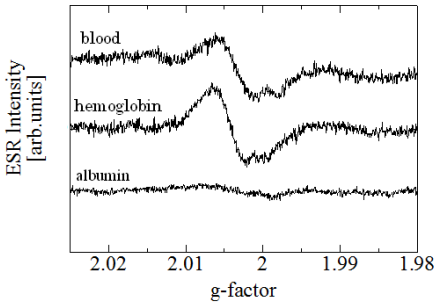

Fig.1 *ESR spectra for blood, hemoglobin, and albumin on the quartz plates measured after plasma treatments for 10 min.*

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.

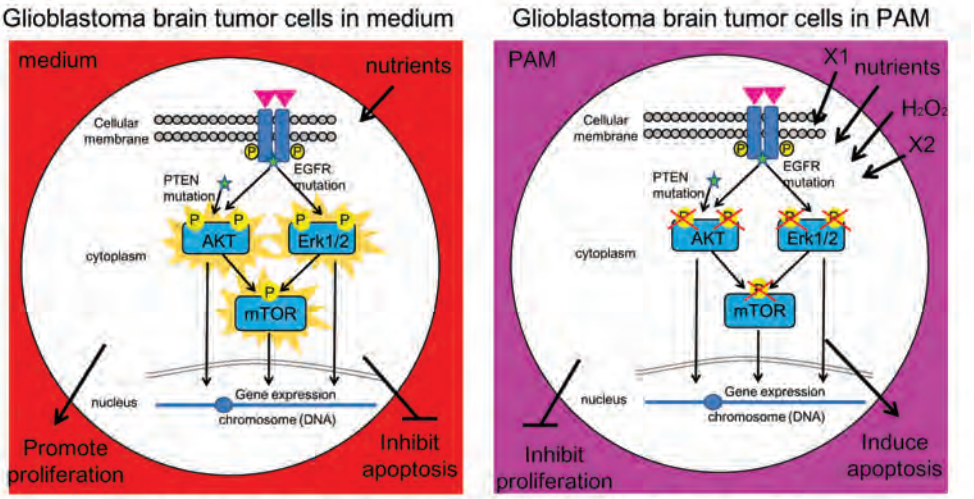

Figure1: Intracellular molecular mechanisms of apoptosis of PAM-treated glioblastoma brain tumor cells

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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).

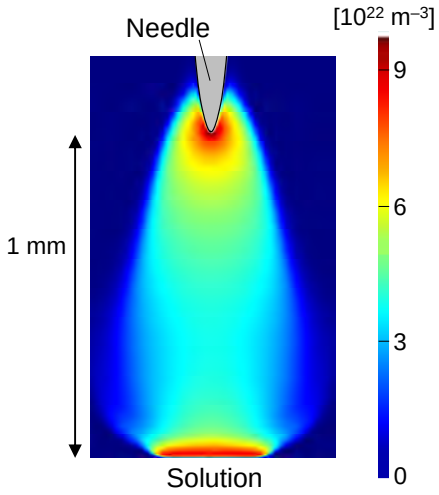

Figure 1: *Distribution of OH radical in gas phase after 100 ns*

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

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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.

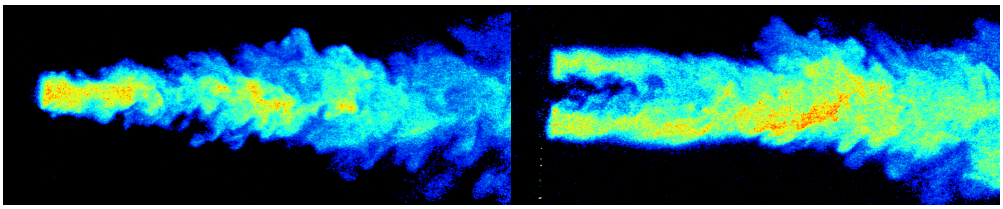

Figure 1: *Gas flux image of a plasma jet nozzle 3 slm (left) and shielding gas 5 slm (right) revealing the turbulent mixture of ambient species with feed gas for the plasma off case. [5]*

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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
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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.

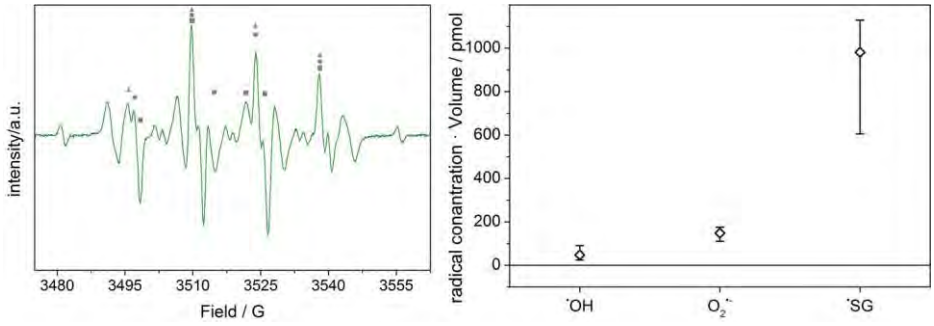

Figure 1: *EPR spectrum after 180 s treatment with VUV radiation of an argon atmospheric pressure plasma jet and hydroxyl (marked in the spectrum with (▲), superoxide anion (●) and glutathione thiyl (■) radical concentrations.*

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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^-.

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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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