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Orléans - France

INTERNATIONAL SOCIETY FOR PLASMA MEDICINE

4th international Conference on plasma medicine

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Book of abstracts

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BOOK OF ABSTRACTS

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Editors: M. MIKIKIAN, H. RABAT, E. ROBERT, J-M. POUVESLE

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Foreword

This fourth International Conference on Plasma Medicine, ICPM4, organized under the leadership of the ISPM, International Society for Plasma Medicine, is a unique opportunity of exchange for all researchers in this rapidly growing field. It comes at a time when the domain becomes fully mature with a better symbiosis between the different communities involved. ICPM4 brings together researchers from very different backgrounds covering all specialties, plasma physics, biology, biochemistry, medicine, pharmacy, and engineering, needed to achieve significant progress in the various relevant topics. Numerous new results have been obtained both on the fundamental level, concerning the interaction of plasmas with cells, microorganisms or living tissues, understanding of process chains involved in the observed therapeutic results, modeling of discharges, and on the experimental one with considerable progress in therapeutic applications, in the areas of sterilization and decontamination, in the development of new plasma sources more appropriated for treatments, or concerning surface modification or functionalization of materials for biological applications.

The abstracts presented in this book evidence the significant progress realized recently in the field of Plasma Medicine and its related topics. The program of ICPM4 is composed of invited talks, solicited oral contributions, selected contributed oral presentations and of two poster sessions after selection and review by the International Scientific Committee whose members are gratefully acknowledge. The venue of ICPM4 in Orléans will give the opportunity to strengthen ISPM through the first general assembly of the society, involving all ICPM4 attendees, during which bylaws will be proposed and voted.

The significant increase in contributions and countries represented in this new edition of ICPM shows the extraordinary vitality of Plasma Medicine and the dynamism of the researchers who devote their efforts to this domain. It's with great pleasure that GREMI, laboratory of the University of Orléans and CNRS, welcomes you for this ICPM4 within one of the oldest universities in Europe, created in 1306, in Orléans, a city steeped in history, ancient Cenabum, Gallic town of Carnutes, or Aurelianium, the Gallo-Roman city. Orléans is the gateway of the "Loire Valley", UNESCO World Heritage, known worldwide for its extraordinary "Châteaux" (Sully-sur-Loire, Chamerolles, Chambord, Blois, Cheverny, Chenonceau, Amboise, Villandry, and tens and tens of others).

We hope that ICPM4 and this book of abstracts will represent a reference for the ongoing multidisciplinary research and will stimulate new developments and collaborations in the Plasma Medicine field. We also hope that you will enjoy your stay and keep wonderful memories of ICPM4.

Jean-Michel Pouvesle

ICPM4 chairman

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Oral Contributions

Evaluating the interaction of low temperature atmospheric pressure plasma with cells for wound healing and disinfection

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Historically, thermal plasmas that generate high levels of heat have been used in certain medical procedures such as electrosurgery, including tissue ablation. Recent advances in plasma science and technology allowed for the generation of biologically tolerant non-thermal plasmas, which could be used in various novel medical applications. The use of nonequilibrium plasmas in diverse experiments ranging from material science to medicine have resulted in efficacious decontamination, wound healing, tissue regeneration, killing of cancerous cells and treating dermatological diseases [1]. However, the mechanisms of action of plasmas at the cellular and molecular level still need to be examined in order to define the general and specific effects of non-thermal plasmas in relation to healthy and diseased host cells.

In order to understand these relationships, our efforts at the Laser and Plasma Engineering Institute at Old Dominion University have focused on a multi-prong approach. The goal of our research is to optimize plasma treatment to effectively kill various bacteria by either inhibiting or destroying biofilm formation, without harming mammalian cells. The same approach has been undertaken to understand how non-thermal plasma affects the molecular machinery in mammalian cells, how these effects differ in disease states of the mammalian cells and ultimately how to modulate a desired cellular phenotype by changing the dose and parameters of non-thermal gas plasmas.

In our research efforts, we utilized the plasma pencil to generate low temperature atmospheric pressure plasma (LTAPP) [2] in order to determine the effect of LTAPP on decreasing or inhibiting bacterial adhesion, proliferation and persistence in different models of infection. The interaction between the reactive species (reactive oxygen species and reactive nitrogen species) generated by LTAPP and the downstream effects are fundamental in understanding how LTAPP decontaminates wounds and simultaneously heals damaged wound tissue. Studying the effects of LTAPP on the coordinated process of bacterial killing at the phenotypic and molecular level provides an important means to study different bacterial signaling pathways. Ultimately, our goal is to understand the effect of LTAPP treatment at the molecular level in both prokaryotic and eukaryotic systems in order to facilitate the subsequent development of LTAPP as a therapeutic against microbial infection of wounds and accelerate healing of damaged or diseased cells. The broad impact of the results from this effort will transform the concept of how low temperature atmospheric pressure plasma affects host-associated microbial infection at the molecular level and provide a model template for future investigations involving LTAPP.

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Plasma Pharmacy: The Use of Plasma-treated Products for therapeutic Purposes

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During the last years, a multitude of findings about plasma-cell and plasma-tissue interactions and its possible use in medical therapy have been provided. One of the key findings is that several biological effects are not result of direct plasma-cell or plasma-tissue interaction but mediated by liquids. It was demonstrated that simple liquids like water or physiological saline, after treatment by atmospheric pressure plasma are antimicrobial active and that these effects are attributable to the generation of different low-molecular reactive species [1] [2]. Plasma treatment of more complex liquids like cell cultivation media result in changes of organic components which could induce various effects on living cells and their components [3] [4]. This focuses attention on a new and innovative field of medical plasma application where the plasma is no applied directly on living structures but is used to generate, optimize and/or stabilize products which contain active agents, above all liquids. In contrast to plasma medicine, what means the direct use of plasmas on or in the living organism for therapeutic purposes, this field – as a specific field of medical plasma application – should be called “plasma pharmacy”. Pharmacy is a branch of health sciences dealing with preparation, dispensing, and proper utilization of drugs whereas drugs in this sense are substance used in the prevention, cure, or alleviation of diseases.

Based on the present state of knowledge, application fields of plasma pharmacy might be: preparation of antimicrobial active liquids for disinfection and antiseptics [1]; modification of complex liquid components to influence cell and tissue behavior, e.g. stimulation of cell proliferation [3]; solubilization and stabilization of poorly soluble or non-soluble substances [5]. Another conceivable possibility is the activation of drugs before application. Finally it might be considered that plasma use for decontamination/sterilization of pharmaceuticals and pharmaceutical packaging materials is also a field of pharmacy.

A main advantage of plasma pharmacy is that direct contact of plasma with living tissue is avoided and, consequently, some possible side effects (e.g. caused by UV radiation) can be excluded. On the other hand, most of such plasma pharmaceutical products have the character of drugs and have to be licensed according to specific regulatory requirements.

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Interaction of Nanosecond and Sub-nanosecond Pulsed Dielectric Barrier Discharge Plasma with Living Systems

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Spatially uniform nanosecond and sub-nanosecond short-pulsed dielectric barrier discharge plasmas [1, 2] are gaining popularity in biological and medical applications [3-5] due to their increased uniformity, lower plasma temperature, lower surface power density, and higher concentration of the active species produced. In this presentation we will compare microsecond pulsed plasmas with nano and sub-nanosecond driven systems and their applications in biology and medicine with specific focus on *wound healing and tissue regeneration*. Transition from negative to positive streamer will be discussed with proposed hypothesis of uniformity mechanisms of positive streamer and the reduced dependence on morphology and surface chemistry of the second electrode (human body) being treated.

Uniform plasma offers a more uniform delivery of active species to the tissue/surface being treated thus leading to better control over the biological results. We will discuss interaction of uniform plasmas with living cells and the biochemical interaction mechanisms leading to angiogenesis, cell proliferation, and differentiation of mesenchymal stem cells. Examples of plasma interaction with living systems will be supported with wound treatment of rats and rabbits and treatment of corneal ulcerations in rabbits.

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Burn wound healing: a role for plasma medicine?

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Burn wounds represent a challenge in terms of the quality of healing. Wounds that extend through the dermis and into subcutaneous layers of the skin often heal with considerable scarring. Also, complications during the healing process such as bacterial colonization and infection will give rise to worsened scar quality.
Improvements in burn care over decades have provided some control over mortality, thereby increasing the focus on morbidity and quality of outcome.
Plasma treatment could provide interesting new treatment modalities for bacterial problems, as well as for scar improvement.
Bacterial colonisation and infection of burn wounds is usually treated with local or systemic antibiotics and/or antimicrobial agents. Clear disadvantages of these treatments are the increasing appearance of resistant bacterial species and cytotoxic effects towards the epithelial elements and cells in the wound bed.
We therefore designed an experimental setup to study these effects, in order to find conditions where bacteria would be effectively reduced and cells necessary in the wound healing process would remain unaffected.
We studied the effects of cold plasma treatment on skin cells and an in vitro burn wound model. A cold atmospheric plasma needle (13.56 MHz micro-jet) was used. Primary cultures of fibroblasts and keratinocytes were treated with plasma. Membrane leakage and proliferation were measured. For the burn wound model, small pieces of human skin were burned and treated with plasma. Samples were incubated air-exposed for 2 to 3 weeks to allow regrowth of the epidermis.

Short treatment times (30-60s) using argon plasma on cell cultures affected cell adhesion and proliferation. Treatment with compressed air or helium plasma for up to 2-4 min only marginally affected membrane integrity and proliferation.
Outgrowth in the burn wound model was reduced by longer plasma treatments (1 min) or with argon or compressed air. Helium plasma seemed to induce proliferation in keratinocytes but this was not accompanied by an increase of newly formed epidermis.

In conclusion, cold plasma can preserve the viability of skin cells. The effects on the cells are highly dependent on the distance between plasma surface and cell sample, the gas used for plasma ignition and the treatment type. Potentially, a contact-free disinfection method for burn wounds could be created.

Effects of a He/O_2 atmospheric pressure plasma effluent and its components on bacteria and bio-macromolecules

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Using the X-Jet introduced by J. Benedikt *et al*. [1] we investigated the effects of a He/O_2 plasma effluent and its components separated into (V)UV radiation and particles on live bacteria and bio-macromolecules. We employed Environmental Scanning Electron Microscopy (ESEM) to investigate the microscopically visible effects of the plasma effluent as well as the emitted (V)UV radiation or particles on vegetative bacteria. The observed ablation of bacterial cell layers was time-dependent and strongest after treatment with the total effluent. Treatment with the particle channel led to intermediate ablation, ablation caused by the (V)UV channel was weakest but significant.
Effects of the plasma effluent and its components on DNA and proteins, two important classes of bio-macromolecules, were studied *in vivo* and *in vitro*. Evidence of plasma-mediated damage to DNA and proteins in living cells was provided by the induction of DNA and protein damage-specific reporter genes upon plasma exposure. To characterize the effects on DNA on a molecular level single-stranded and double-stranded DNA oligomers were dried on glass slides and exposed to the plasma effluent as well as to the emitted (V)UV radiation or particles separately. Raman spectroscopy revealed (V)UV-specific and particle-specific modifications of nucleobases. pUC18 plasmid DNA encoding part of the β-galactosidase enzyme for blue/white selection in *E. coli* DH5α was used as model to study the impact of plasma on DNA integrity and functionality [2]. Transformation efficiencies and mutation frequencies of dried, plasma-exposed pUC18 indicate both significant introduction of mutations and dose-dependent loss of intact plasmid DNA. Two model proteins were chosen to study plasma-related inactivation mechanisms. Glyceraldehyde 3-phosphate dehydrogenase (GapDH) enzyme activity decreased rapidly after exposure to the plasma effluent. Exposure to (V)UV radiation alone had little effect on GapDH activity, while the emitted particles alone efficiently inactivated the enzyme, though much less rapidly than the total effluent. mCherry protein was efficiently inactivated by (V)UV radiation with inactivation being reversible for exposure times up to 5 min. In contrast a 5 min exposure to the particle channel or the total plasma effluent lead to permanent mCherry inactivation.

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Inactivation of microorganisms on skin surface by cold atmospheric plasma

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Eradication of multiresistant superbugs is one of the clinical challenges of the 21st century. In the last twenty years new antibacterial agents approved by the U.S. FDA decreased whereas in parallel the resistance situation of multi-resistant bacteria increased. Thus, community and nosocomial acquired infections of resistant bacteria led to a decrease in the efficacy of standard therapy, prolonging treatment time and increasing healthcare costs.
Successful decolonisation of patients colonised with multi-resistant bacteria is of interest for controlling and preventing bacterial spread in hospital daily routine. The decolonization treatment consisted of mupirocin nasal ointment, chlorhexidine mouth rinse, and full-body wash with chlorhexidine soap. However the success depends on how many body regions are colonised by MRSA, as well as on the compliance of the treatment protocol by the patients and the health care workers. This emphasizes the need for the development of additional strategies for the decolonisation of bacteria.
The challenge of the antimicrobial plasma treatment is to find appropriate parameters which inactivate bacteria without harming the surrounding tissue. Therefore the present study was performed to evaluate the efficacy of two different cold-atmospheric plasma devices for decolonisation of ≥ 3 $\log_{10}$ steps ($\geq 99.9\%$) of *S. aureus,* MRSA and *E. coli*, when these bacteria were applied to an *ex vivo* porcine skin model.
Freshly excised skin samples were taken from six month old female pigs (breed: Pietrain). After application of pure bacteria on the surface of the explants these were treated with cold atmospheric plasma treatment for up to 15 min. Two different plasma devices were evaluated. A decolonisation efficacy of 99.9% was achieved already after 6 min of plasma treatment. Longer plasma treatment times achieved a killing rate of 99.999% independently from the applied bacteria strains. Histological evaluations of untreated and treated skin areas upon cold atmospheric plasma treatment within 24 h showed no morphological changes as well as no significant degree of necrosis or apoptosis determined by the TUNEL-assay indicating that the porcine skin is still vital. This study demonstrates that cold atmospheric plasma is able to very efficiently kill bacteria applied to an intact skin surface using an *ex vivo* porcine skin model.
Cold-atmospheric plasma generated by both new plasma devices are a novel anti-infective to decolonise bacteria, which are applied to intact skin surfaces, very efficiently.
Therefore cold atmospheric plasmas might evolve to a powerful tool for topical use to prevent nosocomial transmission of multiresistant pathogens, like MRSA, in the future.

"SAKAKITA Plasma" as a Novel Hemostatic Technique for Minimally Invasive Surgery

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Recently, plasma medical science has been studied, and medical uses of plasma technologies have also been developed [1-4]. An example device with the atmospheric pressure plasma is an argon plasma coagulator (APC), and is used in an endoscopic submucosal desection (ESD), ablation of residual tumor cells and control bleeding as a medical practice. This plasma is known as a type of arc discharge [5]. Surgical intervention using either APC, electrical coagulator, or laser coagulator causes tissue injury, and scaring problems sometimes are induced. Therefore, a new type of blood coagulator to reduce tissue damages is strongly desired by physicians.

In order to increase the effect of blood coagulation and reduce tissue damages, "Sakakita plasma" (plasma jet based on the dielectric barrier discharge) has been established [6], and tried to treat experimental bleeding of C57BL6 mouse.

1) Helium plasma treatment to the bleeding part by cutting out femoral artery; in this case, coagulation is promptly generated covering on disrupted blood vessel to stop bleeding. Surface temperature is less than 40 deg. during the treatment. Histopathological analysis shows that there is no evidence of either burning or tissue damage by warming.

2) Plasma jet has been applied to adipose tissues on omentum and mesenterium near the stomach of a mouse. After two weeks, the abdomen was cut open again. Histopathological analysis showed that no apparent adhesion and scaring tissue was detected.

Taken together, it is suggested that "Sakakita plasma" which has been used in the present experimental series might be promising as a novel hemostatic technique for minimally invasive surgery. In the conference, experimental results of plasma treatment to the mesenteric artery will be also presented. Moreover, we discuss on the assessment of plasma characteristics in the medical equipment using the plasma.

Acknowledgements

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Molecular dynamic simulation of the transmembrane pore growth under effect of the electric field

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Effect of the plasma upon tissue characteristics has a complex character. First of all one should mention generation of the radicals, ions, excited species, UV emission. Besides of that plasma contains the high intensity electric field stimulating the phenomenon of electroporation (EP) which manifests itself in the growth of pores in a cell membrane under the action of the external electrical field. Therefore the electrical field can be considered as an effective tools promoting the penetration of drugs, genes etc. inside a cell. The main problem arising at the theoretical description of the pore evolution in the presence of the external electrical field relates to evaluation of the time dependent pore size distribution function (PSDF). Two main approaches have been elaborated for solution of this problem. One of those is based on the Einstein-Smoluchowski equation in accordance with which the pore evolution proceeds as a stochastic process and a pore is represented by a few energy parameters [1]. These parameters are chosen usually by an empirical way. The second approach which is based on molecular dynamic (MD) simulation of the pore evolution [2] appears to be more justified. Disadvantages of this approach relate to very high computing time cost. For these reasons the interconnection between the parameters of the electrical field applied to a cell of a specific nature and the size of pores in its membrane remains to be empirical.

In order to overcome the above-mentioned disadvantages of the existing approaches, we apply MD for recovering kinetic coefficients used in the Einstein-Smoluchowski equation for PSDF. All molecular dynamics simulations were performed using GROMACS ver. 4.5.4 program package. OPLS united-atom parameters were used for lipid molecules, while the simple point charge model was used for water.. Two different bilayers of the membrane were considered: POPC membrane with 128 molecules of the lipid, 6606 water molecules and POPE membrane with 340 lipid molecules and 6729 water molecules. After preliminary 1 ns thermalization an external electrostatic field of the magnitude 0.3-0.5 V/nm was applied, so the pore creation process has been observed during next 3 ns. After its completion, the field was turned off, and the pore started to shrink. Then it reached quasi-equilibrium state of minimum radius, and remained stable to the end of the simulation (~2 ns). 10-15 equidistant snapshots from the latter piece of the trajectory were extracted and served as the staring points for all further computations. These simulations permitted us to find non-empirical values of the pores energy parameters which after that are compared with empirical values. Sensitivity of the obtained results to membrane type and used force potential are analyzed and discussed.

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Targeting the cell cycle by cold atmospheric plasma.

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CAP (cold atmospheric plasma) is a technology, which is based on quasi-neutral ionized gas (plasma at low temperatures), which is being evaluated as an alternative or addition to existing cancer therapies. A recent study shows that CAP treatment can cause a significant reduction in tumor size in vivo. Thus the purpose of this study is to begin to identify the mechanism by which cancer cells are killed by CAP.
The studies were performed on normal and transformed epithelial cells. The impact of CAP on cells was evaluated through cell migration studies (microscopy time lapse studies of cells), cell cycle studies using flow cytometry, and viability studies using MTT assays. In addition, cells were synchronized to the same stage of the cell cycle using nocodazole and DNA damage after CAP treatment assessed by evaluating expression of the S-phase damage reporter phospho-histone yH2A.X.
It was found that normal and transformed cells respond differently to CAP treatment. Using a mild CAP treatment, it was observed that migration of normal cells was reduced ~30% ($p<0.001$). While aggressive carcinoma cells showed also decreased their migration rates after CAP (~20% with $p<0.001$), less aggressive papilloma cells did not ($p>0.05$). Flow cytometry studies show that CAP induces a robust G2/M-cell cycle arrest in both types of carcinoma and papilloma cells (double fold increase in G2/M phase in ~24 hours after CAP treatment). Normal epithelial cells showed a more modest cell cycle arrest.
Experiments show a G2/M arrest is induced by CAP treatment in two different types of cancer cells. These data support the hypothesis that the increased sensitivity of cancer cells to CAP treatment is caused by differences in the distribution of cancer cells and normal cells within the cell-cycle. Because more cancer cells are actively proliferating, more are in the S-phase of the cell cycle. Data show that cells in the Sphase are more vulnerable to CAP treatment.

Activities of human cells are modulated by non-thermal atmospheric pressure plasmas

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Non-thermal plasma consists of components such as charged particles, reactive oxygen and nitrogen species (ROS/RNS) including bioactive substances (e.g. OH, NO) as well as radiation (ranging from IR to UV), and free electrons[1]. Due to recent advances in the development of non-thermal plasmas sources, the treatment of living matter with a blend of various plasma components became possible opening numerous possibilities for plasma to influence cells on a molecular/genetic level. Non-thermal plasmas has recently been shown to have broad application potential and therefore promise improvement in treating infected or chronic wounds, superficially skin infections and other demanding skin diseases. While lots of data exists about the killing of microorganisms as well as mammalian cells, the plasma-mediated activation of human cells still remains to be investigated. The aim of this work was to analyze plasma-mediated activation of human cells of different origin (skin, connective tissue and immune system). Therefore human keratinocytes (HaCaT), fibroblasts (MRC5) and immune cells (Jurkat T-cells and THP1 monocytes) were investigated by genomic and proteomic approaches.

Applying a 84 genes wound healing panel the up or down regulation of different genes (ECM and adhesion, growth factors and signaling molecules, inflammatory cytokines and chemokines,) after plasma treatment could be detected. Furthermore, employing liquid chromatography and mass spectroscopy, we were able to identify more than three thousand human proteins, some confirmed by several blotting techniques. The identified proteins displayed a wide range of molecular functions (e.g. antioxidant activities) and a broad spectrum of biological processes (e.g. regulation of cell cycle; cell signaling).

Investigating the cellular responses to non-thermal plasma treatment, we were able to identify several cell specific genes and proteins, which were activated after plasma treatment. Especially cell signaling and pro-proliferative signal molecules were activated after short term plasma treatment indicating stimulatory effects of non-thermal plasmas. However, while all types of cells showed a comparable pattern of activated molecules after plasma treatment, there are some differences in the cellular reactions, displaying diverse sensitivities of the investigated cells towards non-thermal plasma treatment.

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Deciphering non thermal plasma effects on human cell lines by proteomics

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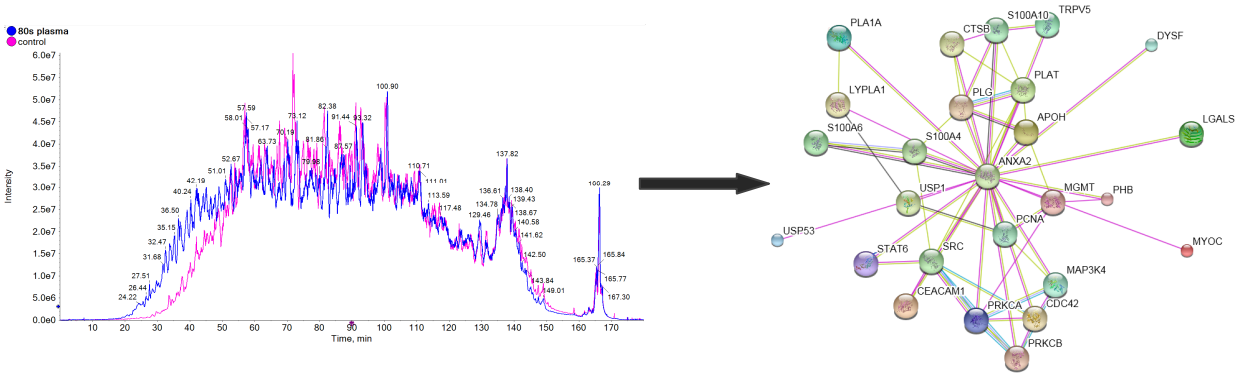
Non thermal plasmas are promising tools for different medical applications. To identify and basically understand the effect of non thermal plasmas on cells and tissues as well as to support plasma tuning efforts a deep insight into plasma – cell interaction is desirable.
The cellular proteome covers the complete protein composition of a biological cell at a given time. Therefore, the snapshot of a cellular proteome – the cell´s toolbox – enables in-depth findings on the cellular status.
To understand the cellular behavior after non thermal plasma treatment by an argon based plasma jet (kinpen, Neoplas Tools) we established a time (hours past treatment) and space (localization within a cellular compartment) resolved protocol to identify and quantify the intracellular proteins of a human keratinocytes cell line (HaCaT). Using an ABSciex TripleTOF 5600 high resolution mass spectrometer, we were able to identify 3000+ human proteins within different cellular compartments in a gel free approach. Proteins detected cover a wide range of molecular functions (e.g. antioxidant activities) and a broad spectrum of biological processes (e.g. regulation of apoptosis; DNA replication).



Measured protein abundances differ specifically between argon jet plasma treated cells and controls. Among the most regulated proteins are proteins involved in reactive oxygen/reactive nitrogen (RONS) metabolism and cell division, indicating an oxidative influence on cellular macromolecules. We also give evidence of the activation of protective and pro-proliferative signal molecules indicating specific (and transient) cell activation after kinpen treatment and encouraging a possible use of such plasmas in wound care.
Together with transcriptomic data the presented overview of HaCaT proteome after non-thermal plasma treatment helps streamlining the identification of both biological and physical key players and the conclusion of further research topics.

This work is founded by German Federal Ministry of Education and Research (grant number 03Z2DN11).

Atmospheric pressure plasma induced changes in cellular phenotypes

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Atmospheric pressure plasma (APP) treatment has gained much attention in biomedical applications due to its selective activation of certain cell types. A number of attempts have been reported on skin therapy using APP to enhance wound healing or to treat cancers, most of which have been unfortunately limited to phenotypic observations. In this study, we compared metastatic cancer to normal cells from liver (SK-HEP-1 vs. THLE-2) and from mammary gland (MDA-MB-231 vs. MCF-10A) to investigate the plausible existence of signature characteristics in cellular responses to plasma in a cancer dependent manner [1].

When treated with APP, human liver cancer cells (SK-HEP-1) and normal cells (THLE-2) exhibited distinctive cellular responses, especially in relation to their adhesion behavior. We discovered the critical threshold voltage of 950 V, biased at the electrode of the micro-plasma jet source, above which SK-HEP-1 started to detach from the substrate while THLE-2 remained intact. Our mechanical and biochemical analyses confirmed the presence of intrinsic differences in the adhesion properties between the cancer and the normal liver cells, which provide a clue to the differential detachment characteristics of cancer and normal cells to the APP. Similar responses to APP were observed in mammary gland cells.

We also exposed the human dermal fibroblast (HDF) and human aortic endothelial cells (HAEC), to APP (970 V, 50 kHz) in vitro to observe dramatic phenotypic changes in relation to cellular transdifferentiation. Our results support the potential use of APP to control the cellular transformation to enhance wound healing or to suppress the growth of tumor mass by controlling the motility of these cell types.

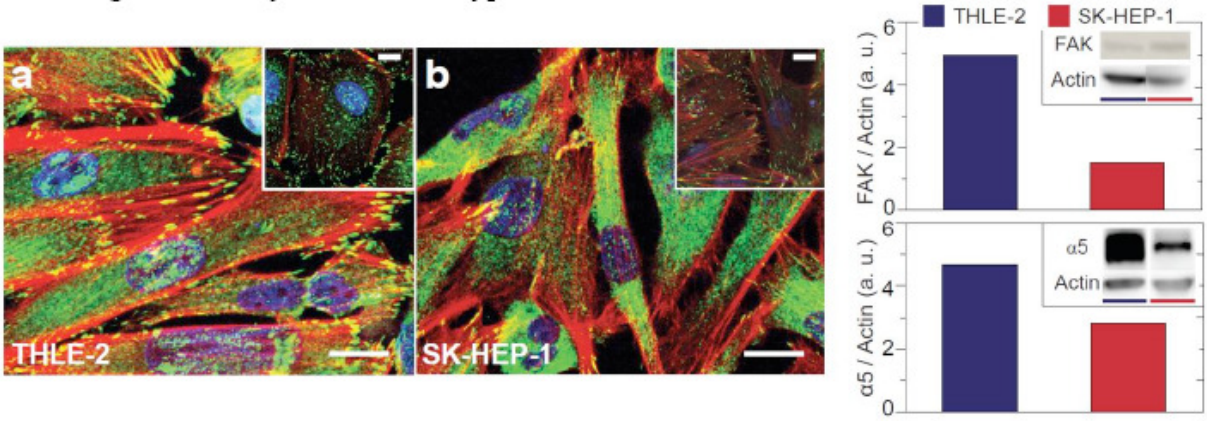

Figure 1: Immunofluorescence image of paxillin dots (green) and actin stress fibers (red) of (a) THLE-2 and (b) SK-HEP-1 (scale bar reads 20 μm). Right: Amount of FAK and α5 integrin proteins. The insets are the Western blot bands of FAK protein [1].

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Preliminary study to evaluate atmospheric pressure plasma jet applicability to disrupt liposomal membranes

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Liposomes (LIPs) consist of phospholipid bilayers and are currently being used as carriers for drug delivery and targeting [1]. Hydrophilic active substances may be loaded in LIPs and for characterization of such formulations a standard method is to disrupt LIP membranes with detergent (as Triton X-100) and measure released active concentration. However, the presence of the detergent may interfere with the analytical procedure used. We have performed a preliminary study in order to understand if plasma jet could be useful for LIP disruption. A jet device similar to the one presented in [2] has been used. The system is fed with high purity helium at atmospheric pressure and driven by sinusoidal high voltage (10 kHz, 11 kV peak-to-peak). As hydrophilic substance we used a highly fluorescent compound, calcein, at a concentration of 100 mM at which its fluorescence intensity (FI) is quenched, permitting easy determination of its leakage from vesicles (since FI is de-quenched due to dilution, when the encapsulated molecules are released in the aqueous dispersion media) [3]. MultiLamellar Vesicle (MLV) and Small Unilamelar (SUV) liposomes, encapsulating calcein (100mM) and consisting of egg lecithin (PC) or saturated lipid DSPC (which forms more rigid bilayers) were prepared by thin film hydration and probe sonication (for SUV) [1]. Vesicle hydrodynamic mean diameter and size distribution were determined by dynamic light scattering (Malvern, Nanosizer). The liposomes were subjected to cold plasma at different lipid concentrations and for different time periods (as seen in Table 1).

Table 1 . Effect of plasma jet treatment on calcein retention in Liposomes

Lipid comp.	Lipid conc. (mg/ml)	Calcein Latency (%)			
Plasma (min)		0	1	2	5
PC(SUV)	1	94.5	94.6	93.7	94.1
	2	93.3	92.9	92.6	92.5
	7	90.5	89.8	89.2	88.5
	12	93.3	-	90.7	83.9
PC(MLV)	1	81.4	-	71.5	22.9
DSPC	10	98.9	-	93.8	91.8

Preliminary results reveal that LIP membranes become more sensitive to atmospheric pressure plasma jet treatment when: (i) their size increases (MLV/SUV); (ii) lipid membrane is less rigid (PC/DSPC); and (iii) at higher lipid concentrations.

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Cold atmospheric plasmas in medicine – where we are today, and where we are heading

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Cold atmospheric plasmas (CAPs) are of considerable interest in medicine due to their versatility in design and their broad application spectrum, especially for in vivo applications in germ related superficial skin diseases. But did they meet the early enthousiastic expectations, or not? 7 years after starting first clinical trials in patients it is time to make a first résumé. Simply spoken: Yes, they did.
Microwave driven cold atmospheric argon plasma demonstrated to be a very safe and effective add-on therapy in patients with chronic infected wounds. A 5 min therapy regimen led to a highly significant higher germ reduction in plasma treated wounds compared to controls (34%, $p<10^{-6}$, 36 patients, 291 applications, MicroPlaSter alpha). [1] Subsequent studies revealed that a 2 min treatment in 2 generations of devices led to significant (40%, $p<0.016$, 14 patients, 70 applications, MicroPlaSter alpha) or highly significant reduction (23.5%, $p<0.008$, 10 patients, 137 applications, MicroPlaSter beta) respectively. [2] The antibacterial effects were independent of the bacterial species and the resistance level. No side-effects occurred and the treatment was well tolerated.
A rapid clinical improvement has also been reported in a patient with Hailey-Hailey disease resistant to topical disinfectants and corticoids with a secondary infection with *Candida albicans* and *Proteus mirabilis* using the MicroPlaStar beta device. [3]
If plasma has beneficial effects in itching diseases is still unclear. A study conducted in 46 patients with different itching diseases did not lead to a significant reduction of itch compared to a control application of argon gas (placebo mode). [4] But both legs led to a significant relief of itch. Further studies with a different approach are necessary to answer this question.
Next generation of cold atmospheric air plasmas already demonstrated their efficacy and safety in a phase I study and are in the starting blocks for further clinical investigations.
To summarize, the future of CAPs in medicine and hygiene is very promising, broadly based and exciting.

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Towards Clinical Trials: Medical Phase I Requirements and Execution

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In the past seven years cold atmospheric pressure plasmas (CAPs) have demonstrated their ability to reduce secondary infections in chronic wounds [1] and alleviate skin diseases (Hailey-Hailey) [2] of patients in clinical phase II studies. Based on these encouraging results a handheld and battery-driven CAP device - using the Surface Micro Discharge (SMD) Technology and the surrounding air for plasma production - was developed, characterized and tested in a medical phase I study:

Efficacy tests *in vitro* and *ex vivo*

To demonstrate the efficacy of the SMD device *in vitro*, different bacteria on agar were treated with CAP: a reduction of 5 log is achievable for all bacteria in 10s. Furthermore the influence of different humidity and angles on the bactericidal efficacy of the SMD device was evaluated. Experiments with bacteria on *ex vivo* porcine skin to simulate *in vivo* conditions revealed a 3 log reduction in 60s of treatment. Furthermore tests with *Candida albicans* on agar (5 log in 30s), adenoviruses in solution (6 log in 240s) [3] and endospores on metal plates (6 log in 3-5 min) were carried out with devices using the same SMD technology.

All required **electrical safety tests** (EN 60601-1) were carried out with a so-called notified body. Furthermore the measurement of **UV** and **toxic gas emission** showed, that - within CAP treatment times of up to 60s - the emission is far below the limits given by ICNIRP and NIOSH/OSHA for inhalation.

Biological safety tests *in vitro* and *ex vivo*

To demonstrate the safe usage of the SMD device, several biological experiments - including *in vitro* cell culture, tissue and *ex vivo* blood and skin tests were carried out:

Cell culture experiments on fibroblasts revealed a "safe therapeutic window" for CAP treatment times of up to 30s. Furthermore times of up to 240s did not induce mutagenicity beyond naturally occurring spontaneous mutations. Histology analysis and gamma H2AX detection of DNA double strand breaks did not show any differences between CAP treated (up to 20 min) and untreated excised human skin. Experiments using mini organ cultures of mucosa did not possess an increase in apoptosis/necrosis for treatment times of up to 60s. Furthermore no increase in DNA double strand breaks were detectable for times of up to 120s. These results were summarized in a medical proposal to get approval for a clinical phase II study on infected wounds. Further medical trials on fungi-related (tinea) and virus-related (herpes, warts) diseases are planned.

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Treatment of chronic venous leg ulcers with a hand-held DBD plasma generator

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In cold plasma medicine, a new field, antiinflammatory, antiitch, antimicrobic, UV, and other therapeutic modalities are combined within one treatment. Generally, two types of cold plasma can be discerned: Direct plasma (dielectric barrier discharge – DBD, corona discharge) and indirect plasma (plasma torch, plasma jet). DBD generates a low temperature plasma under atmospheric pressure and, thus, is a suitable instrument for a non-destructive treatment of biological material. The PlasmaDerm® VU-2010 device is a non-invasive active medical intervention which does not reach direct skin contact. For our medical application, a non-equilibrium, weakly ionized physical DBD plasma is generated by the application of high voltages across small gaps, whereas the electrode is covered by a dielectric. This nonconducting layer avoids the transition of the gas discharge into a hot arc by limiting the current. The biological tissue itself (skin) acts as the second electrode.

Chronic leg ulcers are a major problem in the eldery. The prevalence corresponds to 2-4 % in the population. 80% of chronic leg ulcers are caused by varicosis. Generally, three phases of wound healing (cleaning of the wound ground, granulation, and epithelialisation) can be discerned that are disturbed in chronic venous leg ulcers. Wound debridement, modern wound dressings and compression hosiery comprise methods of standard care. Despite these measures leg ulcers often persist. Additional plasma treatment may have the potential to facilitate wound healing by disinfection, stimulation of tissue regeneration and microcirculation as well as acidification of the wound environment. We are currently conducting a clinical trial with the PlasmaDerm® VU-2010 device to assess safety, applicability, and efficacy of chronic venous leg ulcer plasma treatment. The trial is still ongoing. So far, no profound adverse events of plasma treatment were reported pointing towards a positive outcome of our study.

Nonthermal Dielectric Barrier Discharge Plasma Enhances Skeletal Cell Differentiation and Autopod Development

Theresa A Freeman, Marla J Steinbeck, Greg Fridman, Jun Zhang, Natalie Shainsky, Gary Friedman, Alex Fridman

Non-thermal dielectric barrier discharge plasma (NT-Plasma) is a relatively new physicsbased technology. Although few reports exist with regard to the application of this technology to eukaryotic cells, it is thought that NT-plasma influences cell function mainly through the generation of reactive oxygen and nitrogen species (ROS and RNS). Cell functions including motility, proliferation and differentiation are directed by ROSsensitive kinases, signaling proteins and transcription factors which regulate gene expression. Furthermore, ROS generation enhances development of embryonic structures and initiates the expression of many genes linked to cell differentiation. Based on the involvement of ROS in these processes, we investigated the potential of NT-Plasma generated ROS and RNS to promote mesenchymal cell (MC) proliferation, commitment and differentiation along skeletal lineages. We asked: 1) can NT-plasma promote MC differentiation while maintaining cell viability, 2) what cell signaling pathways are activated by NT-plasma to promote cell differentiation and tissue formation, and 3) can NT-plasma ROS and redox changes enhance developmental factor expression to activate signaling cascades and promote differentiation in organ culture. The results of our study show that NT-Plasma generated ROS can be used to enhance skeletal cell differentiation by increasing intracellular ROS, which lead to the activation of kinases and transcription factors known to influence genes associated with differentiation and skeletal development. Our future goal is to investigate if NT–Plasma can positively influence MC commitment and differentiation *in vivo*. Development of NT-Plasma to amplify MC function will be an invaluable tool for tissue engineering and regenerative medicine.

Electrosurgical Plasma Devices-Some Physics, Chemistry, and Medical Applications

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Electrosurgical devices employing plasmas to ablate, cut and otherwise treat tissues have been in widespread use for decades. Following d'Arsonval's 19th century work on the neuromuscular response from high-frequency excitation of tissue, Doyen treated skin blemishes with a spark-gap generator in 1909. In the late 1920's, physician Harvey Cushing and physicist William Bovie [1] developed an electrosurgical device and power source that eventually became a standard of care for cutting, coagulating, desiccating, or fulgurating tissue. Beginning in the 1990's a new class of electrosurgical devices, employing electrically-conducting fluids were developed by ArthroCare Corp. and other medical device manufacturers. These modern devices are now widely used in many different surgical procedures, including those in arthroscopic surgery, otorhinolayrngology, spine surgery, urology, and gynecological surgery, and others [2].

This talk will include an introductory review of some of the research we have been doing over the last decade to elucidate the physics and chemistry underlying modern electrosurgical devices. I will also show some videos of several procedures employing these devices. Electrical-, thermal-, fluid-, chemical- and plasma-physics all play important roles in these devices and give rise to a rich variety of observations. Experimental techniques employed include optical and mass spectroscopy [3], fast optical imaging [4], and electrical voltage and current measurements. Many of the features occur on fast time scales and small spatial scales, so coupled-physics finite-element-modeling can also be employed to glean more information than has been acquired so far through physical observation [5].

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Non-thermal Plasmas: Potential Dental Applications

Nelson RFA Silva, Van P. Thompson, Paulo G. Coelho

Non-thermal plasmas (NTPs) represent a technology for improving the compatibility of materials and biological systems, e.g. through the chemical functionalization, modification, or sterilization of surfaces as a result of reactions induced by one or more plasma-produced chemical species. In addition, NTP-generated free radicals and ultraviolet (UV) light can be potentially utilized for polymerization purposes. This presentation will focus on the current knowledge and propose possible mechanisms by which NTP technology can be employed to substantially change dental restoration chemical interactions, collagen-cross-linking of dentin substrate and implant surfaces chemistry to improve its wetting response leading and enhance long-term performance of dental resin composites and primary stabilization for implantable devices.

Plasma Health Care: promises and difficulties for haemostasis

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The effectiveness of plasmas in the sterilization process and surface treatment, is now well established. The emergence of the production of a wide range of plasma at low temperature and atmospheric pressure allows various uses in medicine, since it is not possible to place patients in partial vacuum and because that mammalian cells do not tolerate temperature above 50-55°C even for a short period. The future contribution of plasma technology in the food industry is currently probably underestimated. It's a way to increase food resources for the global population, knowing that at present one third of food produced is lost, whether in developing countries or developed countries. For cold plasmas health care, the following fields of application are well identified: 1) dermatology: therapy of skin infections, tissue regeneration, wound healing, sterilisation of infected ulcer. 2) blood coagulation. 3)treatment of cancer cells, pending other applications that we have not yet imagined. In Toulouse, we have initiated studies on the effects of plasma in three areas: cell signaling, treatment of skin infections due to parasites, haemostasis in collaboration with the GREMI (Orleans). We will discuss only the latter subject.

This is Alexander Fridman who first demonstrated the possibility of accelerating blood clotting by using a Floating Electrode Dielectric Barrier Discharge. Other teams have since confirmed its work. The major interest is in the treatment of external bleeding in multiple trauma or in patients with congenital diseases of haemostasis (haemophilia, von Willebrand disease, platelet disorder, etc..). For our part, like other teams, we obtained a coagulation layer surrounding the samples of blood or blood plasma, treated with DBD plasma jets or afterglows, but no clotting in the heart of the sample. But the originality is to obtain here a reversible layer. It is difficult to demonstrate the mechanism: the dosage of coagulation factors including fibrinogen, show no difference in rates of factors, before and after treatment of the sample by DBD plasma. We assume that this is an incomplete polymerization of fibrin monomers linked by weak electrostatic forces, without the intervention of factor XIII, which normally creates covalent bonds. In collaboration with the Gremi, the "plasma gun" causes a statistically significant decrease in bleeding time performed on rat tail. This reflects a stimulation of primary haemostasis, a phenomenon dependent on blood platelets. The metrological study of coagulation has required a methodological adaptation. Indeed, the plasma treatment of blood samples in the measuring wells produces a surface layer. The lower part of the sample remains liquid. So, the analyzers used are unable to measure the clotting time. We therefore used an indirect technique according to a description made in microbiology by Kamgang-Youbi in 2009. We treated calcium chloride by a DBD direct plasma for 30 seconds. Use of this plasma activated calcium chloride resulted in a statistically significant decrease in clotting times measured. Despite these results, we fail to understand the physiological mechanisms behind these effects. Depending on the variation of parameters (plasma source, exposure distance, type of gas mixture, gas flow, etc), we observe contradictory phenomena, sometimes even with longer clotting times. The study shows that blood platelets can be activated or inhibited, as can be seen after exposure to NO.

In conclusion, although these contradictory effects are not really surprising given the existence of many loops of activation or inhibition in haemostasis as in many other biological systems, this situation proves that there is still much work to provide for understanding the physiological mechanisms induced by plasmas, in the haemostatic system. It remains to design experiments in view to isolate the role of each component (ROS, RNS, heat, UV, etc.) and measure a possible threshold effect and then be able to create the most suitable plasma depending on the wanted application.

Preclinical in vivo imaging strategies to boost therapy innovation in cancer research: application to plasma

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Thanks to increasing availability of transgenic models in mice for a variety of tumours and cell lines expressing bioluminescence or fluorescence reporter genes, non invasive in vivo imaging modalities are extremely valuable tools for biomedical research, discovery and development of new therapy strategies in oncology.
Among these, biophotonic and nuclear imaging are likely the most powerful resources to quantitatively assess gene expression, specific functions, efficacy and actual delivery of a labeled drug to the target. This process can be extended to document structure/activity relationship for any therapy including drugs, radiations, microwawes, plasmas etc…

Bioluminescence is based on the non invasive detection of photons emitted by luciferase expressing cells in the living animal. This modality is a unique tool for experimental oncology and is routinely used for a variety of tumour cells to achieve screening or efficacy evaluation of new treatments. In addition to tumour burden determination with usual luciferin, specific pro- substrates that are sensitive to in vivo processing by caspases opens new perspectives for mechanistic studies based on quantitative imaging of apoptosis even for deep foci .
Near infra red fluorescence with a variety of fusion proteins for gene expression imaging and fluorochromes for labeling biomarkers is quite operational for 2D imaging and recent developments make possible to achieve reliable quantitative 3D functional or molecular explorations with great future using enzymatically activatable probes for proteases such as MMPs and Cathepsines and molecular tracers for expression of integrins and exploration of hypoxia via carbonic anhydrase IX. Examples of applications for treatments with fibered plasma (plasma gun) on orthotopically implanted tumours will be presented as well as perspectives for associated in situ examinations by fibroscopy and per-operative fluorescence imaging.

Scintigraphy and Positron Emission Tomography are clinical molecular imaging modalities with satisfactory sensitivity and quantization capabilities, even for deep sites thanks to their 3D capabilities. They offer the most reliable strategy to explore specific biomarkers for tumour proliferation, apoptosis, angiogenesis and hypoxia for the translational research in medium size animals with spontaneous cancers such as cats and dogs in order to improve predictivity of efficacy studies before to move to humans.

Due to recent technological developments, Photo Acoustic Imaging combining 3D high resolution echography to absorption of pulsed laser light by oxy and deoxyhaemoglobin is a new and very promising resource to explore tumour oxygenation. Considering the implication of Reactive Oxygen Species (ROS) in the anti-tumour activity of non thermal plasma, hypoxia is a crucial parameter that should be managed to improve plasma efficiency.

Battery Operated, Room Temperature Atmospheric Plasma Jet for Biomedical Applications

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Atmospheric pressure cold plasmas (APCPs) have received a lot of attention due to novel applications such as surface and materials processing, synthesis of nano materials, and biomedical applications. Among them, biomedical applications of the APCPs, for example, sterilization, are of practical interest. In biomedical applications, a plasma jet which generates a plasma plume in an open space (surrounding air) rather than in a confined discharge gap has many advantages over a traditional dielectric barrier discharge (DBD) device. However, regardless of whether the plasma jet is driven by direct current (DC), kHz alternating current (AC), radio-frequency (RF) current, microwave (MW), or pulsed DC, line AC power of 110 V, 60 Hz (in US, Canada, Japan) or 220 V, 50 Hz (in Europe, Australia, Africa, China) is required to power the device. This has limited the portability of the device and is impractical where line power is not readily available, for example, in rural areas and battlefields. In addition, most of the reported plasma jet devices use noble gases or mixtures of noble gases with a small amount of O_2 as the working gas. There are relatively few plasma jets using ambient air as the working gas, and efficient and portable atmospheric plasma jets using air as the working gas have a large market.

In this report, a battery driven, room temperature atmospheric plasma jet is described. Although the plasma is driven by 12 V DC, the discharge has a frequency of about 20 kHz. Each pulse lasts for about 100 ns with a peak current of about 6 mA. Decontamination experiments show that the plasma emitted from this source not only deactivates the cells on the biofilm, but also penetrates through the 25.5 μm thick *Enterococcus faecalis* biofilm killing the bacteria. This is believed to be the thickest biofilm penetrated by a room temperature plasma jet.

Fig. 1. Photograph of the air plasma flashlight

The Suppression of Hypersensitivity Accompanied by Tooth Bleaching using Nonthermal Plasma

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Tooth hypersensitivity is a common side effect that occurs in the treatment of tooth bleaching [1]. Fluoride treatment has been known to cause less tooth sensitivity [2]. Thus, before or after the tooth bleaching, fluoride is generally applied to teeth. The purpose of this study is to investigate fluoride-coating effect of nonthermal plasma on human tooth.

Human enamel specimens were prepared and randomly divided into 7 groups: N1(2% NaF), N2(2% NaF + iontophoresis), N3(2% NaF + plasma), A1(1.23% APF gel), A2(1.23% APF gel + plasma), V1(5% NaF varnish), V2(5% NaF varnish + plasma). The samples were applied with fluoride products and plasma four times at a week interval. Fluorine content on fluoride-treated enamel was measured using electron probe micro analyzer (EPMA).

Only N3 group was detected to contain fluorine among the N groups, and the amount of coated fluorine decreased as frequency of plasma treatment decreased. More fluorine was detected in A2 than that of A1. Both V1 and V2 groups did not show detectable fluorine on tooth enamel.

This study suggests that combination treatment of plasma and fluoride products is highly recommended to suppress tooth hypersensitivity.

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Plasma meets Dermatology: Clinical aspects in preventive and curative Medicine

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Cold plasma has moved to a promising new treatment tool in medicine and especially in dermatology many applications seem realistic. Up to now superficial treatment is the dominating application mode of cold plasma opening a wide area of treatments on skin targeting epidermal and dermal disorders.
In relation to its distinct ways of action the plasma treatments can be separated into four sections, i) treatment of superficial skin and soft tissue infections ii) treatment of skin contamination and colonization iii) treatment of immunologic disorders and iv) tumor treatment.
The most important clinical indications of cold plasma in category i) are superficial and deep-sited dermal staphylococcal and streptococcal infections, but also parasitic and viral infections (warts) can be treated. Single but also multiple lesions like akne vulgaris can be effectively treated. To predict the plasma effect and the corresponding exposure time before treatment, susceptibility data of all relevant bacterial and fungal species are kept available in a reference data collection with continuous extension. A special goal in plasma therapy is the treatment of tinea and onychomycosis. The category ii) includes the eradication of pathogenic flora like MRSA and other multiresistant pathogens and constitutes a growing relevant factor in preventive medicine. It could be shown that plasma but not conventional antisepsis was able to decontaminate heavily colonized patient skin with MRSA and Pseudomonas. Additionally, cold plasma was shown to disinfect palmar skin in hand hygiene albeit time consuming and therefore not yet competitive to conventional antisepsis. Plasma susceptibility testing of most important clinical species showed excellent performance data and range cold plasma on the level of topical chemotherapy and antisepsis including environmental decontamination i.e. of fungal hyphae and spores. Category iii) includes treatments of diseased skin exhibiting vascular, skin barrier and connective tissue involvement. Cold plasma showed effectiveness in the therapy of psoriatric and sclerotic lesions. Category iv) involves plasma treatment of benign and malignant dermal tumors, like melanoma. In a mouse model plasma exhibits strong potency to destruct melanoma, to inhibit tumor cell spreading and to improve survival. Plasma can act synergistically to bioelectric therapy in curative and palliative tumor management.
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New therapeutic effects of plasma gas containing nitric oxide

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Nitric oxide (NO) is a short-lived signaling molecule that plays an important role in a variety of physiologic functions, including inflammation and wound healing processes [1]. Therapeutic effects of different plasma sources including plasmas generated gaseous nitric oxide (gNO) has been utilized for various clinical applications [2]. Particularly the device ‘‘Plason’’ generating plasma gas containing gNO in atmospheric arc discharge has been used beneficially in treatment of different wound pathologies (trophic ulcers, diabetic foot ulcer) as well as in traumatology, stomatology and other medical areas [3].
At high temperatures realized in the arc discharge (2000 – 4000^0K) in humid air highly reactive radical species like atomic oxygen (O), hydroxyl radicals (OH), atomic hydrogen (H) are formed in addition to NO, NO_2 and other nitrogen and oxygen containing species. We have calculated the concentrations of these species depending on the humidity and gas temperature. According to our simulation the concentrations of O > H > OH could be comparable with the concentration of NO generated in the discharge. The plasma flow after cooling to room temperature is composed of the warm air and the plasma-generated stable molecules like NO, NO_2, H_2O_2 and others. At present the therapeutic action of “Plason” is attributed both direct and indirect gNO regulatory effects on anti-microbe macrophages’ activity, microcirculation and regeneration and nerve conduction. We suggest it also could be intensified by the synergy effects of gNO/H_2O_2 and gNO/O_2 species [4].
Recently the unique composition of NO-containing plasma gas generated by “Plason” has been successfully used in treatment of burns, skin scars and joint diseases. Reduced bleeding and the absence of wound infection at the moment of early necrectomy of extensive and deep burns have been observed after “Plason” treatment in surgical-mode. Moreover, the post operative treatment of burns using “Plason” gNO-therapy mode significantly improved microcirculation and wound healing [5]. As it has been documented in the clinical trial on 160 patients, the neck and facial excessive skin scars after the gNO-therapy undergo flattening and became softer. At the same time the recurrence rate of surgically excised keloids decreased dramatically [6]. The high efficiency of gNO plasma therapy was obtained in treatment of rheumatoid arthritis, osteoarthritis (observed as pain relief, decrease of inflammation and improved join mobility) and sport’s traumas (healing of ligaments and muscles injuries) (E. Karpinskaya, unpublished data).

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Selective killing of ovarian cancer cells through induction of apoptosis by nonequilibrium atmospheric pressure plasma

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Recently, medical applications using nonequilibrium atmospheric pressure plasmas (NEAPPs) have attracted attention in the field of medicine because cells are not vacuum-compatible, and thermal damage to cells is negligible through appropriate tuning of the NEAPP parameters and experimental setup.

In this study, two independent ovarian cancer cell lines and fibroblast controls were treated with the high-density nonequilibrium atmospheric pressure plasma (NEAPP). Most ovarian cancer cells were detached from the culture dish by continuous plasma treatment to a single spot on the dish. Next, the plasma source was applied over the whole dish using a robot arm. *In vitro* cell proliferation assays showed that plasma treatments significantly decreased proliferation rates of ovarian cancer cells compared to fibroblast cells (Figure 1). FACS and Western blot analysis showed that plasma treatment of ovarian cancer cells induced apoptosis.

On the basis of these results, we propose the nonequilibrium atmospheric pressure plasma is a promising tool of anti-cancer therapy for the ovarian cancers.

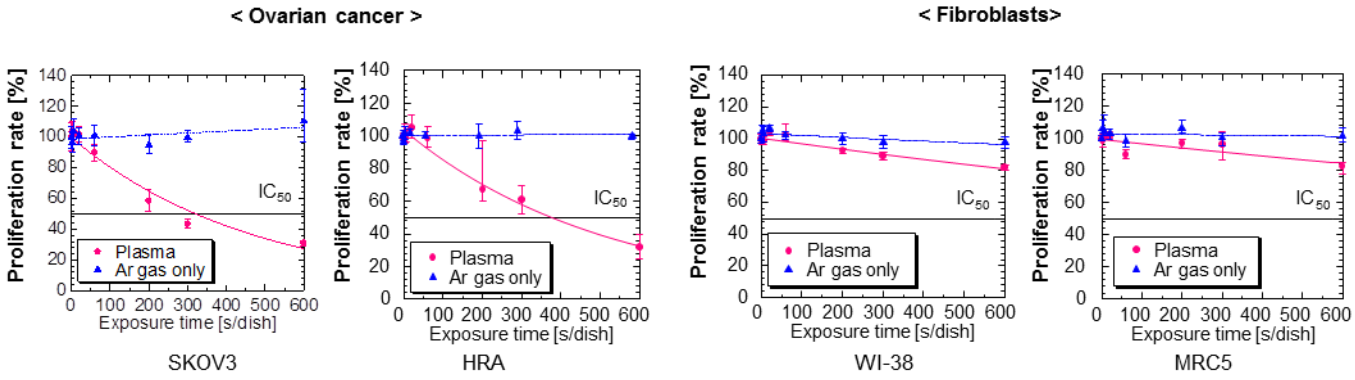

Figure 1: Ovarian cancer (SKOV3 and HRA) cells and fibroblast (WI-38 and MRC5) cells were treated with plasma. After 72 h of plasma treatments, cell proliferation was evaluated by MTS assays

Acknowledgments

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Pulsed Atmospheric-pressure Plasma Streams produced by Plasma Gun: characterization and application for tumor treatment

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The need for remote, controlled delivery of plasma through capillaries in, for example, endoscopic procedures has presented challenges to the plasma medicine community. In response to the need of controllable remote plasma sources, the Plasma-Gun (PG) has been developed. The first part of this presentation will emphasize the detailed experimental analysis of the PG over a large range of parameters including voltage pulse shape, repetition rate, polarity, plasma expansion capillaries [1] and capillary geometry (T-shape, branched). This experimental work and the results from simulations designed to model the fast ionization wave launching and propagation [2], confirm the new moniker of "Pulsed Atmospheric-pressure Plasma Stream" (PAPS) to describe the plasma produced by the PG. These works have also revealed specific features of streamer propagation in dielectric constrained volumes. The key roles of the electron drift in the space charge ionization front and of the impedance of the plasma tail connecting this ionization front with the DBD powered electrode, described by the simulations are in good agreement with experimental data. These results clearly show that plasma expansion and dose delivery can be carefully controlled. This opens up new possibilities for the PG development, including endoscopic plasma delivery strategies.

In the second part of this presentation, we will discuss results from recent studies of both *in vitro* and *in vivo* assessment of helium plasma jet antitumor activity on different cancer cell lines and tumors. In the context of results obtained on the anti-tumoral effect of DBD produced plasmas, the first demonstrations of antitumor activity by PGs will be presented, including the first demonstration of treatment of mouse orthotopic pancreatic carcinoma. In this particular case, the protocol was a three-time plasma delivery, of 10 minutes each, with the PG operating at 2 kHz repetition rate. Four groups of mice have been followed during the five week duration of the study. Tumor growth was monitored through bioluminescence imaging. Besides the control group, a group received a reference chemotherapeutic treatment; a group was treated with the PG, while another one had a combination of both. The PG treatment not only produced a significant reduction in tumor activity and volume, but also led to better results than the chemotherapy alone. Most striking was that the best results were obtained with the group that received both treatments. This is the first evidence *in vivo* for a potential benefit of the association of non thermal plasma and chemotherapy.

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Plasma + Bio – Basics and applications

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The list of possible applications of technical plasmas at low and atmospheric pressure in medical and pharmaceutical branches is still large. It spans from sterilization in ambulances and hospitals to in-line decontamination and sterilization in pharmaceutical production. In addition, wound healing, skin treatment and disinfection of large areas is also an important issue.

From a physical point of view there are some common and some completely different characteristics of low and atmospheric pressure plasmas. For example in most cases ions are absent at atmospheric pressure while in low pressure plasmas their influence is crucial. On the other hand the radical particles concentrations are very often one or two orders of magnitude higher at atmospheric conditions. Looking at VUV (vacuum UV) radiation, both types of plasmas may irradiate treated substrates in this wavelength range depending on gas mixture and the gas channel between the heated plasma zone and the remote or effluent part.

All applications of different plasmas in medical and/or pharmaceutical practice are similar in so far that an extensive and time and money consuming evaluation and certification procedure is necessary in order to transfer them into market. To gain an approval from the American FDA (Food and Drug Administration) or the European EMEA (European Medicine Agency), it is absolutely necessary to understand the basic mechanisms and correlations to the treated objects.

Therefore this talk will show some very basic aspects and results in low pressure plasma treatment as well as in atmospheric situation. From the perspective of plasma technology some principles of plasma diagnostics will be shown in order to reveal the fundamental plasma parameters and the corresponding particle and photon fluxes. From the biology perspective following the îDogmaï of cell biology the talk will show on which parts of cell proliferation and growth those particles and photons may attack or influence their behavior.

As a success story of this approach, the world wide first prototype of a low pressure plasma sterilizer in pharmaceutical production will be shown. In addition, many aspects for applying atmospheric plasmas will also be given.

The design of the PlasmaJet® thermal plasma system and its application in surgery

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The majority of recent papers in the field of plasma medicine have described the potential clinical applications of low-temperature or so-called "cold" plasmas, usually generated by a dielectric barrier discharge. These applications include disinfection and wound healing, but these non-thermal plasmas have low power density and have insufficient power to produce a surgical effect.

By contrast, to create thermal plasma that has a surgical effect requires an arc discharge in a device designed to deliver a much higher power density of 1 – 5 kW/mm^3. In the PlasmaJet® system a multiple electrode array is used to generate a thermal plasma with high power density that can deliver an output power of 20 – 300W using a very low flow of argon gas of typically 0.2- 0.6 l/min. The resulting plasma has the ability to cut and coagulate all tissues including bone.

The PlasmaJet system comprises a console providing an initial ignition pulse of up to 3kV followed by a DC voltage in the 30 – 50V range to maintain the plasma flow. The console also provides control electronics for the user interface and a circulating coolant to maintain the handpiece tip at a low temperature. A range of sterile single-use handpieces for open and laparoscopic surgery complete the system. The PlasmaJet system is both CE marked and FDA cleared for use in surgery, and extensive clinical experience including over 1,300 fully documented cases has confirmed its ability to cut with the precision of the surgical laser but with greater coagulation capability and enhanced safety.

As an electrically neutral energy source, the PlasmaJet handpiece provides a safer alternative to conventional electrosurgery. The thermal plasma can achieve temperatures in the region of 10 – 20,000 degrees Kelvin, but it is short-lived and at the low gas flow employed, the amount of damage to underlying and adjacent tissue is minimal – typically less than 0.2 – 0.5mm, which his less than that found with any other surgical technology.

The ability to cut tissue precisely and with simultaneous coagulation of the cut surfaces and minimal damage to underlying structures makes the PlasmaJet a valuable new energy source for use in surgery. The first papers have now appeared in peer reviewed journals confirming its abilities in a range of surgical applications.

This presentation will describe the PlasmaJet system, discuss the effects of this thermal plasma energy at the tissue level, and illustrate some of its applications in surgery.

Role of reactive species for bactericidal effect in air surface micro-discharge plasma

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The usage of cold atmospheric plasmas has high potential for sterilization because the plasma can produce relevant agents, e.g. reactive oxygen species, UV light [1][2]. In order to achieve an efficient and safe plasma treatment, it is necessary to understand the reactions of reactive species on bacteria. The air plasmas generate a very wide variety of reactive species and the gas phase air plasma chemistry is highly complex. So as a start-up of this topic, we estimated the ozone concentration in our plasma by UV absorption measurements and tested bactericidal property using *Escherichia coli*.

The plasma was produced using a surface micro-discharge (SMD) electrode which has an Al_2O_3 plate sandwiched by a planar metal electrode and a stainless steel mesh grid. By applying a sinusoidal high voltage of 10 kV_{pp} between the metal and mesh electrode, the plasma was produced on the mesh side. To vary the input power, frequency of applied voltage was changed from 5 Hz to 10 kHz. On the SMD electrode, a quartz tube of 10 mm high and 30 mm in diameter was placed. To make an almost closed volume, a ceramic plate was placed on the quartz tube. The ozone concentration in this closed volume was measured. For testing the bactericidal property from the plasma, *E. coli* inoculated on agar plates was placed instead of the ceramic plate.

When the input power was low, the ozone concentration was monotonically increased in the closed volume. Using very high specific input powers, the performance of ozone production was drastically changed and the ozone concentration was kept low (ozone-less mode) [3]. In the presentation, we will report time evolutions of ozone concentration in the closed volume and discuss the relation between the ozone concentration and bactericidal property.

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Conformal Atmospheric Pressure Plasmas for Biomedical Applications: Along Surfaces, Inside Tubes and Penetrating Cracks

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The direct use of atmospheric pressure plasmas (APPs), such as dielectric barrier discharges (DBDs) and ionization waves (IWs), in biomedical applications rely on delivery of active species to non-planar surfaces and remote locations. In the treatment of human tissue, the surface is rarely flat and may have convex, concave or sloping topography. In plasma sterilization of surfaces or delivery of plasma to remote locations, surfaces range from branched tubes and channels, to deep cracks as might be encountered in deactivating bacteria or viruses on industrial contaminated surfaces. The common feature of these APPs is the ability, or need, for the plasma to propagate in a conformal manner along the surface. In this paper, results from computational and experimental investigations of conformal propagation of APPs for biomedical applications will be discussed. The computations were performed using a 2-dimensional plasma hydrodynamic model.[1] The experiments provide ns resolved images of the conformal propagation of APPs.[2]

We found that pulsed APPs in the form of IWs, as might be launched in DBDs, conformally propagate along surfaces in a manner determined by the capacitive charging of those surfaces. Propagation is slowed in regions of high capacitance and speeds up in regions of low capacitance. As gas phase streamers charge surfaces, components of the electric field are produced parallel to the surface which directs the now surface wave to uncharged regions. Model results and imaging of the propagation of surface hugging IWs in branched tubes are explained by the reliance on surface charging to split IWs and turn corners. This also contributes to DBD filaments which strike sloped surfaces, such as human tissue, being able to fairly uniformly treat the surface. (See Fig. 1.) The IW propagating along the surface is directed by electric fields oriented towards uncharged regions. Results will also be discussed for plasma propagation into high-aspect-ratio features such as cracks.

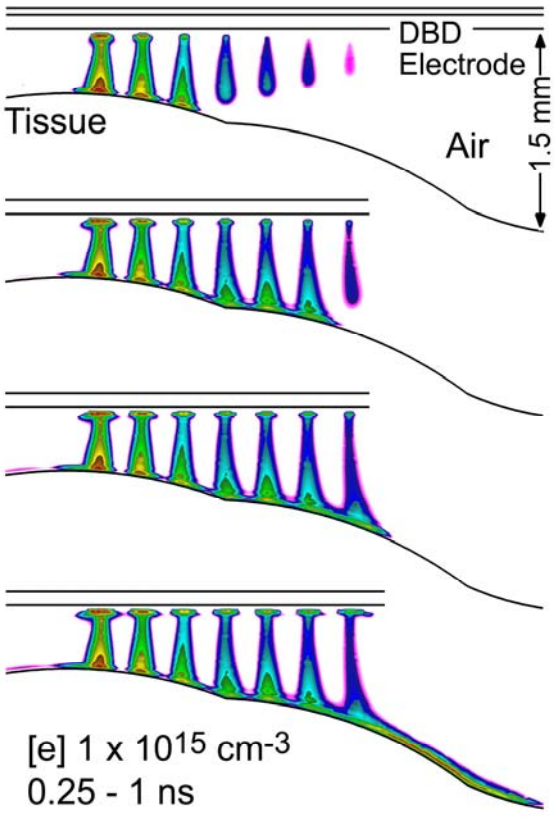

Figure 1: *Electron density for simultaneously launched DBD filaments in air striking human tissue, and conformally propagating along the surface.*

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Helium plasma microjet for combined RF radiation and plasma treatment

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The estimation of electrical characteristics of radiofrequency (RF: 13.56 MHz) He plasma jets in open atmosphere without any shielding is complicated by the existence of emitted RF radiation which disturbs electrical measurements. In this work we generate a plasma jet by applying rf power to a hollow needle through which there is a flow of helium. In this configuration, the radiated RF power exceeds the power deposition in the plasma (see fig. 1), and the classical technique for determining I (V) through a phase shift analysis is inaccurate. We present an alternate method for determining the ratio between the power dissipated in plasma jet and the radiated power.

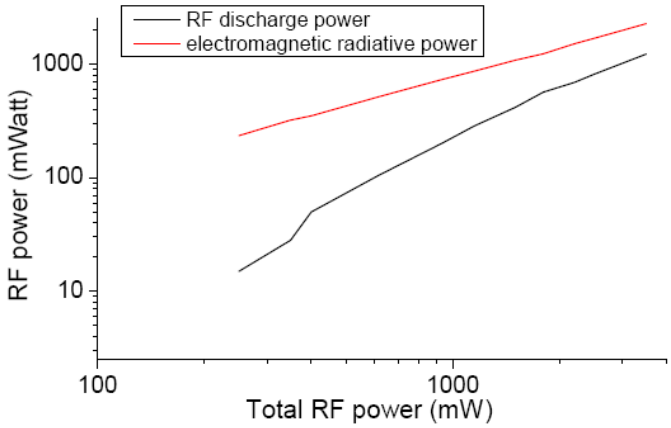

Fig 1: *RF power deposited in the helium plasma jet compared to radiated RF power.*

In term of applications, this work demonstrates that plasma jets generated by radiofrequency excitation are always accompanied by RF radiation. This last point is not necessarily a negative because some treatments use radiofrequency to treat tumors [1].

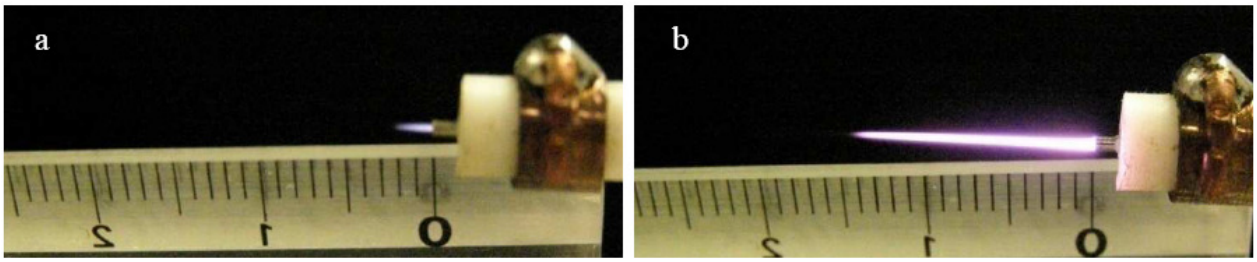

Fig 2: *RF He plasma jet: a) 15.6 mW, b)1.8W accompanied respectively by irradiated powers of 135mW(a) and 3.2 W(b)*

Supported in part by ANR through the project PAMPA.

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kinpen MED: a plasma source for clinical trials

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Atmospheric pressure plasmas are very promising tools for biomedical applications and are expected to bring new therapeutic options in surgery, dentistry and dermatology [1]. Currently, many scientific activities are related to basic research to study plasma-cell interaction or to explore the application spectrum in different fields of medicine. Meanwhile, a series of clinical trials have started to show efficacy and safety, but only few atmospheric pressure plasma sources are available as approved medical device.

Within the joint research project "Campus PlasmaMed II" the device "kinpen 09" is widely used as standard plasma source for research on biomedical applications. Very promising pre-clinical results have shown advantages of plasma based therapies in skin disease treatment [2, 3, 4]. The next step towards an accepted therapy and a medical device is a clinical trial. A device to be permitted for clinical trials has to fulfill specific technical requirements. Therefore the "kinpen 09" plasma source has been redesigned and the new type "kinpen MED" was constructed. Basic plasma properties were kept whereas special attention has been paid to safe and easy operation. The device was successfully tested (IEC 60601-1 and EN 60601-1-2) and approved for clinical trials.

In this contribution the "kinpen MED" and its basic characterization concerning gas temperature, ultraviolet irradiance and ozone concentration, obtained in three different measurement conditions, will be discussed. Furthermore specific aspects related to the development of a plasma source as medical device are given. Currently, the "kinpen MED" is used in a clinical trial at "Charité" for ulcus cruris therapy. First results of this study will be presented.

Acknowledgements: This study was realized within the joint research project "Campus PlasmaMed II" supported by the German Federal Ministry of Education and Research (grant no. 13N11188)

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Controlling aqueous phase reactive chemical species generated by air surface micro-discharge

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It is well established that atmospheric pressure air plasmas generate a wide variety of reactive oxygen and nitrogen species (RONS) in gas phase. For biological and medical applications, aqueous-phase chemical reactions and mass transfer are also thought to play crucial roles because cells and tissues to be treated are mostly immersed in or contact with aqueous solution.

Several recent reports demonstrated that plasma-treated water or other solution has antimicrobial activities [1-5]. In our recent report [4], we showed that plasma-activated water (PAW) maintains the sterilization activity at least for 7 days after PAW generation. Interestingly, the concentration of chemical species in plasma-treated water shows surprisingly dynamic behavior during the 7-day period. This is a clear indication that gas/aqueous-phase reactions and mass transfer is quite important. A challenge is to find a way to precisely control the plasma chemistry and to identify RONS stored in plasma-treated liquid medium.

In this study, we focus on aqueous-phase RONS generated by surface microdischarge (SMD) in air [6]. 150 μl of phosphate buffered saline (PBS) with E. coli K12 was added to a small glass vial (15 mm in diameter and 45 mm in height), covered by the SMD device, and treated for 0.5-5 minutes. After plasma treatment, suspension were plated on agar and incubated overnight, after which colonies were counted to determine the number of viable cells. Hydrogen peroxide was measured with an electrochemical probe. Nitrite and nitrate were quantified using UV absorbance spectroscopy. Our results showed that RONS stored in plasma-treated PBS shows three different modes, depending on power consumed in plasma. At high power density (> 0.2 W/cm^2) nitrite was dominant, while nitrate became dominant over nitrite around 0.1 W/cm^2. When power decreased further (< 0.05 W/cm^2), nitrite and nitrate concentration monotonically decreased. However, the inactivation rate was highest at the low power mode and the antimicrobial efficacy significantly dropped when the power density was higher than 0.1 [W/cm^2]. Our gas-phase spectroscopic measurement suggests that ozone plays a key role at the low power mode. These results clearly show that air plasma chemistry is quite dynamic and that RONS can be modulated by carefully controlling discharge conditions.

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Plasma chemistry in atmospheric pressure plasmas with varying air and humidity

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For sensitive surface treatments in bio-medicine/bio-plasma applications, one of the most essential species is reactive oxygen species. Therefore, in the experiments using micro-scale atmospheric pressure plasma jets (μAPPJs), a small amount of oxygen is added to a carrier gas, helium. In order to further understand the underlying operating principles of the μAPPJ system and to optimize its performance in applications, it is important to know the chemical kinetics of the He-O_2 plasma containing a moist ambient air as an impurity. We describe the influence of humid-air on reactive species in a He-O_2 plasma for wide air fraction of 0-500 ppm with the relative humidity of 0-100% as determined through a zero-dimensional time-dependent global model. Comparisons made with experiments using an rf driven μAPPJ and one-dimensional simulations [1, 2] suggest that the plausible air impurity level in the experiments is not more than hundreds ppm. The evolution of species concentration and its complex chemical links are described for reactive oxygen species, metastable species, radical species and positively- and negatively-charged ions (and its clusters) (Fig. 1). Effects of the air impurity containing water humidity on electronegativity and chemical activity are clarified with particular emphasis on reactive oxygen species.

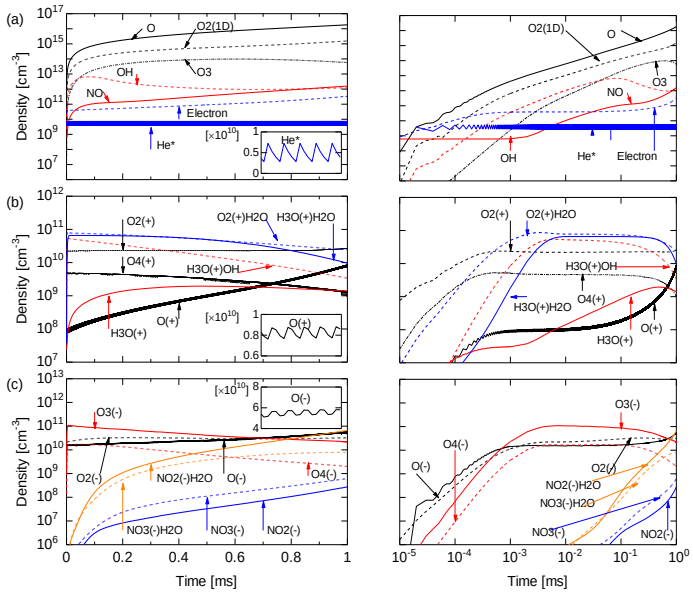

Figure 1: *Temporal evolution of (a) neutral species, (b) positive ions and (c) negative ions. Left-hand side: linear-time-scale plots. Right-hand side: log-time-scale plots.*

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Plasma-generated reactive oxygen species for biomedical applications

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Reactive oxygen species (ROS) are well known to play an important role in several biological systems. However, the production of ROS exceeding the ability of the organism to mount an antioxidant defense results in oxidative stress. If the amount of oxidative damage overcomes the repair capacity of a cell, this can ultimately lead to cell death, which is very important to be taken into account for biomedical applications of plasmas. To get a better insight into the effects of ROS on cellular components, fundamental studies are essential to determine firstly the nature and concentration of plasma-generated ROS, secondly the chemistry induced in biological liquids by those ROS, and finally the ability of this "cocktail" of reactive species (plasma-generated ROS and the others created within the liquid by those ROS) to damage biomolecules. In this context, we have measured the absolute density of two of the main ROS created in three different atmospheric pressure plasma sources: two geometrically distinct radio-frequency-driven microplasma jets (μ-APPJ [1] and kinpen [2]), and an array of microcathode sustained discharges [3]. Optical diagnostics of the plasma volumes and the effluent regions have been performed: ultra-violet optical absorption for ozone (O_3), and infra-red emission for singlet-delta oxygen ($O_2(a^1\Delta_g)$) [4]. High concentrations of both ROS (10^{14}–10^{17} cm^{-3}) have been efficiently obtained in the gas phase at low gas temperatures (~300 K). The effect of different parameters, such as gas flows and mixtures, and power coupled to the plasmas, on the production of both ROS has been studied. Opposite trends for the different ROS densities within the operational range of each plasma have been observed. Thus, the control of the operating conditions enables to tailor the ROS composition of both plasmas towards different biomedical applications. For plasma medicine, the determination of the reactive species present in plasma-treated liquids is of great importance. In this work, we focused on the measurement of NO_2^-, NO_3^- and OH•, generated in physiological solutions like sodium chloride solution, cell culture medium, and phosphate buffered saline. The detection of these reactive species has been done via electron paramagnetic resonance spectroscopy and colorimetric assays. Additionally, the pH value and concentration of H_2O_2 have also been measured by electrochemical detection.

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"Plasma bullets" propagation inside of tissue and agarose tissue model

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Non-thermal plasma jets in open air have been previously shown to be composed of ionization waves commonly known as 'plasma bullets' propagating at high velocities [1,2]. One of the obvious and exciting applications of this type of plasma is treatment of internal organs, for example lung cancer treatment. However, if the conductivity of a tube in which plasma bullets are propagating is relatively large, the plasma formation extinguishes and consequently treatment is impossible. Here we show the possibility of plasma bullet propagation inside of tissues and agarose tissue models with ionic type of conductivity. We study the effects of agar conductivity and tube diameter on length and velocity of propagation, and emission spectra, as well as production of hydrogen peroxide in the agarose gel.

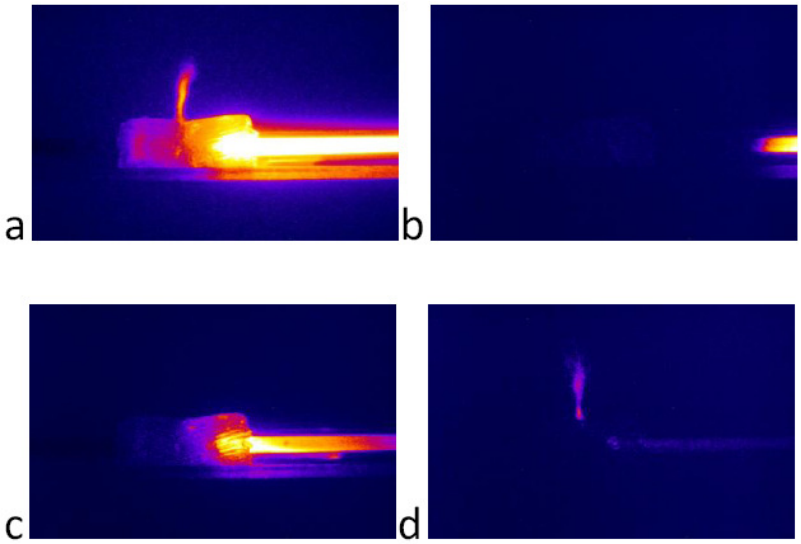

Figure 1: *Plasma bullet propagation in agarose gel with an L-type tube: images taken with a high-speed ICCD camera a) exposure time 20 us b-d) exposure time 100 ns, delays b) 0.3 us, c) 0.6 us, and d) 1.5 us.*

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Remote and direct plasma processing of cells: how to induce a desired behavior

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The interplay between plasma processes and the biological environment is a long and intriguing story that spans different applications from surface modification of biomaterials to the direct interaction of plasma with cells. This makes plasma processes a very powerful tool in such distant biomedical fields as tissue engineering and sterilization. In vitro cell culture experiments represent the best way to fully understand the more subtle and fundamental interactions between the chemical species produced by plasma processes and cells. In this presentation the use of cell lines will be highlighted since it allows a high reproducibility and control of results[1]. Three main items, ranging from low pressure plasma modifications of 2D and 3D materials to Dielectric Barrier Discharges (DBDs) directly on cells, will be addressed.Due to their versatility in tailoring surface properties of materials used in different applications, cold plasma processes are utilized to dictate the interactions of proteins, cells, and biological tissues with biomaterials, membranes and biomedical devices, to induce desired cell responses. For example, the behavior of Saos2 osteoblast-like cells on surfaces with different roughness and morphology, produced by RF glow discharges, fed with hexafluoropropylene oxide[2], will be shown. In case of 3D objects, such as scaffolds for tissue engineering, the aim of the surface modification is to allow cell colonization of the entire substrate since usually a higher cell colonization at the scaffold periphery and inadequate colonization at its core have been reported [3]. It will be shown how this has been achieved using a sheath with chemical characteristics different from those of the core. Porous polycaprolactone scaffolds, fabricated by solvent casting/particulate leaching technique, were plasma-coated with PEO-like coatings, having different cell unfouling characters [4]. Finally, the direct effect of DBDs applied directly on two different cell lines will be shown. For the same process applied to different cell lines; a primary line: NHDF fibroblasts, and an immortal line: Saos2 osteoblasts, varying results on cell proliferation and morphology have been observed. A stimulatory effect was observed on NHDF cells while in case of Saos2 inhibition of cell adhesion and growth was directly dependent on the increase of plasma doses. The obtained results demonstrate that by properly tuning the dose of exposure of cells to air plasma, it could be possible to induce selective effects on cell growth of different cell types. This would in turn be useful in different fields of medicine such as treatment of cancer, wound healing and tissue regeneration.

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Immobilization of Biomolecules onto Magnetic Nanoparticles Functionalized by RF Plasma and Its Medical Application

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Magnetic nanoparticles have many great interests in potential to biomedical application such as high-sensitive virus detection, drug delivery system, hyperthermia treatments, magnetic resonance imaging contrast enhancement, etc [1]. Carbon coating of the magnetic nanoparticles can leave the toxicity out without detracting their magnetic properties and stabilize the nanoparticles so that compatible to be used in bioapplications. Among various functional groups for medical application, the introduction of amino groups composed of primary amines to the particles surface achieves enhanced wettability and improves its adhesion. However, this modification has not been deeply studied on carbon encapsulated magnetic nanoparticles. In fact very few information can be found on the topic of graphene layer-encapsulated iron nanoparticles related to the plasma surface treatment in order to introduce nitrogen-containing group functionalities, such as amino group.

In this study, we functionalize the graphene layer-encapsulated magnetic nanoparticles fabricated by dc arc discharge[2] by using Ar and NH_3 inductively-coupled RF plasmas[3]. After plasma treatment, the biomolecules are immobilized to the particles to test the role of the nitrogen-containing group as a linker to the biomolecules. The schematic sequence of the experimental steps is illustrated in Fig. 1. The amino functional groups produced in the second step are expected to serve as an anchor for covalent bonding with aldehyde groups of oxidized dextran. This step is followed by chemical derivatization method using 4-(trifluoromethyl)-benzaldehyde ($C_8H_5F_3O$) to determine the amount of free amino groups on the surfaces. For comprehensive discussion, X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS) and high resolution-transmission electron microscopy (HR-TEM) are used to characterize and analyze the results. These experimental results are presented and discussed at the conference.

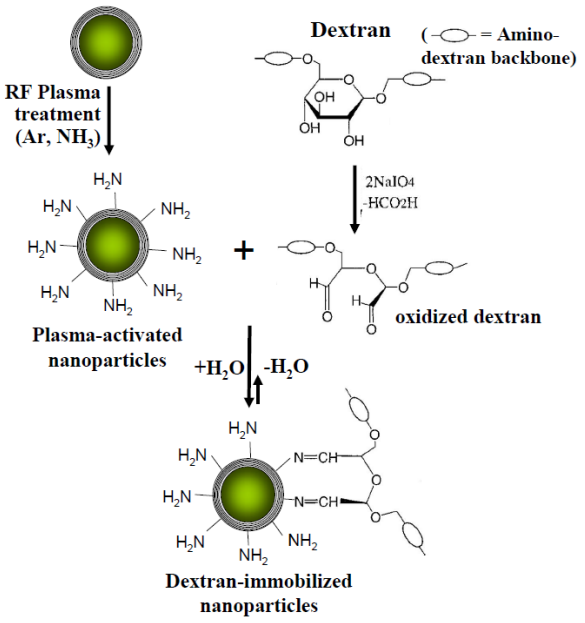

Figure 1: *Schematic illustration of plasma surface modification of nanoparticles.*

Acknowledgments

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Enhancing the bioactivity of the surfaces using atmospheric plasma deposited coatings

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Surface properties play a key role in the biocompatibility of implant medical devices. Atmospheric plasmas provide a means for tailoring surface properties at the biointerface through the deposition of coatings. This presentation provides an overview of a wide range of plasma polymerised coatings, which can be deposited using atmospheric plasmas. It details how both plasma processing conditions and precursor type influences the chemical and physical properties of the deposited coatings. The effect of coating properties on protein adhesion is also described.

Coatings were deposited from both gaseous and liquid precursors, using both the PlasmaTreat (air) and PlasmaStrean (helium) atmospheric plasma jet systems. The coatings were deposited from siloxanes, fluorosiloxanes, fluoorpolymers and quaternary ammonium salt precursors. Techniques such as in-situ reflectance IR and optical emission spectroscopy were used to monitor and control the deposition process. The adhesion of bovine serum albumin (BSA), immunoglobulin G (IgG) and fibrinogen (Fg) proteins, onto a number of the coatings, was evaluated under flow conditions, using a spectroscopic ellipsometry technique. The level of protein adhesion was determined in phosphate buffer solution on both uncoated and coated silicon wafer substrates.

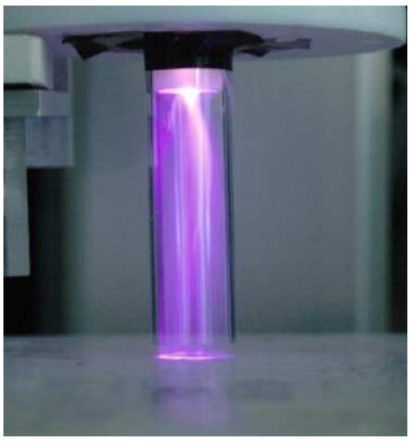

Figure 1: *PlasmaStream helium atmospheric pressure plasma jet system*

The deposited coatings were tailored to yield a range of properties as required, such as water contact angles of less than <10° (superhydrophilic) to >150° (superhydrophobic). Coating roughness (Ra) was altered from smooth (Ra <5 nm) to rough (Ra >100 nm). By controlling the deposition conditions of a HMDSO precursor for example using the PlasmaStream system
(Figure 1); coatings exhibiting either a flat surface morphology, an array of spherical clusters (up to 500 nm in diameter), or a densely packed arrangement of aligned fibres were obtained. In the case of the latter, individual fibres with diameters of up to 300 nm and fibre lengths of up to 12 μm were deposited. The level of protein adhesion was found to be dependent on both the coating and protein type. Extremely low levels of protein adhesion were observed on superhydrophobic coatings. While on hydrophobic surfaces BSA was found to adsorb via a single step mechanism, while Fg was shown to undergo multistage adsorption, indicating a structural rearrangement of the protein layer.

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Cell adhesion and morphology on plasma copolymerized PEG-PCL thin films

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Poly (ε-caprolactone)-poly (ethylene glycol) (PCL-PEG) copolymers have great potential applications in the fields of tissue engineering, pharmaceutics and medicinal chemistry. In the present work we have developed, amphiphilic biodegradable PCL-PEG copolymer coatings by catalyst free ROP of ε-caprolactone (ε-CL), in presence of 2-Methoxyethylether (DEGME). A low pressure inductively excited RF (13.56MHz) plasma reactor, designed for the deposition of copolymers [1], [2], was operating in the pulsed mode with Argon as the carrier gas. Experiments were performed at different ε-CL/PEG monomer feed ratio and effective power.

Cellular adhesion tests have been performed using 3 different cell types: human ovarian carcinoma cell line (NIH:OVCAR-3), human bone marrow endothelial cells and human fibroblast (3T3) (see. Fig. 1). Cells were cultured in the RPMI-1640, which was supplemented with 1% (v/v) antibiotics (10,000 U/ml penicillin-G sodium, 10mg/ml streptomycin), 2mM L-glutamine and 10% fetal bovine serum. All cells were expanded by routine cell culture technique in 25 cm^2 cell culture flasks which were incubated in a humidified atmosphere of 95% air and 5% CO_2 at a constant temperature of 37 °C for 24 hours.

The results have shown that it is possible to control and achieve good cellular response for PCL-PEG 33:67.

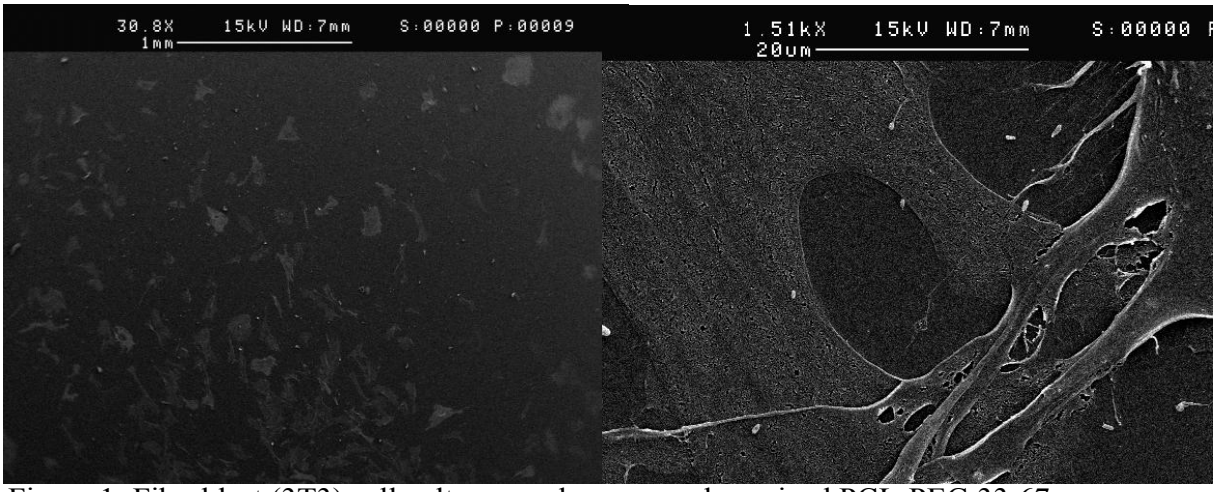

Figure 1: Fibroblast (3T3) cell culture on plasma copolymerized PCL-PEG 33:67.

The authors gratefully acknowledged the Institut de Biologie Intégrative (IFR 83, UPMC / INSERM) for SEM analysis.

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Plasma deposition of thermo-responsive films of N-isopropylacrylamide using DBD at atmospheric pressure

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Thermo-responsive polymers are of great interest as functional coatings in biomedical applications. A widely used compound is poly-N-isopropylacrylamide (PNiPAAm) which switches its surface properties from hydrophilic to hydrophobic by passing the lower critical solution temperature (LCST) of 32 °C. PNiPAAm coated substrates can be used e. g., for controlled attachment and detachment of cells [1, 2].

In the literature, the deposition of PNiPAAm films by graft polymerization on activated substrates or by low pressure plasma polymerization has already been described [3-5]. In this paper plasma deposition of N-isopropylacrylamide on polypropylene foil was performed using a dielectric barrier discharge (DBD) at atmospheric pressure. High monomer retention, which is a precondition for thermo-responsive behavior, was achieved under pulsed plasma deposition with duty cycles $D= t_{on}/(t_{on}+t_{off})$ of $D = 0.02 – 0.1$. At room temperature a water contact angle < 10° was observed on plasma polymerized NiPAAm (pp-NiPAAm) films which rose to 25° for films deposited with $D = 0.05$ and $D = 0.02$, resp. While these films show measurable thermal response, the stability after storage in water was insufficient.

Film stability was improved by copolymerization with glycidyl methacrylate (GMA), leading to higher contact angles of the freshly prepared films. Due to cross-linking within the plasma polymerized film no sharp LCST, but a temperature range between 30 and 40 °C was observed (Fig. 1). The total increase of the water contact angle of a pp-NiPAAm-co-GMA film was typically about18°.

Future work has to be done to ensure long-term stability of these films, particularly in aqueous environment.

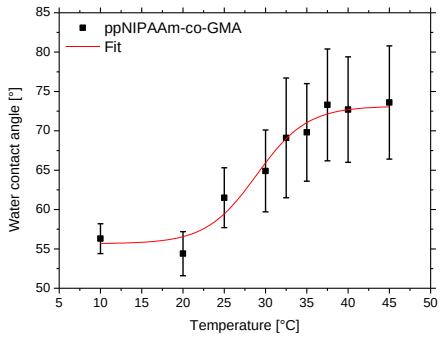

Figure 1: *Water contact angle measurements of pp-NiPAAm-co-GMA in dependence on the different temperatures.*

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Plasma Modification For Designing Reversible Mechanoresponsive Bioactive Surfaces

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The design of responsive materials and in particular mechanosensitive materials is now thoroughly investigated and it emerges as an extremely hot topic [1]. We present here two examples of mechanoresponsive surfaces designed *i)* by using plasma polymers as platforms to attach materials sensitive to the mechanical stimuli or *ii)* by exploiting intrinsic properties of plasma polymers to change their performances under stretching.
The first example concerns chemo- and cyto- mechanoresponsive surfaces (figure 1) which become proteins adsorbent or cell adherent under stretching in a fully reversible way. Our strategy is based on grafting ligands directly on a plasma modified elastomeric substrate embedded in a PEG brush [3]. By stretching the substrate, the ligands become accessible to proteinic receptors. Retunrning to the non-stretched state, the pressure exerted on the proteins induces their expelling assuring a full reversibility of the process.

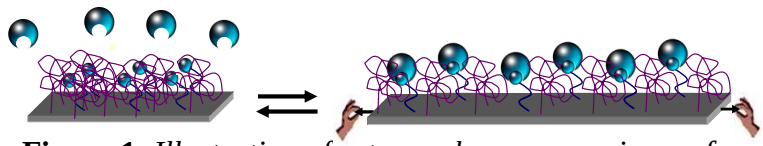

Figure 1: *Illustration of cyto-mechanoresponsive surface*

The second example concerns the effect of mechanical stimuli on the release of bioactive agents (here antibacterial agent, figure 2) from a plasma multilayer matrix [4]. Owing to differences between mechanical properties of plasma-polymer thin films and the elastic bulk substrates, tensile strengths generate cracks within the plasma polymer, which might be used as diffusive channels for bioactive substances located between two plasma polymer thin films. The originality of this system is that the aperture of the crack can be controlled mechanically in a reversible way.

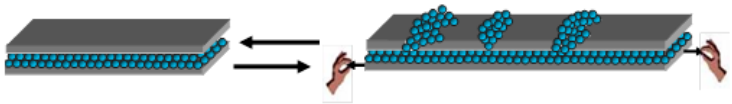

Figure 2: *Illustration of Mechanoresponsive bioactive surface*

Such surfaces would not only be of fundamental interest but could also present numerous potentialities from a technological point of view.

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Thromboelastography cups and pins for improved blood coagulation testing: Surface modification by plasma coating

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One of the current challenges to render the performance of bio-technologies such as thromboelastography (TEG) more reliable is the development of new materials; in the case of TEG, control of the coagulation properties of anticoagulated blood plasma and unmodified whole blood are currently not satisfactory. Plastic cups used to hold the blood sample being analyzed are made of "Cyrolite", a type of methacrylate, in which the clotting processes starting between 15 to 60 min are uncontrollable. The contact coagulation pathway can be triggered by selective adsorption of Factor XII to negatively charged surfaces, such as silicates, which alters the protein conformation to expose a serine protease active site. We began with the hypothesis that surface-modification of TEG cups and pins will change the coagulation behavior of recalcified, citrated human blood plasma and of unmodified human whole blood: The presence of (positively-charged) amine groups should inhibit clotting, while (negatively-charged) carboxylate ($-COO^-$) groups should accelerate it [1,2]. Therefore, surfaces of TEG cups and pins were modified so as to change their properties, for example their surface chemistry and free-energy, and thereby to promote reproducible, rapid clotting time. This is being examined by using four different types of glow-discharge plasma deposits: (i) "low-pressure N-containing plasma-polyethylene" (L-PPE:N), rich in primary amine groups [3]; (ii) its O-containing counterpart (L-PPE:O) with COO^- groups; (iii) plasma-polymerized hexamethyldisiloxane (PP-HMDSO), to create a hydrophobic surface [4]; and (iv) silica (SiO_2, glass-like coating) from a HMDSO/O_2-Ar mixture. TEG cups and pins with these different surfaces were tested for the coatings' influence on TEG performance. Custom-made metal electrode-moulds assured complete contact with the cups' slightly tapered outer walls, while silicon wafers placed inside the cups and XPS analyses were used to monitor uniformity and composition of deposits. Preliminary data show that anionic coatings tended to accelerate coagulation of recalcified human plasma, while cationic ones slightly delayed coagulation time. These observations are consistent with differential interaction of the modified TEG cups with clotting factors of the contact pathway such as Factor XII.

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Differentiation of Motor neurons Derived from Embryonic Stem Cellson a Polymerized Surface by Plasma

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The embryonic stem cells (ES) are undifferentiated cells capableof renewing themselves and under certain physiologic or experimental conditions; they can be induced to become tissue or organ specific cells with special functions [1]. Spinal motor neuronsrepresentasubtype of CNS neuronstothespecificationwhichneuronalpathwayshave been defined. Ectodermal cells take on a rostral initial neuronal through the regulation of BMP, FGF, and Wnt signaling. Rostral neural progenitor cells acquire an identity of spinal position in response to signals inducing caudalización RA (retinoic acid). Subsequently, spinal progenitor cells acquire the identity of MN progenitors in response to the action of ventralization of Sonic Hedgehog (Shh). [2,3].

Once a specification of motor neuron gain is dispensable to be carried to the morphological differentiation process, in which the contact surface wherein the differentiation is carried must provide an adequate anchorage to allow the morphology characteristic of motor neurons.An alternative to improve the adhesion properties of the surfaces of seed is used the polymerization by plasma using apyrrole monomer, where modified the surface characteristics of materials to increase their seeding adhesion properties, has been found that the polypyrrole synthesized by plasma creates a surface layer rich amine which promotes cell adhesion [4].

In this paper evaluates the properties of pyrrole as growth substrate promotes cell adhesion and therefore the differentiation of motor neurons derived from embryonic stem cells, cells will grow and then be differentiated on cover slips coated with polypyrrole synthesized by plasma, were tested for cell viability on the polymer that tried in good faith the proper functionality of motor neuronsand to verify that the polypyrrole synthesized by plasma in a good substrate biocompatible growth that does not alter physiological properties of motor neurons and allowing cell differentiation.

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Plasma Treatment for Dental Restoration Application

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Polymethacrylate-based dental composites have received widespread clinical acceptance as alternative restorative materials to dental amalgam amid concern regarding the potential health risks associated with mercury release. As supported by results from multiple clinical and laboratory studies, however, the current dental composite restorations suffer from much reduced longevity mainly due to interfacial failures of dental adhesives to the surrounding tooth structures, which cause microleakage, sensitivity, recurrent caries, and composite restoration failure [1,2]. **A**dequate dentin/adhesive bonding requires dispersion of the adhesive throughout the dentin surface and micromechanical interlocking of adhesive with collagen fibrils in decalcified dentin [3].

The objective of this study is to investigate the non-thermal atmospheric plasma treatment effects on dentin surfaces for oral bacterial disinfection, dentin surface modification, adhesive wettability improvement, and composite restoration bonding enhancement. Oral bacteria of *Streptococcus mutans (S. mutans)* and *Lactobacillus acidophilus (L. acidophilus)* with an initial bacterial population density between 1.0×10^8 and 5.0×10^8 cfu/ml were seeded on various media, which including porous filter papers, smooth glass slides, hydroxyapatite disks, and dentin slices from extracted human teeth. The survivability of these oral bacteria with plasma exposure was examined and evaluated. The plasma exposure time for a 99.9999% cell reduction was less than 15 seconds for *S. mutans* and within 5 minutes for *L. acidophilus*. Scanning electron microscopy (SEM) was used to examine the cell structural changes upon plasma treatment. It was found that the plasma treatment induced a significant alteration in cell size and morphology when compared with the untreated controls.

To evaluate the dentin/composite interfacial bonding, extracted unerupted human third molars were used by removing the crowns and etching the exposed dentin surfaces with 35% phosphoric acid gel. The teeth thus prepared were sectioned into micro-bars as the specimens for tensile test. Student Newman Keuls (SNK) tests showed that the bonding strength of the composite restoration to peripheral dentin was significantly increased (by 64%) after 30 s plasma treatment of the dentin surfaces. Fourier transform infrared (FTIR) spectra of plasma treated dentin surfaces showed two major structural changes of the demineralized dentin after plasma treatments as compared with the untreated controls. First, a new shoulder peak around 1,760 cm^{-1} associated with carbonyl stretch was found. Second, an amide II shift of ~10 cm^{-1} was observed (1,543 cm^{-1} before to 1,533 cm^{-1} after), which might indicate the secondary structural changes of dentin collagen after plasma treatment. These chemical changes of the collagen fibrils may allow more interactions with the adhesive resins applied subsequently. The findings from this study indicated that non-thermal atmospheric plasma technology is very promising for dental clinical applications.

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Plasma deposited Poly Lactic Acid- like films containing elastin by means of an aerosol assisted DBD

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The ability to impart desirable mechanical, physical and chemical properties makes natural and synthetic polymers well-suited for biomedical purposes. The main obstacle in engineering optimal biomaterials is to match and to align the required properties in shape and composition using one specific material or strategy. As an example, biocompatible synthetic polymers (*e.g.* PLA, PCL, etc.) lack of specific binding sites for cells attachment and tissue regeneration and therefore several approaches have been developed to introduce bio-related functionalities. Among these, elastin-based materials are becoming popular thanks to the remarkable biomechanical and biological properties of elastin in cellular activity [1]. Various technologies for surface modification, including physical adsorption and dry low pressure (LP) plasmas [2, 3], have been tested to bind peptides or proteins to the material surface. Nonthermal plasmas at atmospheric pressure (AP) can represent a feasible alternative approach with some advantage over low pressure plasmas [4]. An expensive and time consuming vacuum system is not necessary and the technology has the ability to be scaled-up and integrated in inline processes.

Our approach consists in a one-step immobilization process in an atmospheric pressure plasma. In this process, elastin is dissolved in a water solution of lactic acid (LA) used as the precursor for the coating. The system for coating deposition consists of a parallel plate dielectric barrier discharge coupled with an atomizer for the LA/elastin aerosol generation. The liquid precursor acts as a protective shell for the biomolecules preserving its structure and functionalities from the plasma active species [5]. The effect of the aerosol amount in the gas feed was found to be the key parameter influencing the retention of the monomer chemical structure in the coating, i.e. the highest content of carboxylic (and/or ester) groups directly involved in the (bio)degradation process is obtained at high aerosol concentration. The aim of the work was also to combine the (bio)degradation properties of the organic matrix with bioactivity promoted by the presence of elastin, which is able to interact with polar functionalities of the growing coating thanks to its hydrophilic domain. Several complementary surface analysis techniques, e.g. ATR-FTIR, XPS and UV-VIS, were used to investigate the chemical composition of the deposited films. When the highest elastin concentration is used during coating deposition, the protein is clearly embedded in the PLAlike film. Amino and amide functionalities (found in both ATR-FTIR and XPS measurements) are in fact ascribed to the presence of the protein since the PLA-like film deposited without elastin is nitrogen free.

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Plasma Surface Modification of Artificial Bones for Bone Regeneration

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In recent years, plasma technologies for biomedical applications have been extensively studied because of their potentially large future market [1]. Clinically, various types of porous hydroxyapatite (HA) have been used as materials for bone substitutes in the field of orthopedics because of their superior properties as scaffolds for osteogenic cells, such as high osteoconductivity, high biocompatibility and sufficient mechanical strength [2]. However, the properties as a bone substitute may be further improved for wider clinical applications if the surface is further biofunctionalized by plasma treatment. It has been found that a dielectric barrier discharge (DBD) plasma treatment promotes hydrophilicity of interconnected porous calcium hydroxyapatite (IP-CHA) surfaces [3], which indicates the possibility of further increase of the osteoconductivity. In the present study, we have investigated effects of plasmas on surface modification of artificial bones made of IP-CHA both *in vitro* and *in vivo*. Several results in the animal experiments have shown plasma-treatment can improve bone healing by IP-CHA, enhancing hydrophilicity of IP-CHA and its osteogenic potential in vitro (Figure1). The study has indicated that appropriate plasma application is a potent tool for modifying biological functions of artificial bones.

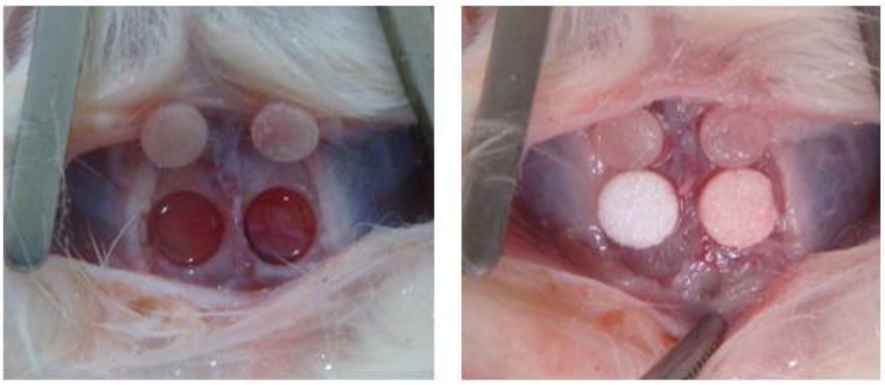

Figure 1 : Critical calvarial defects in rats were implanted with untreated IP-CHA in left and plasma-treated IP-CHA in right. Plasma-treatment increases the blood inflow into the IP-CHA.

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Resistance of plasma polymers to sterilization techniques used in biomedical applications

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Thin films of plasma polymers have been frequently suggested for use in biomedical applications. Versatility of precursors used for plasma polymerization allows modification of surfaces with interfacial layers of very different bioresponsive properties. It is also generally recognized that artificial materials to be used in contact with biological media should undergo preliminary sterilization to eliminate any form of microbial life. Sterilization techniques, however, may induce irreversible changes in plasma polymers and may be detrimental for their performance as bioactive coatings. This problem is rarely addressed to [1, 2] and can be considered as overlooked in the literature.

This work studies the effect of three most commonly used sterilization techniques (UV treatment, dry heat and autoclaving) on physical, chemical and cell adhering properties of plasma polymers. Hydrophobic fluorocarbon, bioadhesive amino-containing and non-fouling PEO-like plasma polymers were prepared by rf magnetron sputtering and plasma-assisted thermal vapor deposition. Their thermal stability, tolerance to hydrolysis and ability to maintain biological performance after sterilization was studied. It was found that the fluorocarbon and amino-containing films were most chemically prone to autoclaving due to hydrolysis whereas the PEO-like plasma polymers exhibited the strongest chemical changes after the dry heat treatment due to thermal degradation/oxidation. It was concluded that no universal sterilization method exists that assures preservation of the properties of all kinds of plasma polymers. Resistance of each plasma polymer towards sterilization methods has to be tested individually.

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Polymerization of acrylic acid by atmospheric pressure plasma jet for cell adhesion applications

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The stability of coatings obtained from plasma polymerization of acrylic acid(AA) has been widely studied at low pressure [1] and is an issue for its use in the biological applications. It is even more difficult to obtain stable coatings from AA by atmospheric pressure discharges. In this paper we report on thin film coatings obtained from AA with an atmospheric pressure plasma jet, an original and fast technique to grow organic thin films. Liquid acrylic acid was introduced directly in a nitrogen plasma jet which moved above a glass substrate to grow the thin films. OES has been used to follow the fragmentation of the precursor in order to obtain the maximum retention of the carboxylic coatings in the coatings. Several parameters where investigated such as the speed of the jet which defines the treatment time as well as the frequency of the discharge which monitors the power injected in the plasma. The typical treatment time to grow a film of roughly 1µm thick on a large surface (dozens of cm^2) is in the order of only 30 seconds. FTIR and XPS have shown that the deposited films have typical chemical functions of acrylic acid. As the energy input in the plasma and in the growing film increases the retention of functional groups decreases. However the retention of carboxylic groups is always high and XPS shows that around 30% of the carbon atoms can be bonded to carboxylic groups (theoretical maximum of 33%).The stability of the coatings in water has been studied by gravimetric measurements. It appears that coatings deposited with lower energy are less stable. Moreover *after soaking in water for 24 hours*, a part of the thickness of the micrometer thick films is removed, as observed by weight loss measurements without any remarkable change in the chemical composition of the films.
NIH:OVCAR-3 cancer cells were cultured in physiological conditions and were seeded in a microplate which was loaded with autoclaved coated glass cover slips for 24, 48 and 72 hours. The cell adhesion to the surface was determined by using an inverted microscope. Our results were correlated with the chemical structure of the films, as well as the important parameter which was the jet speed (Fig.1). The present study shows the possibilities to monitor the cell adhesion on surfaces presenting different carboxylic groups on the surface.

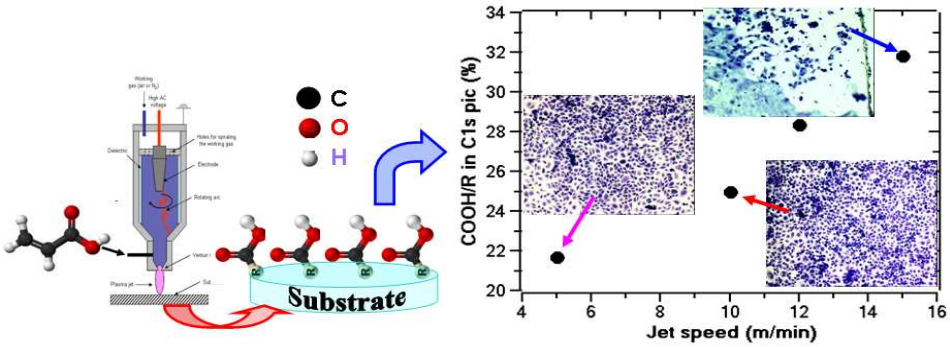

Fig. 1: *COOH groups percentage of XPS C1s peak, as a function of the jet speed and Optical microscopy images of NIH:OVCAR-3 cells on the resulting poly-acrylic thin film coatings.*

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On mechanism of inactivation of bio-particles by the plasma exposure and evaluation of the toxicity using single DNA molecules

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Effect of Non-Thermal Plasma on bio-particles has been studied on *B. subtilis*, *E. coli* and bacteriophages. Plasma-jet with Ar, or dielectric-barrier-discharge in room air has been used. The bio-particles suspended in liquid or dried state were exposed to NTP, and states of different biological components were monitored during the course of the exposure. For spore of *B. subtilis* in liquid, turbidity of the suspension changed quickly and became transparent after the exposure to DBD. This finding suggests the cell surface has been modified to be more hydrophilic with the plasma exposure. Analysis of green fluorescent protein, GFP, introduced into *E.coli* cells proved that NTP causes a prominent protein damages without cutting peptide bonds.

NTP can also inactivate viruses. In the previous research, critical damages for the inactivation of λ phage were done on coat proteins and M13 phage on DNA. These results were obtained with wet phage sample [1]. We also tried to use dry sample. We used bacteriophage φX174 which is resistant to drying. When the bacteriophage is inactivated by NTP, the damage should exist either on viral nucleic acids or coat proteins or both. It is possible to extract the nucleic acids from the bacteriophage so that the damage to the DNA can be separately analyzed to exclude the effect of the proteins. The DNA extracted from the plasma-exposed phage can be assayed its plaque forming activity by transfection. The coat proteins were analysed by SDS-PAGE. Using these assays, the damages of viral DNA and coat proteins have been determined separately. The results for the dry φX174 phage inactivated by the exposure to DBD showed both DNA and coat proteins were damaged and the critical damage were done to the coat proteins. This is similar to the result of the wet sample.

We also report a single-molecule-based analysis of strand breakages on large DNA molecules induced by the plasma exposure. Single-molecule observation of DNA that involved molecular combing was used to measure the length of individual DNA molecules. The measured DNA length showed that plasma exposure caused a marked change in length of DNA molecules. The rate of plasma-induced strand breakage on large random-coiled DNA molecules was determined using a simple mathematical model. The measured rate shows good relation with the plasma exposure time, and could be used for safety evaluation of the plasma treated water.

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Bactericidal effects of non-thermal plasmas on causative agents of nosocomial infections

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Non-thermal plasma was proven to possess unspecific bactericidal activity. Effectiveness of bacterial eradication is dependent on both plasma and bacterium features. We compared the effectiveness of three non-thermal plasma sources in eradication of causative agents of nosocomial infections, i.e. infections acquired by patients in the course of staying in a hospital. Clinically isolated bacterial strains characterized by multiple antibiotic resistance were used. The sources of microwave argon plasma, ferroelectric discharge in argon or ambient air and pin-to-plane positive and negative DC coronas in air were applied to treat bacteria placed on the agar surface and in biofilms. Chlamydia trachomatis was used to evaluate effectiveness of non-thermal plasmas against intracellularly persisting bacteria.

Bacterial eradication with microwave argon plasma produced by the MicroPlaSter β device was dependent on a bacterial species and strain. In general, Gram-negative bacteria were more sensitive than Gram-positives. Bactericidal effects on bacteria in biofilms were dependent on biofilm thickness. Microwave plasma was effective against pathogenic bacteria infecting slash wounds in rats. Moreover, it was effective against intracellular bacteria. Microwave argon plasma included UV radiation, charged argon particles, free radicals and chemically active molecules from the ambient air, argon metastables and microwave radiation and its bactericidal effect was a superimposition of all types of antibacterial agents as no one of them did not cause a bactericidal effect as significant as the effect of whole plasma. The afterglow of the ferroelectric discharge included neutral active species only. Ferroelectric discharge in air produced air-borne active particles including O3 and NO radicals in concentrations comparable with described above MicroPlaSter β. Still its effect on bacteria was 2 to 3 log10 lower that confirmed the importance of a synergetic effect of microwave plasma components. The afterglow of the ferroelectric discharge in argon was not bactericidal. The pin-to-plane positive and negative DC coronas in ambient air generate predominantly the charged particles and neutral active species and UV radiation. Fission of charged particles and neutral species reduced a bactericidal effect.

Taken together, obtained results demonstrated that both charged and neutral active particles contribute essentially to the whole plasma bactericidal effect on causative agents of nosocomial infections, including those in biofilms, on the wound surface and within mammalian cells, and underscored the importance of synergetic effects of plasma active components [1, 2].

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Plasma Applications to Sterilization and to Aesthetics

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Low-temperature plasmas interacting with living tissues or germs have shown their best applications to sterilization in the past decade or two. Their commercial attractiveness will be enhanced greatly with the use of compact low-cost nonthermal air plasmas using less hydrogen peroxide [1]. The ozone reduction issues in air plasmas have to be resolved beyond the present level of using charcoal or catalyst. Compared with lasers, plasmas also have many advantages in other applications such as aesthetics: oral and skin cares. Plasma treatment to bacterial plaque located in oral tissues is effective in killing only pathogens without damaging normal tissues [2]. Since oral diseases are not caused by only one pathogen, Ar or He atmospheric plasma should demonstrate the removal of various oral pathogens at the same time. Along with sterilization, plasma can also enhance blood coagulation and wound healing, relating to cell stimulation. Treatment with compact microwave plasmas showed enhanced expression of anti-aging genes in skin cells, collagen, fibronectin and vascular endothelial growth factor without causing cell death with reduced E-cadherin [3]. The characteristics of these plasmas [4] driven by various power sources, esp. from dc to portable microwave modules are modeled by various methods [5]. Plasma devices with several pending issues resolved can have great potential for oral and skin cares as well as for sterilization. The challenging plasma issues are oriented around the design of compact low-cost Ar or He plasmas with sufficiently low gas consumption or air plasmas with drastically reduced ozone production. The biomedical issues are equally formidable.

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To die or not to die, that is the question: the paradigm of sterilization

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Sterilizing, at first glance, seems like a straightforward all-or-nothing process. An item is sterile or it is not. As such, any new technologies developed as alternatives to autoclaving should meet the same requirements: the complete inactivation of a standard load of bacterial spores that guarantees that all microbes will also be killed by the process. A burden of proof is thus imposed on the assessment of sterility, which is based on survival curves. However, the correct interpretation of these curves is of paramount importance, both to elucidate the mechanisms of bacterial inactivation and to be sure that the inactivation is irreversible. How then do we interpret the biphasic nature of some curves, and what are the reasons for and implications of the tailing often observed? When assessing sterilization potential, researchers rely on cell cultures, which are still the gold standard. However, cell cultures rely on the inability of a large population of spores to "revive," that is to (1) germinate, (2) proliferate, or (3) produce "visible" colonies on nutrient agar. Should superdormant spores that require higher priming by germinants be taken into consideration in experimental designs? A good understanding of the mechanisms involved in the inactivation of spores and vegetative bacteria by plasma-based technologies is thus vital, from the design stage through to the approval of new types of sterilizers. Is irreversible blocking of spore germination an indication of successful sterilization? How can this be assessed? And, how specific should the sterilization target be? Logically, the more specific the target, the fewer the number of susceptible microorganisms. Targeting DNA is a good example. Spores and vegetative bacteria can both repair damage to their DNA very quickly. However, since their DNA is damaged in different ways, they use different strategies to repair the damage. In addition, some microorganisms are more efficient in repair than others. This thus raises another question: are spores the best biological indicators when DNA is targeted? My goal is not to provide precise answers to the questions I have raised, but to lay a foundation for discussions and reflections on key sterilization paradigms that have an impact on experimental designs and, ultimately, on the acceptability of new technologies as alternatives to current standard sterilization processes.

Production of atomic nitrogen in (100-x)%Ar-x%N_2 flowing afterglows at reduced pressure. Implications for the sterilization of the medical instrumentation.

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Reduced pressure (1-20 Torr) flowing afterglows are able to selectively produce large amounts of atomic species at room temperature and for low cost. As, in this pressure range, these extremely reactive species can homogeneously diffuse in volumes of a few tens of liters, flowing afterglows appear to be a promising alternative to the high temperature autoclaving for the sterilization of the medical instrumentation.

In our previous works, we have demonstrated the possibility to obtain a complete sterilization (i.e. a 6 log reduction of an initial bacterial concentration) by exposure to a pure nitrogen flowing afterglow [1-3]. In this case, the key parameter is the concentration of the nitrogen atoms in the operating chamber. The same inactivation rate can be reached either at room temperature with a high microwave power (P_{MW} = 300 W) injected in the discharge [3], or for an operating temperature of 60°C with a lower injected microwave power (100 W) [2].

In the present paper, we have tried to increase the absolute concentration of the nitrogen atoms in the late afterglow by the use of (100-x)%Ar-x%N_2 mixtures. The first part will be devoted to the optimisation of the N-atoms concentration with the operating parameters of the flowing afterglow : nitrogen percentage, pressure, total gaz flow rate, injected microwave power. In the second part, inactivation rates obtained with Ar/N_2 mixtures will be presented and compared to the one previously obtained in pure nitrogen.

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NanoSIMS50 analyses of *Escherichia coli* exposed to 18O and 15N afterglows

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Biomedical applications of cold plasmas need a better understanding of plasma interaction with biological tissues or organisms. This knowledge can be acquired not only through plasma modeling and diagnostics but also through fine characterizations of plasma treated tissues or organisms. This work is presenting a new method of characterization based on nano secondary ion mass spectrometry allowing the detection and imaging of isotopic atoms present on or in the microorganisms [1].
Isotopic R18OS and R15NS are produced in a microwave discharge at reduced pressure by using isotopic process gases and are transported in an uncharged region by the gas flow, in the so-called afterglows. These isotopic reactive species are then interacting with living bacteria.
More precisely, this study deals with the production of oxygen, nitrogen and NOx afterglows by using mixtures of Ar, 18O2 and/or 15N2. *Escherichia coli* bacteria have been exposed to these reactive media by varying several parameters like gas composition and duration of treatment. NanoSIMS analyses of the treated samples are realized to clarify the way oxygen, nitrogen and NOx species act on bacteria.
The results show that it is possible to detect and localize isotopic atoms (18O, 15N) coming from the plasma in the plasma treated bacteria. Reactive species can pass through membranes and interact with bacteria cytoplasm. A saturation of the isotopic atoms fixed on the bacteria structure is observed after a critical plasma treatment time of few minutes.

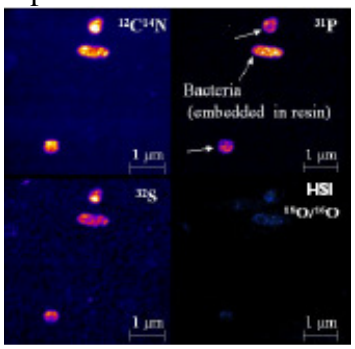

Figure 1: *Cross-section cartography by NanoSIMS50 of 12C14N, 31P, 32S and HSI 18O/16O for* E. coli *exposed to Ar/18O2 microwave afterglow during 15 min at 2mbars [2].*

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Innovative disinfection for dental and surgical therapies combined with the plasma treated water and the reduced pH method

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With the intention of disinfecting human bodies in dental and surgical applications, sterilization experiments in water have been conducted with low-temperature atmospheric-pressure plasmas. For the disinfection in the body fluid, we have successfully developed the reduced pH method that efficient bactericidal activity can be achieved if the solution is sufficiently acidic [1]. It is interesting that a critical pH value of about 4.7 exists for the bactericidal activity. The critical pH value may be associated with p*K*a of the dissociation equilibrium between superoxide anion radicals (O_2^-•) and hydroperoxy radicals (HOO•), which is known to be approximately 4.8. O_2^-• of reactive oxygen species generated in gas penetrate into liquid via plasma–air–liquid interactions [2, 3]. For the enough long lifetime of O_2^-• in gas as air ion, O_2^-• can be supplied into liquid by non-contact plasma. The non-contact plasma with 400 cm extra tube has strong bactericidal activity only with the reduced pH method. In addition, we found that the plasma treated water has strong bactericidal activity for a few minutes with the reduced pH method, as shown in Figure 1. This suggests that the disinfection can be done by the plasma treated water which contains short-lived active species (cannot supplied by chemical reagent) and it would bring safety plasma medicine considering usual contact or non-contact plasmas to human body. Currently, animal experiments for root canal therapy in dentistry [4] and surgical site infection prevention in surgery have been done with indirect plasma disinfection techniques of non-contact plasma and plasma treated water.

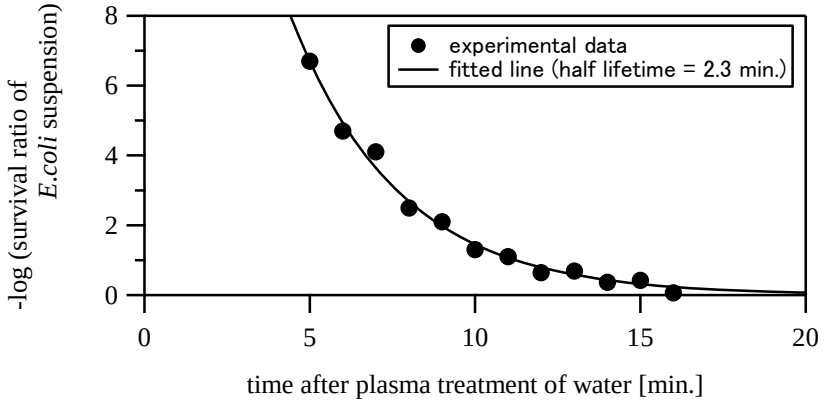

Figure 1: *Bactericidal activity of the plasma treated water with the reduced pH method.*

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Managing cytotoxic activity and etching effect of plasma for biomedical applications

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Discharge gas generated from plasmas has been widely used and investigated in many industrial and medical applications for sterilization and decontamination [1]. It is believed that reactive species and UV photon generated in the plasma all have a direct impact on the microorganisms, especially on their outmost membranes and on the cell walls [2].

We found that discharge gases with distinct chemical properties had strong activity to pre-formed biofilms from various bacterial strains on biomedical devices [3]. Further studies revealed that two different mechanisms were involved in discharge gas mediated biofilm inactivation. The first involved the diffusion of discharge gas in biofilms which causes the erosion of bacteria cells and resulted in severe damages to the cell membrane. Bacterial damage at the cellular level could happen even at low discharge power and after short time exposure. The second mechanism involved the etching effect associated with discharge gas generated at high discharge powers or after long time exposure. Discharge gases caused a chemical break down in biofilm extracellular polymeric matrix (EPS) and released bacteria and biofilms from the substrate surface. Biofilms were completely removed after long time exposure and the damage becoming more severe. We also found that different reactive species in plasma were responsible for etching and antibacterial activities of plasma. By controlling plasma device and plasma generation conditions, the etching and cytotoxic activity of plasma could be manipulated and tuned to meet the needs of various biomedical applications. Related studies also represent opportunities to promote the basic plasma research and will enrich our knowledge on plasma chemistry and physics.

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Rapid Destructions of Biological Species in the Air using Atmospheric Pressure Non-thermal Plasma

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Airborne biological particles are ubiquitous in the environments, including a variety of microorganisms (bacteria, fungi, and viruses), allergens, plant debris, endotoxin, glucans and skin scales. Exposure to those pathogenic microbes or derivatives was shown to cause numerous adverse health effects. In addition, the contamination of the environments as a result of either intentionally or accidentally released biowarfare agents can induce great harm and fear among the public as manifested by the anthrax events in 2001 in the United States. Biological aerosol exposure has become one of the major concerns for the residential, healthcare, and government sectors. The outbreaks of SARS in 2003 and influenza H1N1 viral infections across the globe in 2009 prompted worldwide attention for effective biological monitoring and control measures.

Here, non-thermal plasma generated by a dielectric barrier discharge (DBD) system was applied to inactivating aerosolized *Bacillus subtilis* and *Pseudomonas fluorescens* as well as indoor and outdoor bioaerosols. The culturability, viability, and diversitylosses of the microorganisms in air samples treated by the plasma for 0.06-0.12seconds were studied using culturing, DNA stain as well as polymerase chain reaction-denaturing gradient gel electrophoresis (PCR–DGGE) methods. In addition, the viable fraction of bacterial aerosols with and without the plasma treatment was also quantified using qPCR coupled with ethidium monoazide (EMA).

It was shown that less than 2% of B. *subtilis* aerosols survived the plasma treatment of 0.12 s, while none of P. *fluorescens* aerosols survived. Viability tests, EMA-qPCR results and Scanning Electron Microscopy (SEM) images demonstrated that both bacterial species suffered significant viability loss, membrane and DNA damages. Exposure of environmental bacterial and fungal aerosols to the plasma for 0.06 s also resulted in their significant reductions, more than 95 % for bacteria and 85-98 % for fungal species. PCR-DGGE analysis showed that plasma exposure of 0.06 s resulted in culturable bacterial aerosol diversity loss for both environments, especially pronounced for indoor environment. The results here demonstrate that non-thermal plasma exposure could offer another highly efficient air decontamination technology.

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Electron Spin Resonance (ESR) Measurements of Free Radicals Produced by Atmospheric Nonthermal Plasmas

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The use of nonthermal gas discharge plasmas for biomedical applications has been validated in multiple studies over the last two decades. Although both so called 'direct' or 'indirect' methods of plasma application have been shown to be effective in pathogen inactivation, the actual mechanism of sterilization is still poorly understood. Recent inquiry regarding these processes has shown the significance of the interaction of reactive oxygen species with the cellular lipid bilayer which results in the radical mediated lipid peroxidation cascade [1]. However, the specific manner in which this process of cellular destabilization is accomplished depends strongly on the reactive species present. Although, various spectroscopy techniques have recently provided quantification of chemical species produced in a wide range of plasma discharges, knowledge of the gas phase concentrations is insufficient since subsequent chemical reactions on the surface can produce new active species. For example, peroxone chemistry [2] can create OH radicals from ozone and hydrogen peroxide. Direct measurement of free radicals such as OH is difficult because of the short lifetimes. An effective technique for quantification of short lived free radicals is ESR which utilizes a technique known as spin trapping. The resulting spin adduct (for example, but not limited to, DMPO-$OH^{\cdot}$) is spectroscopically analyzed to yield concentration measurements of reactive species as well as information regarding the chemical environment [3]. We perform ESR tests on a novel plasma device that utilizes the indirect delivery approach coupled with hydrogen peroxide additives which has demonstrated strong clinical potential [4].

The efficacy of inactivation is coupled to the engineering parameters (e.g. distance to surface, flow rate, application volume, and so on) of the design. Therefore, in order to optimize such a device for clinical application, correlation between treatment protocol and active chemical species is of upmost importance. This work demonstrates the direct investigation of free radical chemistry using electron spin resonance measurements.

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Efficient Ar/O2 and/or N2 plasma inactivation of infective human adenoviruses

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Over the last decade, numerous studies have described the lethal activity of plasma treatments for successful inactivation of a large variety of microorganisms, including Gram-positive and Gram-negative bacteria, bacterial and fungal spores, yeasts, molds and viruses [1]. Human adenoviruses (HAdV), commonly causing respiratory, ophthalmic and gastrointestinal infections, withstand number of sterilization processes, owing to their robust protein capsid. Two recently published reports indicate the potential of plasma applications for reducing the infectivity of adenoviruses [2-3]. However, these plasma treatments often do not indicate structural damage of the viral capsid, thought to be the main cause of inactivation rather than DNA destruction [2], or remain long with still reduced inactivation efficiency.

In the present work, exposure of HAdV-2 suspensions to Ar/O_2 and/or N_2 plasma afterglows under reduced pressure (2 mbars) and 35°C during less than 5 minutes, enabled up to 6-log reductions of the infective viral titer, as shown by infectious state assays performed on HEK 293A host cells (**Fig. 1**). This reduction range is highly suitable for an efficient decontamination process. In parallel, transmission electron microscopy observations of plasma-treated HAdV-2 particles, currently under investigation, will help clarifying the effects of the plasma-active agents on the structural integrity of the viral capsid. Variation of ROSRNS involved in the decontamination process will be evaluated depending on gas mixtures.

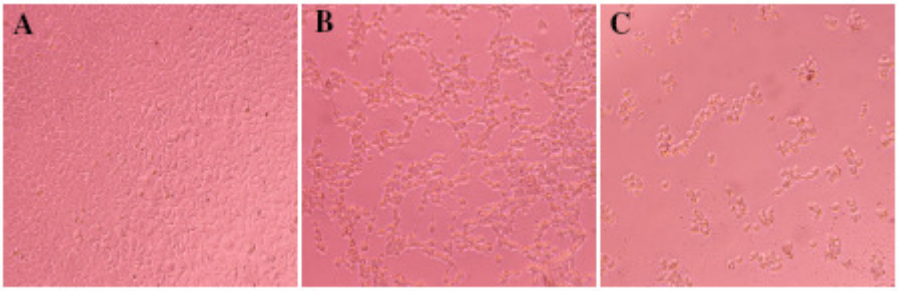

Figure 1: *Infectivity assays of human adenoviruses with HEK 293A cells; light microscope images show host cell layers (A) infected by HAdV-2 particles, resulting in progressive cell death (B) until complete plaque lysis (C)*

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Inactivation Mechanism of Single-Stranded DNA Bacteriophage Treated with Atmospheric Pressure Cold Plasma

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It is essentially important to understand interactions between atmospheric pressure cold plasma and living organisms for promoting the bio-medical application of the plasma. Bacteriophages are suitable for the study of such interactions because of their simple composition and structure [1] [2]. We have analyzed biological damages of both single strand and double strand DNA of M13 phage exposed to the plasma. The plasma caused damage to the phage DNA not only single strand break but also some chemical modification. The damage of double strand DNA was repaired *in vitro* by DNA repair enzymes, and ascorbic acid prevented the DNA degradation. DNA transfection assay revealed that single strand DNA is extremely susceptible to the plasma. Coat proteins of M13 phage proved to be more robust for the plasma treatment than the DNA using recombinant DNA experiments technique. We reached the conclusion that DNA damage is responsible for the plasma-inactivation of M13 phage.

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Atmospheric surface micro-discharge air plasma disinfection against *Clostridium difficile* spores

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Introduction: According CDC *Clostridium difficile* is a germ that causes diarrhea and other intestinal problems linked to 14,000 U.S. deaths annually. *C. difficile* infections cost at least $1 billion in extra health care costs at all types of medical facilities including hospitals, nursing homes, and outpatient facilities annually. The transmission of highly-resistant *C. difficile* spores occurs fecal-orally *via* contaminated surfaces in the environment and directly *via* the hands of the personnel. According to the EPA-registered product list, there is no specific disinfectant for the inactivation of *C. difficile* spores [1].
Cold atmospheric plasmas (CAP) are under investigation as promising gas-borne disinfectants or sterilants [2]. Recently, Tseng et al. used a helium radio-frequency cold plasma jet to show the sporicidal effect against *C. difficile* [3]. However, this device cannot be used for surface disinfection of large areas. We are confident that the surface micro-discharge (SMD) plasma technology - a scalable and robust technology - has this capability.
Method: We evaluated the disinfecting effect of SMD air plasma on spores of *C. difficile* NCTC 13366. Spores of *Bacillus subtilis* ATCC 6633 as well as vegetative bacteria of *Enterococcus faecium* ATCC 6057 served as references. Initially 10^6 spores or 10^8 vegetative bacteria were inoculated on dry stainless steel test specimen (30×6 mm^2) either with or without a small burden of 0.03 % serum albumin and wrapped in Tyvek. These samples were CAP-treated inside a small box (FlatPlaSter2.0) with ~35 mW/cm^2 plasma power (1 kHz, 10 kV_{pp}, sinusoidal wave form). Furthermore different CAP treatment times and open/closed volume conditions were used. The CAP-treated samples were microbiologically analyzed according to disinfection testing standards.
Results: Survival Curves and decimal reduction values revealed that a rapid surface disinfection takes place for closed volume conditions (10^3 spore reduction within few minutes). The role of diverse SMD air plasma species, particularly of oxidizing agents like ozone, is discussed in the microbial inactivation. Potentials and limitations of the technology in the field of disinfection are discussed.
Conclusion: Our study clearly showed that SMD air plasma can effectively disinfect dry surfaces contaminated with *C. difficile* spores and therefore, can serve as an alternative disinfection method. Further investigations simulating the clinical practice are necessary.

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Decontamination of teeth and plastic surfaces from biofilms and spores with DC and pulsed corona in air

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Biofilms or bacterial spores can cause a variety of infections and exhibit increased tolerance towards commonly used antimicrobial agents. Cold air plasmas at atmospheric pressure provide an alternative to conventional sterilization methods, because they are efficient even for biofilms and spores and do not cause degradation of termo-sensitive materials or human tissues. We investigated bactericidal effects of the atmospheric pressure air corona discharges combined with electrostatic spraying of water applied on plastic and *ex-vivo* human teeth surfaces contaminated by *Streptococci* biofilms and *Bacillus cereus* spores.
Teeth and plastic foils were contaminated by oral biofilm cultivated on *Streptococci* selective agar or directly by *Bacillus cereus* spores. We applied positive and negative corona discharge generated by either DC or pulsed power supplies. With DC power, positive corona formed streamers with frequency 5-16 kHz and maximum amplitude 30 mA and negative corona formed Trichel pulses with frequency 20-100 kHz and current amplitudes up to 7 mA. The pulsed discharge with frequencies up to 300 Hz was generated by rotary spark gap system. Exposure times were 5 min for teeth and 2-10 min for plastic foils.
The discharge set-up contains hypodermic injection needle as a high voltage electrode opposite to a grounded stainless steel mesh or plate [1]. Polypropylene plastic squares (1.5×1.5 cm) and extracted human teeth with *Streptococci* biofilms or *B. cereus* spores were placed on the grounded mesh (plate). The gap between the needle electrode and a sample (teeth or plastic) was 0.5 cm.
The use of pulsed power showed that negative corona was more efficient in decontamination of spores than positive one. With 5-10 min treatment, the efficiency of 96-97% was reached, which was slightly more than with DC power (80-90%).
In some experiments with biofilms treated by DC corona, tap water was electro-sprayed on samples from the HV electrode through the discharge. This significantly improved the decontamination effect both on teeth and plastics: from about 1 log reduction without spray to 3-4 logs with the spray. At water flow rate 0.01 mL/min, negative pulses were the most efficient (~4 logs). Decontamination of biofilms on plastic foils exposed for 2 min was slightly stronger with negative Trichel pulses than with positive streamers but not statistically significantly different.
DC positive and negative, as well as pulsed corona was proved as efficient methods of biofilm and bacterial spore decontamination from teeth and plastic surfaces. Electro-spraying of water on the treated surfaces at low flow rates significantly improved the effect.

This work was supported by VEGA 1/0668/11, Slovak Research and Development Agency APVV SK-CZ-0179-09 and SK-FR-0038-09.

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Poster - Session 1

Plasma gas temperature effect on survival ratio of human cells

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In recent years, atmospheric non-thermal plasma sources have attracted much attention in medical field because of its effectiveness for sterilization of medical devices and wound area. Previously, obvious difference of sterilization effect changing plasma gas species was observed [1]. It is expected as effective sterilization of medical devices or human body.

Note that plasma gas temperature should be sufficiently considered, if targets are sensitive to temperature such as human cell (required below 43℃). Because atmospheric pressure plasma source generates plasma by an electrical discharge through a gas supplied at around room temperature and the gas temperature of the generated plasma is somewhat higher than room temperature. Conventionally, to bring plasma irradiation to human cells, input power of plasma generation should be reduced.

In our laboratory, new plasma generation system in which the gas temperature of the plasma can be accurately controlled from below freezing point up to a high temperature without reducing power have been developed [2]. Using this system, survival ratio of plasma irradiated HeLa cells was observed under gas temperature variation of helium plasma. The helium plasma gas flow rate was 5 slm and electrode was connected to power supply with a frequency of 16 kHz and high voltage of 9 kV. As shown in Figure 1, plasma of high gas temperature (over 43℃) killed human cell at 60 s. Taking this point carefully into account, biological effect was investigated by various gas plasmas at room temperature. The details of the plasma source and the results of these experiments will be presented.

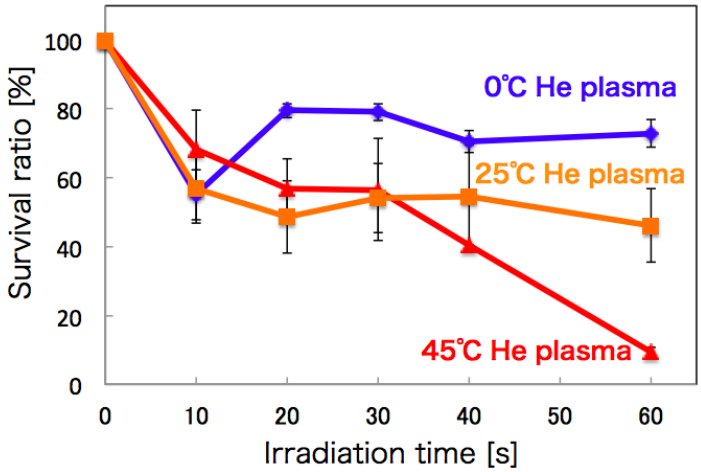

Figure 1: *Survival rate of HeLa cell*

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Treatment of glioblastoma: New application for CAPs?

Julia Köritzer[1], Tetsuji Shimizu[1], Tobias G. Klämpfl[1], Yang-Fang Li[1], Veronika Boxhammer[1], Jin Jeon[1], Gregor E. Morfill[1], Julia L. Zimmermann[1], Jürgen Schlegel[2]

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Glioblastomas (GBM) represent the most common primary brain tumors in adults. GBM is a highly aggressive tumor and is associated with extremely poor prognosis. Although options for initial treatment have improved, nearly all GBMs recur and treatment options are limited. So far, the median survival remains approximately 15 months for glioblastoma patients [1]. Current standard therapy for GBMs consists of surgery followed by radiotherapy combined with the alkylating agent temozolomide (TMZ). A crucial challenge is to develop and deliver effective drugs to cure this deadly disease.

Treatment of glioblastoma cell lines

Cold atmospheric plasma (CAP) displays features that are favorable in tumor biology. In this studie we were able to demonstrate that CAP - using the Surface Micro Discharge (SMD) technology for plasma production [2] - is able to block cell proliferation, to induce S/G_2-phase cell cycle arrest and mediate apoptosis to a lower extend in three different glioma cell lines. Very short treatment times of about seconds strongly inhibit human glioma cell proliferation. Cell cycle arrest in S/G_2- phase was induced after sixty seconds and persists at least for 72h when cells were treated with CAP once. In combination with the standard chemotherapeutic, temozolomide, CAP treatment is more effective in inhibition of cell viability and clonogenicity compared to TMZ alone. This data on glioblastoma might open new applications in brain tumor biology using cold atmospheric plasma.

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Inactivation of Bacteria in Solution by Atmospheric Pressure Plasma: Density Effects

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Bactericidal, fungicidal, virucidal and sporicidal effects of cold atmosphere pressure plasmas were under intensive investigation in the past few years. Reactive species (besides charged particles) produced by the plasma are believed to play a crucial role in this [1, 2]. Most of the recent studies focused on occurring reactive oxygen species (ROS) during the plasma application and on oxidative stress on microorganisms. The generation of ROS and the resulting lipid peroxidation is believed to cause a loss in membrane integrity [3]. The purpose of this study is to take into account not only the involvement of ROS but also reactive nitrogen species (RNS). Additionally influences of initial cell densities and different plasma treatment times were taken into consideration. E.coli were treated in solution for up to 8 minutes with initial cell densities between 102 and 108 cells per 20 µl with a plasma device, which uses the Surface Micro Discharge (SMD) technology and the surrounding air for plasma production [2]. The products of a few chemical reactions between the reactive species produced by the plasma and the liquid (with and without bacteria) were examined and analyzed. During the first 2 minutes of plasma application hydrogen peroxide and reaction products of NO rapidly occurred. The evidence of NO uptake by bacteria and further reference experiments with hydrogen peroxide clearly showed that the bactericidal properties of plasmas are a combination of oxidative and nitrosative effects.

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Intracellular or Extracellular: a Way to Plasma Protection

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With the development of plasma medicine, atmospheric pressure cold plasmas have shown promising results in various biomedical applications. However, safety concerns need to be addressed when a plasma is applied directly to living cells or tissue. The reactive oxygen species (ROS) produced by plasmas are considered to be the key constituents that induce biological effects, but they can also induce oxidative stress and consequently cell death, if the dosage is not properly controlled. As a result, anti-oxidative defenses must be taken to protect the nearby vulnerable tissue in plasma treatments.

In this study, both intracellular (genetic engineering) and extracellular (scavengers) measures were tested in an effort to evaluate anti-oxidative protection for cells against atmospheric pressure cold plasma treatment. As we know, oxidative stress pathway plays a very important role in the resistance to plasma processing for the eukaryotic cells. Hereby, for intracellular protection, we constructed two overexpression mutant strains through recombinant plasmid (pACT2-SOD1 and pACT2-SOD2) transforming the wild *Saccharomyces cerevisiae* strain. Superoxide dismutase (SOD) catalyzes dismutation of superoxide anion (O2 -·) to less harmful hydrogen peroxide (H2O2), which is then decomposed by Catalase into H2O and O2. SOD in concert with Catalase form the first and most important line of antioxidant defense. A series of scavengers: SOD, L-Histidine and D-Mannitol with different concentration gradient were employed as extracellular protection.

The intracellular protection, wild-type and extracellular protection strains are respectively exposed to a direct current, atmospheric pressure, cold air plasma mircojet. The intracellular ROS is measured with dichlorodihydrofluorescein diacetate (DCDHF diacetate) through fluorescence microscopy. Relative survival rate of plasma treated strains was performed using XTT assays. To evaluate the protection effects in the long term, full growth curves of each strain were obtained by tracking the cell growth after plasma treatment up to 28 hours. Reactive oxygen species (ROS), such as hydroxyl (·OH), singlet oxygen (1O2) and O2 -· were detected by end-on optical emission spectroscopy (OES). A promising precautionary measure in future clinical applications of plasmas will be provided in this work.

Cell Proliferation Activated by Micro-Spot Atmospheric Pressure Plasma

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Atmospheric-pressure plasma is essential not only for sterilization, disinfection, decomposition of hazardous substances, and surface modification but also for exploration and development of new composite fields that are based on multifaceted nanotechnology, biotechnology, and medical sciences.[1,2] In recent research on modification and regeneration therapies using pulsed plasma, plasma exposure was found to have a healing effect on burns and cutaneous wounds caused by diabetic necrosis. However, although there are several hypotheses, the mechanism underlying the regeneration of tissues through short exposure to plasma has not yet been elucidated despite its increasing practical applications.[3,4] In order to improve the situation, it may be important to clarify the mechanism from multilateral standpoints including plasma science and engineering, molecular biology, and biochemistry. Therefore, we conducted a basic experiment on direct irradiation of cells by using a micro-spot atmospheric-pressure plasma generated from helium (He) gas, which is hardly harmful to living bodies both thermally and electromagnetically. Mice embryonic fibroblast cell line (NIH3T3), which is usually used for cell experiments, was used in this experiment, and the effect of plasma on the cultured cells was investigated. It was revealed that cell proliferation is activated by plasma exposure. In response to the result, we considered growth factors related to the proliferation. Although there are many factors involved in cell proliferation, we focused on angiogenesis considered vascular endothelial growth factor (VEGF) and basic fibroblast growth factors (bFGF and FGF-2). [5]

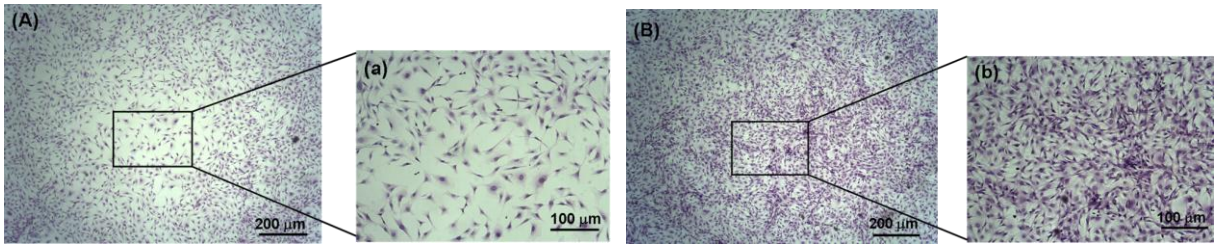

Figure 1: *HE staining of NIH3T3 cell line. (A)He gas flow only (×10), (a) He gas flow only (×40), (B) Plasma irradiation (×10), (b) Plasma irradiation (×40).*

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The separation of photons and reactive particles in the effluent of He/O_2 atmospheric pressure plasma jet

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The radiofrequency glow discharges operated in He at atmospheric pressure with small admixture of oxygen are known to be effective sources of reactive oxygen species (ROS) such as O atoms, ozone molecules, or $O_2(a^1\Delta_g)$ metastables, and of VUV and UV photons. It is well known that the treatment of bacteria with the effluents of these plasmas leads to their effective inactivation. A specially constructed atmospheric pressure plasma jet source, so-called X-Jet, allows effective separation of plasma-generated ROS and photons and can be used to study their separate and combined effect on bacteria [1,2].

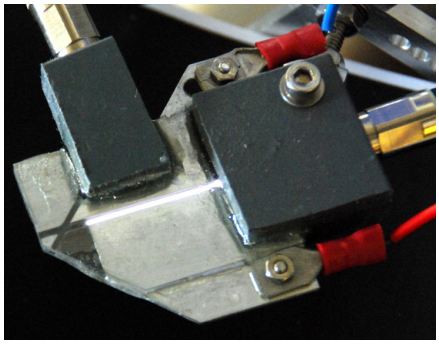

Figure 1: *Photograph of the X-Jet*

Additionally, the possible photo-chemical reactions can be studied with its help. Here, we present the characterisation of the X-Jet performance by means of mass spectrometry of neutral and ionic species, VUV optical emission spectroscopy, etching experiments, shadow photography, and fluid model of the gas flow and chemical kinetics in the jet. The measurements are correlated with the effects of the plasma effluent on *E. coli* cells on agar plates. It is demonstrated that i) ozone dominates the inactivation of bacteria at larger distance from the jet, ii) atomic oxygen can etch the biological material including bacteria, iii) the direct radiation damage induced by plasma generated photons is much less effective than the effect of ROS, and iv) the photons generate in the photochemistry reactions some reactive species (probably ions), which also inactivate bacteria. The X-Jet source is an ideal source to study the interaction mechanisms and synergistic effects of different reactive components of the plasma effluent on bacteria [2,3]. The results of more detailed studies on the effects of the plasma effluent and its components on bacteria and bio-macromolecules will be presented in the talk of Julia Bandow.

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Towards a plasma treatment of ocular surface infections

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The ocular surface is continuously exposed to microorganisms that can cause or aggravate infections like bacterial conjunctivitis and keratitis. In particular, the latter is considered an ocular emergency that requires immediate treatment to limit corneal morbidity and vision loss. In this contribution we present a study aimed at developing a plasma-based treatment of these infections, which exploits the bactericidal effects of the plasma. A low-power, atmospheric pressure plasma source specifically developed for plasma medicine applications has been used for the study [1]. In this source, a plasma is created ionizing a helium flow, mixed with ambient air, in the space between two grids. The effluent coming out of the most external grid, composed of helium enriched by reactive chemical species, is sent to the surface to be treated. *E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans*, and *A. fumigatus* cultures, all possible agents of ocular infections, displayed a treatment duration-dependent inactivation, with decimal reduction times of tens of seconds for *E. coli*, *S. aureus*, and *A. fumigatus*, of 209 s for *P. aeruginosa*, and more than 300 s for *C. albicans*. To determine whether the treatment affected the viability of keratocytes and conjunctival fibroblasts, primary cells isolated from conjunctival or corneal tissues were cultured and treated for the same time intervals used with microorganisms (from 0 to 5 minutes). Cell viability, analyzed one hour after the treatment through the MTT test, was initially reduced for 5-minute treatments, unlike the case of 2-minute ones, but in all cases it significantly increased after 24 hours. The cells retained their typical morphology. A significant rise in intracellular Reactive Oxygen Species (ROS) levels was detected in all bacterial and fungal strains tested, as well as in kertocytes and fibroblasts. The burst of intracellular ROS was dampened by cell pretreatment with NAC, a ROS scavenger currently used in ophthalmic surgery. Treatment of *ex-vivo* human corneas for 2 minutes did not induce any change in the corneal stroma, but caused a partial epithelial cell detachment. Evaluation of DNA fragmentation (TUNEL test) performed in parallel did not reveal significant apoptotic effects in corneal tissues. *Ex vivo* human corneas infected with *E. coli*, *S. aureus* or *P. aeruginosa* were also studied. A 2-minute treatment significantly decreased the survival of microorganisms recovered from infected tissues, thus confirming the disinfectant power at the tissue level. The oxidative burst and functional recovery observed in cultured corneal cells was also confirmed. The formation of thymin dimers at DNA level, which would indicate a detrimental effect of UV radiation emitted by the plasma, was tested and no effect was detected. The results presented in this study indicate that a 2-minute treatment with our plasma source substantially inactivates ocular pathogens without causing significant tissue and DNA damage [2].

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Characteristics of reactive oxygen and nitrogen species during nonthermal bioplasma interactions with biological cells immersed in fluids and its influences on the biomolecular surface electron energy structure

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We have investigated the characteristics of reactive oxygen species of OH and $O2^{*-}$ as well as reactive nitrogen species of NO by using the ultravilioet absorption spectroscopy in the atmospheric pressure nonthermal argon bioplasma sources interacting with the biological cells immersed in the fluids like water. It is noted in this study that these radical species play a very important role in the interactions between the bioplasma and biological cells, where their densities are found to be 3.7×10^{15} cm^{-3}, 1.3×10^{15} cm^{-3}, and 3.2×10^{14} cm^{-3}, respectively, for the OH, $O2^{*-}$, and NO radical species, respectively, under argon gas flow rate of 200 sccm. The secondary electron emission coefficient (γ) induced by a Auger neutralization of slow He ion beam, has been increased by nontherma plasma exposures, which might be caused by a damage on cell surface. It is also found that the molecular electron energy band structure for the bioplasma-treated cells has been shifted toward the vacuum surface energy since the biological surface cells might be oxidized by these reactive oxygen species.

Non-thermal Plasma-Induced Free Radical Effluent with Hydrogen Peroxide Additives

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A plasma medicine technology comprised of dielectric barrier discharge and hydrogen peroxide additives has been shown to be effective in deactivating pathogens on time scales of tens of seconds even though it involves the so called “indirect” exposure technique. *In vitro* deactivation tests of gram negative bacteria (*Pseudomonas aeruginosa)*, gram positive bacteria (*Staphylococcus aureus)*, bacteria spores (*Bacillus atrophaes*) and biofilms (*Escherichia coli*) have been performed. Optical frequency comb spectroscopy shows the detection of free radicals and other chemical species including O3, H2O2, N2O, and NO2 with evidence of OH· radical production through surface reactions and secondary chemical dynamics. Histology performed on murine skin exposed to the plasma induced effluent does not show adverse affects and compares favorably with alcohol and Silvadene treatments that are currently the mainstays of live tissue disinfection. The technology has been implemented in a new device that allows for application of the effluent to porcine wound healing models and can be used as a bedside burn wound treatment (Figure 1). We use an established porcine burn injury model inoculated with both *Staphylococcus aureus* and *Pseudomonas aeruginosa* to compare prevention of burn wound infection and burn wound sterilization to standard-of-care topical treatments.

We acknowledge support from the National Institutes of Health (USA) , Air Force Office of Sponsored Research (USA), and the Unviersity of Colorado Denver Center for Faculty Development.

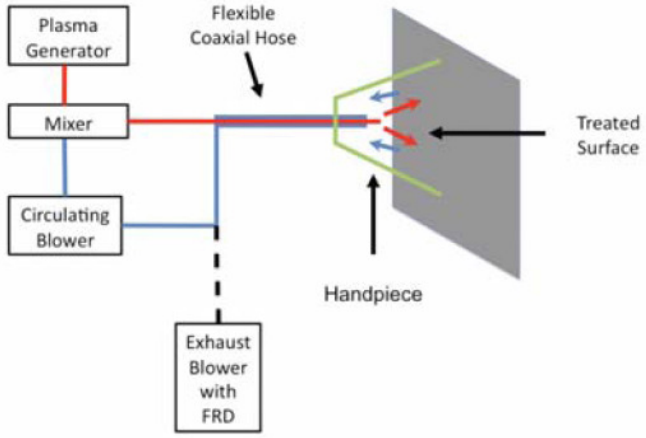

Figure 1: *Schematic of representation of non-thermal plasma-induced free radical delivery system*

Validation of Plasma Irradiation Effect on Gene Transfection by Using Microplasma Jet from Capillary Nozzle

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On a unique gene-transfection technique using plasma irradiation developed by some of us (Sato *et al.*), we have been studying the transfection mechanisms with various plasma sources. In this work, the effect of a microplasma jet ejected from a capillary nozzle is evaluated by irradiating plasma onto living cells in a localized area. The effect of electric field caused by the applied voltage on the sharp capillary nozzle is investigated by changing the voltage across the breakdown voltage to extract the effect of plasma irradiation.

Figure 1 shows the outline of the experimental setup. A copper capillary nozzle of 70 μm outer diameter was placed 3 mm above the sample in a Petri dish containing COS 7 cells and pCX-EGFP DNAs. The capillary nozzle worked as the high voltage electrode, while the grounded electrode was a metal wire of 160 μm diameter placed under the Petri dish. Sinusoidal voltage of 20 kHz was applied on the nozzle, which was pulse-modulated at a frequency of 25 Hz with a duty ratio of 1%. The working gas was helium with a flow rate of 100 sccm. In higher applied voltage region, filamentary plasma was generated between the capillary and the grounded wire as schematically shown in Fig. 1. The plasma was irradiated onto the sample for 4 s. In the lower applied voltage region below the breakdown voltage (see Fig. 2), the sample was only exposed to the gas flow and the high electric filed. After 24 h incubation of the treated sample, the transfection and survival rates were measured by fluorescence observation.

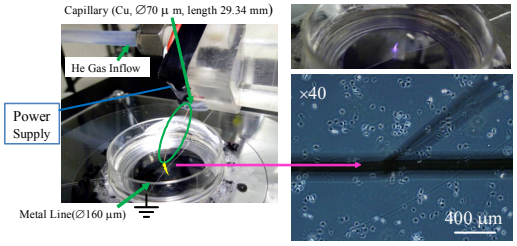

Figure 1: *Experimental setup.*

Figure 2 shows the transfection and the survival rates as a function of the applied voltage. Discharge plasmas were generated when the applied voltage was over 12 kV (peak-to-peak). The transfection occurred in this condition and the transfection rate increased exponentially with the applied voltage. These results imply that the high electric filed without plasma is not effective for the transfection. Note that the fairly high transfection rate with almost 100% cell survival is realized with this technique.

This work was partly supported by the Grant-in-Aid (22654070) from JSPS.

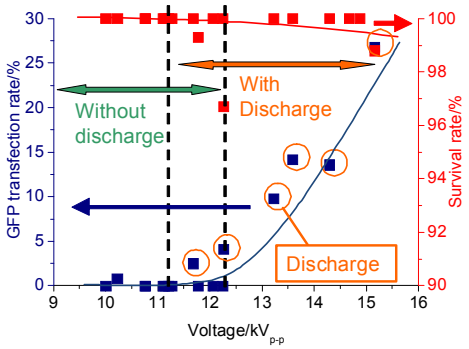

Figure 2: *The transfection and the survival rate vs. applied voltage.*

Gene Transfection by Electric Charge Injection Using Electrospray

Hideki Motomura[1], Tadashi Okihiro[1], Jun Matsuda[1], Kentaro Ikeda[1], Masafumi Jinno[1], Kunihide Tachibana[2], Susumu Satoh[3], Noboru Saeki[4]

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On a unique gene transfection technique using plasma irradiation developed by some of us (Sato *et al.*), we have been studying transfection mechanisms with various plasma sources. These studies imply that the electric charges have important roles for transfection mechanisms. In this study, we have evaluated that the electric charges need to be supplied on both cells and DNAs by an electrospray technique.

Figure 1 shows a schematic of experimental setup. Sample solution of 0.52 ml containing both COS 7 cells and pCX-EGFP DNAs was filled in a syringe and the piston was pushed down at the constant rate (344 µl/s or 34.4 µl/s, i.e. 1.51 s or 15.1 s for whole sample exhaust) and the sample was dropped onto a Petri dish through the metal nozzle of 0.60 mm outer diameter and 0.43 mm inner diameter. At the same time, positive DC or sinusoidal AC (14 kHz) high voltage was applied to the nozzle. The grounded electrode of metal mesh was placed under the Petri dish. The gap distance between the nozzle tip and the metal mesh was adjusted from 5 to 25 mm. By applying the high voltage to the nozzle, the sample solution obtains the electric charges at the nozzle tip and is sprayed onto the Petri dish breaking into small droplets by the mutual repulsive force. After 24 h incubation of the treated sample, the transfection rate was measured by fluorescence observation.

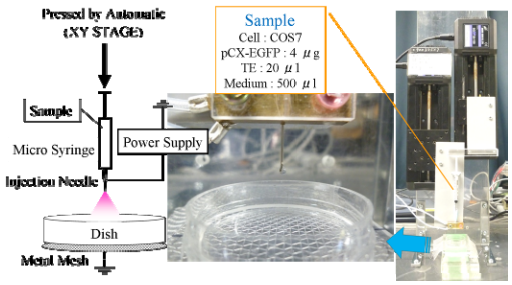

Figure 1: *Experimental setup.*

Figure 2 shows the transfection rate as a function of the applied voltage and the gap distance. In the present condition, the average value of 0.2% transfection rate is obtained. At the same voltage and gap distance conditions, DC voltage and longer spraying time (smaller flow rate) is preferred due to a larger charge injection amount. Compared to the plasma transfection method, this method has an advantage that the cell fusion does not occur as shown in Fig. 2, although the transfection rate is low. The optimization of the applied voltage, the gap distance and the flow rate is now in progress.

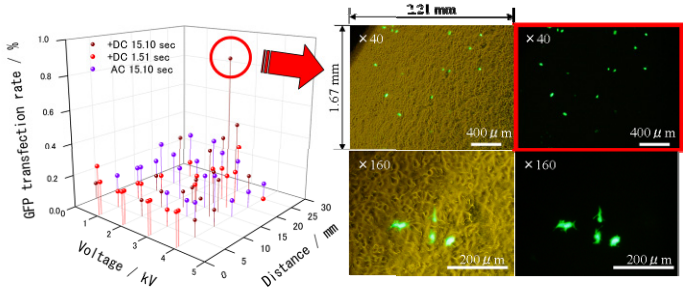

Figure 2: *The transfection rate vs. the applied voltage and the gap distance, and an example of fluorescence image.*

This work was partly supported by the Grant-in-Aid (22654070) from JSPS.

Quantitative Investigation of Energy Injection and Gas Flow Rate for the Plasma Used in Gene Transfection

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On a unique gene-transfection technique using plasma irradiation developed by some of us (Sato et al.), we have been studying transfection mechanisms with various plasma sources. In this work we have quantitatively compared the performances of two sources, i.e. arc plasma and plasma jet, on the effects of the injection energy and the gas flow rate.

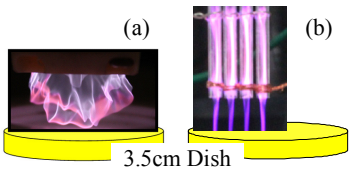

Figure 1: *Schematic of (a) arc and (b) plasma jet heads.*

Figure 1 shows schematic photos of the two plasma source heads in gene transfection: (a) arc plasma and (b) plasma jet. A sample solution containing COS 7 cells and pCX-EGFP DNAs in a Petri dish rotating at 75 rpm was exposed to each plasma for 0.2–4 s (arc head) or 10–100 s (plasma jets). The 20 kHz sinusoidal voltage of 5.8–8 kV peak to peak amplitude was applied with pulse-modulation at a frequency of 10–200 Hz and a duty ratio of 7–100% for arc head, while the 14 kHz sinusoidal voltage of 6–9 kV p-p was applied for the plasma jet head. The working gas was argon (16.8–28 slm) for the arc head and helium (0.8–9.8 slm) for the plasma jet head. After 24 h incubation, transfection rate and survival rate η were measured by fluorescence observation.

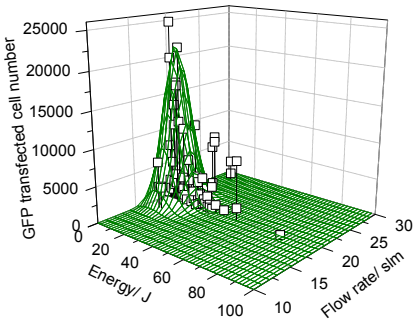

Figure 2: *Transfected cell number vs. injection energy and gas flow rate for the arc head.*

Figure 2 shows the transfected cell number n vs. the injection energy E and the gas flow rate F for the arc head. For both plasma heads, n and η have optimal values. These tendencies are fitted by Gaussian functions against E and F,

$$n = n_0 \exp\left[-\frac{(E-E_0)^2}{2w_E^{\,2}} - \frac{(F-F_0)^2}{2w_F^{\,2}}\right], \quad (1)$$

$$\eta = 0.15 + 0.85\exp\left(-\frac{E^2}{2w_E^{\,2}} - \frac{F^2}{2w_F^{\,2}}\right). \quad (2)$$

Table 1: *Fitting results.*

(a) Transfected cell number

Head	n_0	E_0/J	w_E/J	F_0/slm	w_F/slm
Arc	22 000	9	6.5	20	1.6
Jet	940	145	30	3.6	1.15

(b) Survival rate

Head	w_E/J	w_F/slm
Arc	19	10 000
Jet	450	10

Table 1 shows the specific numbers obtained by the above fitting for both plasma heads. From this result, it is found that larger injection energy (E_0) is required for the plasma jet source for the optimum n and η due to the inherent nature of localized (thin) plasma and too short duration. Moreover, the survival rate dependence on the gas flow rate (w_F) is very small for the arc head. Detailed analysis is shown at the conference.

This work was partly supported by the Grant-in-Aid (22654070) from JSPS.

Effect of a non-thermal atmospheric pressure plasma effluent on both growth media and PC-3 prostate cancer cells

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As an emerging field in medicine, plasma treatment offers many opportunities and challenges. Critical to any future implementation of plasma devices in medical therapy are in-depth studies of the end effects that such plasma sources induce in a liquid environment. Of particular interest is how the changes in the liquid affect biological matter contained within it [1]. Thus studies on plasma induced liquid chemistry combined with cellular response assays such as those for cell viability and death pathway analysis are required.
In this study the influence of the effluent of an rf driven microscale atmospheric pressure plasma jet [2] operated in gas mixtures of helium and oxygen, on PC-3 prostate cancer cells and their growth media are investigated. Absolute densities of relevant reactive oxygen species - atomic oxygen, ozone and metastable singlet delta oxygen have been measured and simulated in the plasma jet [3, 4]. Analysis of growth media exposed to the plasma effluent via a two step nitric oxide quantitation assay [5] showed high concentrations of nitrite produced in the media. This suggests large amounts of nitric oxide - a molecule of critical importance in both cell death and proliferation [6] - is being delivered from the plasma source. Additionally clonogenic assays were carried out and showed a decrease in the surviving fraction of PC-3 cells that is correlated with increasing exposure time to the plasma effluent. Furthermore studies of cellular proteins via western blotting, after exposure to the plasma effluent, showed evidence of cleavage of key caspase proteins, suggesting that cell death induced by the plasma effluent exposure occurs via apoptosis, and is likely caused by nitrosative or oxidative stress.

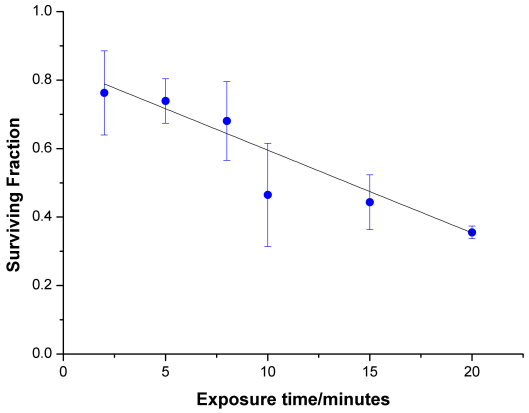

Figure 1: *Plot showing result of clonogenic assays*

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Influence of cold atmospheric plasma treatments on mammalian cells

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There is growing interest in the application of cold atmospheric pressure plasma treatments in wound healing. The objective of this study is to compare the effect of the plasma treatment on cell survival and metabolic activity of two different mammalian cell lines **-** Chinese hamster ovary (CHO) and osteoblast (MG63) cells. Parameters evaluated included cell survival, viability and metabolic activity. In addition to comparing the effect of the helium plasma on the two types of cell a further objective was to quantitatively compare how treatment duration, input voltage and frequency affected the cell response.

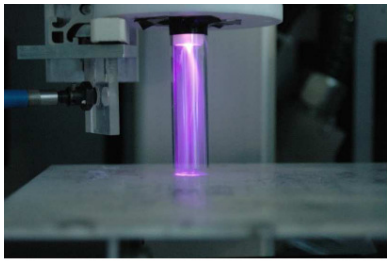

Figure 1 Atmospheric Pressure Plasma Jet formed in the 6 cm long quartz applicator

The helium atmospheric plasma jet (Figure 1), was generated using a duel-pin parallel tungsten electrode source. This source was powered using a C2000 Redline generator. The electrode pins were spaced 12 mm apart and positioned at one end of a quartz reactor cylinder of length 6 cm and nozzle exit area of 2 cm2. Applied voltage and frequency were varied between 50-300 Volts and 50-450 kHz respectively. The helium flow rate was kept constant at 10 L/min and treatment times were in the range 10 to 360 seconds. The jet to substrate distance was fixed at 15 mm. The mammalian cell lines investigated were CHO and MG63 which were both cultured in the appropriate medium. Treatments were performed on cells attached to polystyrene 6-well plates, with a diameter of 36 mm. Prior to the plasma treatment the medium was removed. Note that no deleterious effect was observed for cells with medium removal (no plasma treatment) for a period of up to 30 minutes.

The effect of the plasma was evaluated with respect to cell number, viability, cell cycle, ATP and apoptosis. The early stages of the research focused on quantifying the effects of varying treatment times on cellular responses. As expected cell death increased with increased plasma exposure times up to the 360 seconds investigated in this study. For all treated well plates a significant level of cell viability was observed 24 hours post treatment in culture. This study indicated that for the treatment conditions used, the most significant effect of the plasma was to reduce the level of cell proliferation. The effect of increasing the plasma power (voltage and frequency) resulted in an accumulation of cells within the G2 phase of the cell cycle process i.e. prevents mitosis. Analysis of apoptosis induction was carried out using Annexin V assay and flow cytometery, the results of which demonstrated a distinct progression of viable cells towards the apoptotic phase. Finally, using a Roche ATP Bioluminescence Assay Kit, a marked increase in ATP production within the cells was detected with increasing plasma power.
This study also indicated that MG63 cells were more sensitive than CHO cells to plasma exposure. Both cell types exhibited a dose dependent relationship with the intensity of the plasma applied.

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Enhanced germination characteristics and seed vigor by Cold Atmospheric Plasma

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Cold Atmospheric Plasma (CAP) [1] was exposed to seeds of *Cicer arietinum* in different doses. For each case, germination percentage, speed of germination, seedling length, seedling dry weight and seed vigor [2] were evaluated compare to unexposed control. An optimum result was obtained at 1 mint CAP exposure with increased percentage of germination from 60.83%(control) to 89.16%. The speed of germination enhanced from 3.922 ± 0.125/day (control) to 7.125 ± 0.107/day. The increased level of germination percentage, speed of germination and seed vigor and the de- creased level of mean germination time, and the time to get 50% germination of seeds were observed for CAP exposure at 1mint. This advantage and promising role of CAP to increase seed germination suggested that CAP technology could be an easy to handle technology for farmers and people in food industry to enhance seed germination characteristics in a simple way.

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Liquid Mediated Effects on Cells, Bacteria, and Model Membranes by Plasma-born Reactive Species

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The recently developed cold atmospheric pressure plasma sources opens the possibility to treat thermosensitive living tissue in a non-destructive beneficial way. One successful application is the plasma treatment of wounds including stimulation of cell growth, killing of bacteria, and endotoxin inactivation. For all applications stable and controlled plasma-parameters are essential for reproducibility and optimized effects. By development of an shielding attachment for a non-thermal plasma-jet, kinpen, fluctuating environmental influences can be eliminated and plasma chemistry can be controlled. This attachment produces a shielding gas curtain that isolates the plasma effluent from ambient air including all its impurities. As a result, plasma-treatment leads to different concentrations of active species (e.g. reactive oxygen or nitrogen species (ROS or RNS, respectively), nitrate/nitrite, or peroxides.

Even if the effects of plasma treatments are well known, the deeper understanding of molecular mechanisms still needs improvement. The cell-surrounding liquid, an interface between plasma/gas-phase and biological object, is mediating these effects and gets more and more into focus of interest recently. In these liquids, reactive species are either introduced directly by the plasma phase into the liquid phase or are created within the liquid.

Because every externally applied substance that creates a cellular effect, has to interact with the envelope of the cell -the membrane. Lipids and proteins are the constituents of it; the first mainly responsible for the structure, the last mainly responsible for membrane function. Normally protein interaction requires a high specifity in binding. Due to the unspecific interaction of radicals, it is unlikely that they interact specifically with any biological molecule. The externally applied agent can either overcome the membrane to get access to intracellular targets or interact directly with the membrane. This direct interaction can result in activation of a cellular signal path (e.g. intrinsically apoptosis pathway) or in direct lipid interaction like lipid peroxidation. Lipid interaction can result in pore or lesion forming which allows a "self-promoted uptake" into intracellular space. The intracellular concentration of the agent depends therefore of its ability to form pores. If the pores are big enough, this can result in depolarization of cells and therefore cellular death. Furthermore, the lipid reorganization can result in fusion processes. Therefore, the membrane is the primary target of plasma-treatment of cells (pro- and eukaryotic).

Here we are presenting models to verify if the observed effects are directly membrane related. For this we use different liposome models to measure membrane effects (e.g. fusion). Neutral lipids and lipopolysaccharides (LPS) are used to mimic eu- and gram-neg. eukaryotic membranes. Because LPS aggregates are the active form of Gram-negative endotoxin, it is tested, if this disordering ability results in LPS inactivation preventing a septical shock.

Feed Gas Humidity: A Hidden Parameter affects Cold Atmospheric Pressure Plasma Jet and Plasma-Treated Human Skin Cells

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Gas humidity is an important parameter in cold atmospheric pressure plasma treatment of biological systems [1, 2]. This is especially true for humidity change in the feed gas of a cold argon plasma jet since the humid working gas is transported through the active plasma zone entirely. Hence, water molecules become dissociated and are significant for the active plasma component composition. Besides the intended admixture of water vapor, working gas humidity can originate from different unknown or hard to control sources. Polymeric feed gas tubes are prominent examples for a hidden humidity source.
In this work the humidity amount introduced by feed gas tubes is measured and the impact of the resulting working gas humidity on the active plasma agents is investigated. An example of the humidity-plasma interaction is given in figure 1. It displays the spectrally integrated optical emission signal of a plasma jet (kinpen, neoplas GmbH, Germany) in dependence on the humidity and the axial position. Two excited plasma species are evaluated, namely argon atoms (figure 1a) and hydroxyl molecules (figure 1b). In the case of argon the highest intensity is detected for dry working gas. When humidity is artificially added to the working gas, the emission of argon decreases while the emission of OH increases. The increase of OH emission continues until the maximal OH emission is reached at a humidity admixture of 400 ppm. From here the OH emission decreases steadily. In addition to plasma diagnostics humidity influence on plasma treated HaCaT skin cells is presented. The treatment effect on cells is tested in an in vitro model using proliferating human keratinocytes. Cell viability is determined using the Alamar Blue Assay. A strong effect of the water admixture to the feed gas on cell viability is shown, which correlates to the plasma diagnostic findings.

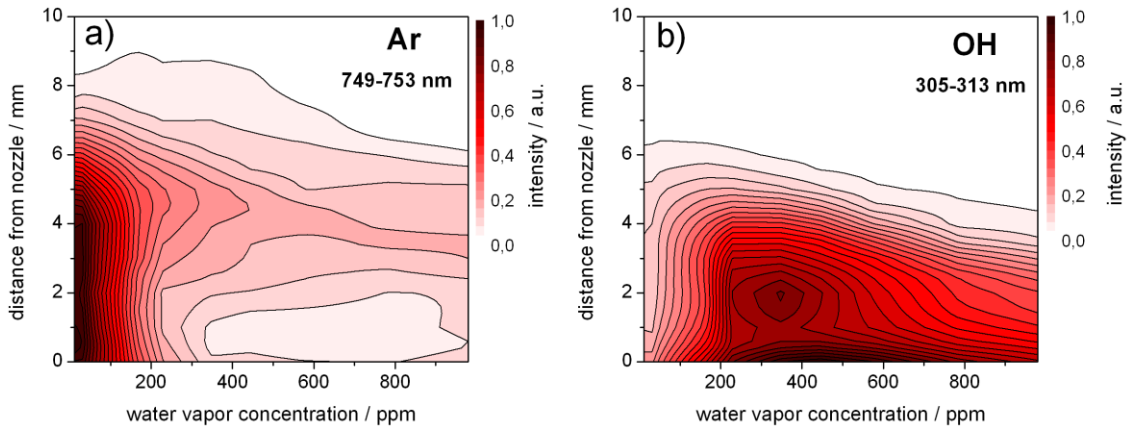

Figure 1: *Integrated optical emission in dependence on water vapor concentration and axial position for excited argon atoms (a) and hydroxyl molecules (b).*

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Genotoxicity of Atmospheric Pressure Cold Plasma Evaluated with Yeast Reporter Assay System

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To understand the interaction between plasma and living system is essentially important for supporting medical and sanitary use of low temperature plasma. One of the effective approaches to the plasma and living system interaction is to study influences of atmospheric pressure plasma to gene expression. Yeast *Saccharomyces cerevisiae* is suited to such a investigation because it is unicellular eukaryotic organism, easily manipulated and may respond to various DNA-damaging factors in a manner similar to mammalian cells.

Improved yeast-based genotoxicity tests have been established in several laboratories by harnessing cellular responses to DNA damage. We have constructed a yeast-based genotoxicity test system using reporter assay linked to DNA damage-inducible promoter gene. Our yeast test system responded to many type of carcinogenic reagents [1].

Figure 1 shows the concept of the yeast genotoxicity test system. *RNR2* (ribonucleotide reductase subunit gene) is involved in cellular DNA repair reactions. DNA damage on the chromosomes works as a signal to elevate the level of *RNR2* gene expression. Therefore, the reporter *lacZ* (β-galactosidase) gene under control of *RNR2* promoter sequence is induced by DNA damage. Extent of DNA damage is evaluated by the reporter β-galactosidase activity in the plasma treated yeast cells. Application of argon or helium plasma jet to the yeast-based system induced high levels of the reporter gene expression. It is suggested that atmospheric pressure cold plasma has not only DNA damaging activity, but also carcinogenic activity. The remarkable genotoxic property of the plasma may play an essential role when the plasma is used as medicine.

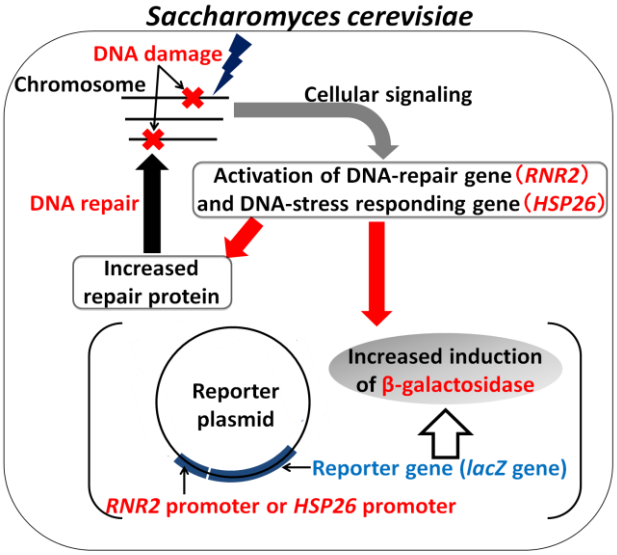

Figure 1. Yeast reporter assay system for detection of genotoxicity.

Reference

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Effects of low-temperature atmospheric pressure plasma on cell physiology *in vitro*

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The application of physical plasma to living tissue is expected to have many uses in the future, e.g. wound healing by plasma-disinfection, stimulation of tissue regeneration, dental applications, treatment of skin diseases and cancer treatment based on the specific induction of apoptotic processes.[1,2] In contrast, there is little knowledge on how physical plasma interacts with living tissues, in particular with cells. Studying the characteristics of plasma and its interactions with cells *in vitro* is essential.
The experiments were carried out using an argon plasma jet (kINPen®09, INP Greifswald) to gain insights into time-dependent plasma effects on cell attachment, viability and tight junction formation *in vitro*. Murine epithelial cells mHepR1 were suspended in complete cell culture medium and were irradiated with argon plasma (direct approach) for 30, 60 and 120 s. Suspecting that physical plasma may exert its effect via the medium, cell culture medium alone was first treated with argon plasma (indirect approach) and immediately afterwards, cells were added and also cultured for 24 h. Cell morphology and vitality were verified using light microscopy and an enzyme-linked immunosorbent assay. Already after 30 s of treatment the mHepR1 cells lost their capability to adhere and the cell vitality decreased with increasing treatment time. Interestingly, the same inhibitory effect was observed in the indirect approach. Furthermore, the argon plasma-treated culture medium induced large openings of the cell's tight junctions, verified by the zonula occludens protein ZO-1, which we observed for the first time in confluently grown epithelial cells.

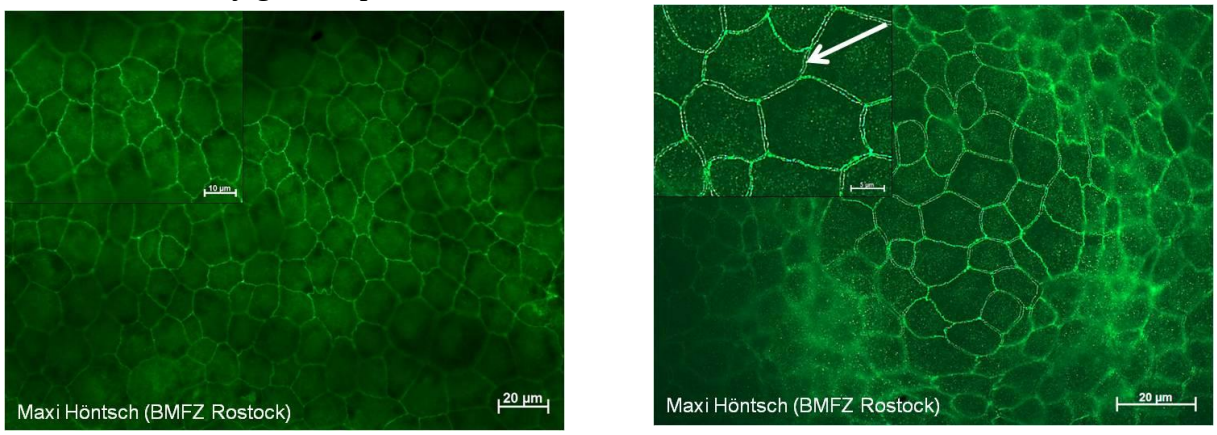

Figure 1: Immunofluorescence staining of the tight junction protein ZO-1 in normal, untreated mHepR1 cells indicating the strong cell-cell contacts at the cell margins, represented by continuous ZO-1 bands (left) and the tight junction protein ZO-1 in confluent mHepR1 cells after 24 h incubation with plasma-treated DMEM. Note the large openings between two cell margins (right, arrow) indicating a loss of the tight cell-cell contacts. AxioObserver.Z1, Carl Zeiss, 63x magnification, bars = 10 (inset) and 20 µm.

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In vitro anti mitogenic and apoptotic effects by using plasma jets

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Since approximately ten years now, numerous studies have been investigated on the very important potentialities of non thermal plasmas for biomedical applications. Ionised and excited gases are produced at atmospheric pressure and many developped systems are thus conceived to form gaseous reactive mediums at mean temperature closed to the physiological temperatures of living organisms. Therapeutic medical applications are observed for blood coagulation, cicatrisation of wound healing and cancerology [1-4].
But what about the interaction mechanisms between the numerous species formed in such plasma devices and living organisms? ROS and RNS are produced in gaseous plasma phases as well as electrical charges, photons, electric local fields, thermal low variations, and all these energies levels may individually or synergetically interact with living cells. Although plasma was recently shown to induce cell apoptosis and cell cycle arrest in malignant cells [5], little is known about the effect of plasma exposure on non malignant, connective cells. Here, we tested the effect of plasma jets on periodontal ligament cells (PDLC), that have been shown to be mesenchymal-like progenitors cells, involved in periodontal healing and homeostasis. Our results showed that plasma jets induce cell apoptosis, cell cycle disturbances and inhibition of osteogenic differentiation, effects largely dependent on time exposure and distance from the plasma source. Interestingly, the use of foetal bovine serum partially rescue the apoptotic and anti proliferative effects plasma-induced. These results clearly showed that plasma jet may interfere with connective tissue wound healing.

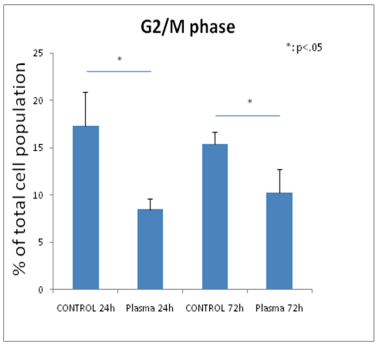

Figure 1: *Decrease of PDLC percentage in G2/M stage 24 hrs and 72 hours after 30s plasma jet exposure*

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Atmospheric Plasma Jet Exposures: Beyond the Skin

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The primary function of the epidermis is the production of the Stratum Corneum (SC) that effectively protects our body from desiccation even in dry environment as well as from external invasion of injurious agents, although it is a thin (less that 20 µm thick) biological barrier membrane [1]. The SC is comprised of two-compartment system of corneocytes, flattened dead cell bodies of epidermal keratinocytes and intercellular lipid lamellae.
The field of plasma health care is now the subject of a broad interdisciplinary research effort involving medicine, biology, physics, chemistry and engineering [2]. A huge number of works have been dedicated to the understanding of the mechanisms involved during cold atmospheric plasma skin exposition by exploring deeper layers than the SC itself. Surprisingly, there is a poor interest in studies relating to interactions which may occur in the near-surface region (i.e. ~ 10nm depth) of the SC. Such understanding is essential in a fundamental point of view but also present great interests for cosmetic applications.

In this work, we concentrate our efforts to characterize the physicochemical modifications of SC after exposition to helium atmospheric plasma jets. We work with sheets of SC isolated from normal skin and usual surface characterization tools as IR, Raman, XPS are used. Besides equilibrium proton-transfer reactions are used as probes [3] to define in a fundamental point of view the nature of the plasma interactions with the surface of stratum corneum.
The helium plasma jet is produced by using a pulsed high voltage power supply consisting in chopping a 10kHz sinusoidal waveform. Plasma jets at mean temperature closed to the room temperature are thus formed and contain numerous reactive oxygen and nitrogen species (ROS and RNS). Distance between SC samples and the jet and exposures times are modified in order to analyse physico-chemical modifications.

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Intravital Fluorescence Microscopy for the Assessment of Microcirculation and Leukocyte-Endothel Interaction after Application of Tissue Tolerable Plasma in the HET-CAM

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According to current knowledge, low-temperature atmospheric pressure plasma, so-called tissue tolerable plasma (TTP), seems to be a promising therapeutically option for the treatment of chronic wounds [1]. Several in vitro and in vivo studies demonstrated wound healing aspects of plasma treatment [2]. So far, the exact mechanisms and effects on the microcirculation are not elucidated.

The chorioallantoic membrane (CAM) of embryonated hen's eggs represents a vital, vascularized tissue and can be considered as a crossing from *in vitro* and *in vivo*, because tests are performed on living tissue, but not on the embryo itself. Therefore these tests are not classified as animal experiments and in terms of the 3R they may help to reduce animal testing. In previous studies it could be shown in a modified HET-CAM (Hen's Egg Test on the chorioallantoic membrane) that TPP induces aseptic inflammations that are suitable for the modification of chronic inflammations in wounds [3].
By means of intravital fluorescence videomicroscopy it was possible to detect detailed effects of the plasma treatment on the CAM. The method allows the assessment of the microcirculation including the capillary blood flow and the qualitative and quantitative analysis of dose-response relationship. Both haemostatic and vasoconstrictive or-dilatory effects could be assessed. Moreover, the method allows the quantitative analysis of dynamic processes and variables, such as leukocyte-endothelial interaction (LEI) or functional vessel density (FVD).

Method: After 10 days of incubation, the CAMs of fertilized hen's eggs were dissected in microsurgical technique. New is the microscopically assisted puncture of a micro-vessel with a specially made glass micro-cannula (inner diameter 10 microns) using a hydraulic micromanipulator (MO-203, Narishige, Japan) for injection of approximately 5 µl 0.05% rhodamine 6G (Sigma-Aldrich, Germany) as a fluorescent dye for in vivo labeling of autologous leukocytes. Intravital fluorescence microscopy studies to quantify the FGD and LEI were made 5 min after meandering application of TTP (HF plasma jet, neoplasms GmbH, Germany; argon as carrier gas) and the non-activated gas as control.

Results and conclusions: TTP induced a reduction in the FGD while increasing the LEI as a sign of increased immunological reaction. The results are presented in detail in a video clip. The proposed model is suitable for qualitative and quantitative analysis of the effects of tissue tolerable plasma on the functional vessel density (FVD) and the leukocyte-endothelial interaction (LEI) in the vital tissue. It will help to understand the basics of the plasma effects and to develop new therapies for the treatment of chronic wounds by TTP.

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Microsecond-Pulsed DBD Plasma Induces Osteogenic Differentiation in Mesenchymal Cells

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Intracellular reactive oxygen species (ROS) is a known activator of cell signaling promoting differentiation. Our goal was to determine if Non-thermal Microsecond-Pulsed Dielectric Barrier Discharge plasma (NT-plasma) could induce cell differentiation. In this study, we applied NT-plasma to mesenchymal cells and evaluated osteogenic differentiation. First, we compared media containing ß-glycerophosphate (ß-GP), a known inducer of osteoblast differentiation, to NT-plasma treatment or H_2O_2 treatment, to evaluate redox effects. Using qPCR we measured gene expression of the osteoblast-specific differentiation genes (RUNX2, BMP2, COL I, OSTRX and ALKP). Both NT-plasma and H_2O_2 treatment resulted in only a 30% induction of ß-GP's osteogenic gene expression, so a potential synergism between ß-GP and NT-plasma was investigated. We cultured the mesenchymal cells for 24 hrs. in ß-GP and then applied NT-plasma or Sham treatment. NT-plasma significantly enhanced both early and late osteoblast differentiation gene expression as compared to ß-GP; from 2 - 20 fold depending on the gene. Taken together, these results indicate that that NT-plasma alone is not sufficient to initiate significant changes in osteogenic differentiation. However, once differentiation has been initiated, NT-plasma enhances osteogenic differentiation at both early and late time points.

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Apoptosis induced by Nanosecond Dielectric Barrier Discharge Plasma

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Application of non-thermal plasmas on cultured cells [1] and in vivo [2] was shown to be a promissing strategy in cancer treatment. Recently, many groups reported on apoptotic effect of different plasma sources based on direct exposure to Dielectric Barrier Discharge Plasmas (DBD) operated in ambient air and multiple plasma jet devices allowing to deliver plasma in a flow of noble gases. DBD plasma sources have large and scalable active area and together with high Reactive Oxygen Species (ROS) production rate and low operating cost seem to be the most convenient tool for skin and subcutaneous cancer treatment. Nanosecond repetitively pulsed DBDs demonstrate better homogeneity compared to AC driven DBDs [3] and, hence, result in more uniform effect.

A dielectric barrier discharge with a cylindrical electrode covered by glass (very similar to the DBD devices described in [1,2]) is used for treatment of immortalized HMEC cell lines. HMEC cells were incubated following standard procedure during 4 days at 37 C with 5% CO_2. The confluent cell layers were obtained at the bottom of a plastic well (Falcon 24-well). The gap between dielectric and cells was 2mm. The high voltage pulses of 40 ns and 10 kV in amplitude were applied with a frequency of 500 Hz. The treatment time varied between 10s and 120s and resulted in 25-300 J of total dose. The micrographs performed 24 hours after treatment show a number of detached cells, cells in the process of detachment, elongated and bloated cells. The media with detached cells and the cell layer were then collected after trypsinisation. Flow cytometry analysis was performed to distinguish normal, apoptotic and necrotic cells using Annexin V and Propidium iodide labeling. A significant dose effect was demonstrated on the number of apoptotic cells (Figure 1) with almost 100% of apoptotic cells corresponding to 120s of treatment time. The number of necrotic cells was of the order of 1% and was found to be independent of the dose. The experiments with Jurkat cell lines are now in progress.

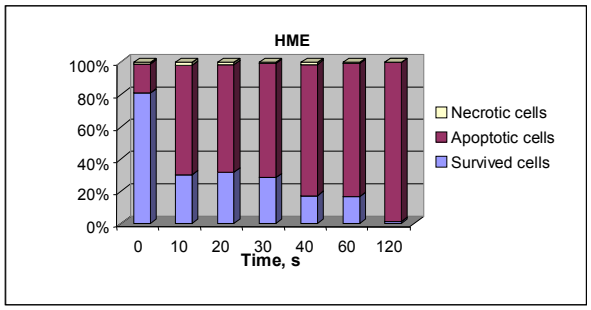

Figure 1: *Apoptotic, necrotic and survived cells 24H after plasma treatment*

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Cold plasma for bacterial inactivation and its effects on keratinocytes

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Plasmas have been used for many years for different applications. Atmospheric pressure plasmas deliver electrons, ions, UV radiation and an electric field, which together are effective in killing bacteria. Cold atmospheric pressure plasmas might provide additional means to reduce the bacterial load in a burn wound. It is however important to keep a balance between inactivating the bacteria and maintaining the wound healing potential. [1,2]

We studied the effects of cold argon plasma treatment on in vitro inactivation of *Pseudomonas aeruginosa*. For the treatment, a pulsed cold atmospheric plasma jet (13.56 MHz micro-jet) was used. Bacteria were diluted in culture broth (LB), phosphate buffered saline (PBS) or physiological salt (PS) and were treated with plasma for different times in microtiter plates. Growth or the absence thereof was recorded after overnight incubation. Alternatively, surviving bacteria were counted by plating dilutions.

The use of microtiter plates was a convenient way to test many different aspects in plasma treatment. However, it proved not to be useful when LB or PBS were used. Growth was absent after treatment but this appeared in part to be due to inhibition of growth rather than bacterial killing. In contrast, bacterial inactivation upon plasma treatment in PS was immediate and reached a log 6 reduction after 1 min. Our results indicated that a low pH during plasma treatment is critical. In addition, distance, duty cycle, plasma dissipated power and treatment time are important.

Because reactive radicals in cold plasma can interfere with the healing process, cell cultures of keratinocytes in PS were also treated with cold plasma. Two hours after treatment, the release of lactate dehydrogenase was determined as a measure of membrane leakage due to the treatment. In addition, activity of the cells was quantified with a tetrazolium based assay after 24 hours. Short treatments (10-60s) with argon did not result in extensive membrane damage or loss of activity when immediately after treatment culture medium was added.
In conclusion, non-thermal argon plasma can be used to kill bacteria and yet preserve the viability of epidermal cells.

Acknowledgment
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Cold Atmospheric Plasma in the Treatment of Malignant Glioma

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Recent investigations into cold atmospheric plasma (CAP) technology have revealed very promising results in various malignancies [1,2]. In specific, we previously demonstrated a unique selectivity of CAP for cancer cells *in vitro* and *in vivo* [1]. This attribute would be especially useful in the treatment of glioblastoma multiforme (GBM), a very aggressive and invasive primary brain malignancy which continues to carry a poor survival despite multi-modal therapies. We investigated the role of CAP in the treatment of glioma *in vitro*. Three glioma cell lines (U87, A172, U373) were grown and exposed to CAP for various time points between 15 to 180 seconds. The impact of CAP on cell growth was assessed with microscopy and MTT assays. Additionally, we evaluated the cytotoxicity, the role of caspase activation, and various intracellular messengers (i.e. cGMP).
Treatment with CAP resulted in a dose-dependent decrease in cell proliferation (Figure 1). Caspase activity and cGMP levels were also altered. These results further characterize the role of CAP as a promising therapy in the treatment of GBM.

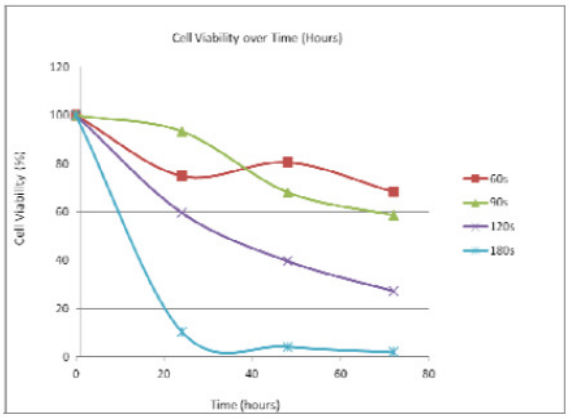

Figure 1: *Cell viability over time for various time exposures of U87 cells. The exposure times ranged from 60 seconds (60s) to 180 seconds (180s).*

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Comparison of the effect of cold atmospheric plasmas on mammalian and bacterial cells

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In the application of non-thermal plasmas to wound treatment both skin and bacterial cells are affected. This study presents a comparison between the behavior of different mammalian and bacterial cell types, on exposure to weak atmospheric plasmas. Three different types of mammalian cells were investigated – SW480, Chinese hamster ovary (CHO) and osteoblast (MG63) cells. The behavior of these cells when exposed to the plasma was compared with the bacterial cells *Staphylococcus aureus*, Coagulase-Negative *Streptococci* and *Pseudomonas aeruginosa*. A further objective is to quantitatively compare how treatment duration and frequency affect the cell response.

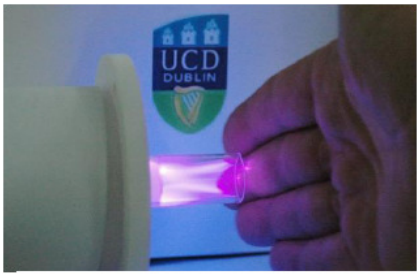

Figure 1 Helium atmospheric Pressure Plasma Jet system

The plasma treatments were carried out using the helium plasma jet system shown in Figure 1. This is generated using a dual-pin parallel tungsten electrode source powered using a C2000 Redline generator. The quartz reactor cylinder has a length of 6 cm and nozzle exit area of 2 cm2. In this study the treatment frequency was varied systematically between 150-450 kHz. Due to the differences in the relative size of the mammalian and bacterial cells, the cell concentrations exposed to the plasma were 2x 10e5/ml in the case of the mammalian cells and 10e9/ml for the bacterial cells. Exposure to the plasma was carried out either directly on polystyrene 6-well plates or by passing the cells through the plasma jet. This was achieved by passing droplets of the suspensions into the plasma using a pneumatic nebulizer. The effect of the plasma treatments was evaluated with respect to cell number, viability, cell cycle, ATP and apoptosis. Lactate dehydrogenase measurements were used to quantify plasma membrane damage of sheared cells.

Passing the cells through the nebulizer system even in the absence of the plasma was found to give rise to a significant level of cell death. This may be due to the harsh mechanical effect of the nebulisation process on the cells. The lethal effect was correlated with the nebulized droplets size (typically in the 3 to 5 μm range). The plasma intensity was assessed using emission spectroscopy and the cell treatments were carried out at the frequencies which yielded intensity maxima and minima within the 150-450 kHz range investigated. As expected cell death increased with increased plasma exposure times, with apoptosis is the predominant mechanism in mammalian cells. The level of ATP in CHO cells following plasma treatment was increased after incubation of 24 hours. It is suggested that the increased ATP following treatment might play a major role in energy provision when cellular repair processes are able to operate. The different sensitivity of mammalian and bacterial cells may be related to different cellular and molecular mechanisms in cell types. These findings suggest that such difference in sensitivity could be clinically exploited to improve the use of plasmas in wound healing.

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In vitro antitumor activity of plasma Gun

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Our group has recently showed a marked antitumor effect of DBD plasma treatment *in vivo* on U87 glioma bearing mice. Beside these results *in vivo*, various studies have showed an antitumor effect of plasma treatment on various cancer cells lines *in vitro* by apoptosis induction using DBD or plasma jet. In our lab, development of a plasma jet so called plasma gun lead to new very interesting perspectives in a context of tumor treatment *via* small catheter. The main goal of this work was to investigate the *in vitro* cells sensitivity to plasma and major cells mechanisms induce by plasma gun treatment. Experiments were performed using the plasma gun previously described by our group. Antitumor activity was evaluated on HCT116, Mia Paca and H460 cancer cells which are representative models of colorectal carcinoma, pancreatic ductal adenocarcinoma and lung tumors respectively. These cells lines are potential future target for *in situ* plasma application. *In vitro* effects of plasma treatment on proliferation and viability were assessed by bioluminescence imaging (BLI).

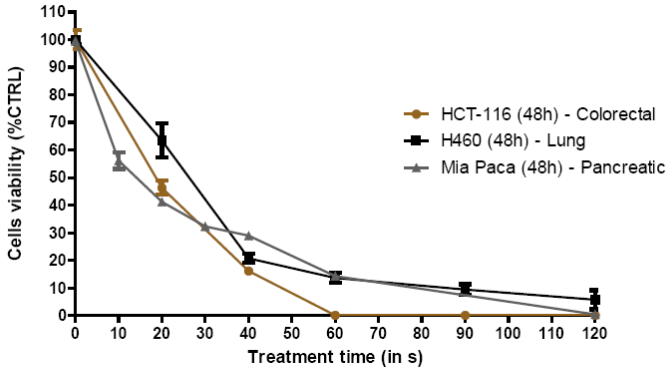

Figure 1: *Effect of plasma gun treatment on different cells lines in vitro.*

Plasma treatment induced a significant antitumor effect the different cells lines. The most sensitive cell line to plasma treatment was Mia Paca cells with a LD_{50} of 12s, then HCT-116 (LD_{50} 18s) and H460 (LD_{50} 28s) (figure 1). In HCT-116, plasma induces a decrease of cell proliferation and a decrease of cell mobility. This decrease of cell proliferation was the consequence of a massive cell cycle arrest in G2/M after plasma treatment. Moreover, an increase of apoptosis was observed in the treated cells. These results obtained with the plasma gun were closed to previous results obtained with our DBD and to other research group. Recent studies suggest the major importance of p53 in response to ROS generated by plasma treatment which is implicated in the cell cycle control. Our results on various cell lines including intracellular mutations like p53 or PTEN could explain the difference of cell sensitivity. Indeed, the most resistant cell line (H460) has various mutations including p53, KRAS and PIK3 while a Mia PACA cell has KRAS and p53 mutations. Implications of theses mutations in plasma cell sensitivity need to be further investigated.

Non thermal plasma applied with plasma appears to be very promising and experiments are ongoing to evaluate antitumor activity of this strategy on representative cancer models *in vivo*.

Numerical Simulation of Reactive Species in Liquids in Contact with Atmospheric Pressure Plasmas

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Generation of chemically reactive species that may affect biochemical reactions in liquid have been studied with the use of numerical simulations for a global chemical reaction model. When a living tissue is exposed to a low-temperature atmospheric-pressure plasma, there is almost always a liquid layer, such as blood or other body fluids, that separates the gas phase and the tissue. Therefore chemically reactive species generated by a plasma discharge in the gas phase need to be transported through the liquid phase before reacting with the tissue surfaces. During this transport process, some of the chemically reactive species may react with other species and, therefore, the observation of gas-phase chemically reactive species does not directly convey information on abundant chemically reactive species in the vicinity of the tissue. In the present study, we have performed numerical simulation of reaction equations that govern time evolution of the densities of various species in pure water under the global-balance (i.e., zero-dimensional) conditions. The liquid phase is assumed to be in contact with the gas phase and the density of each species between the gas and liquid phases are connected via Henry's law. Figure 1 shows time evolution of various species dissolved in water if the initial densities of NO and electrons are 10^{-10} mol/L each.

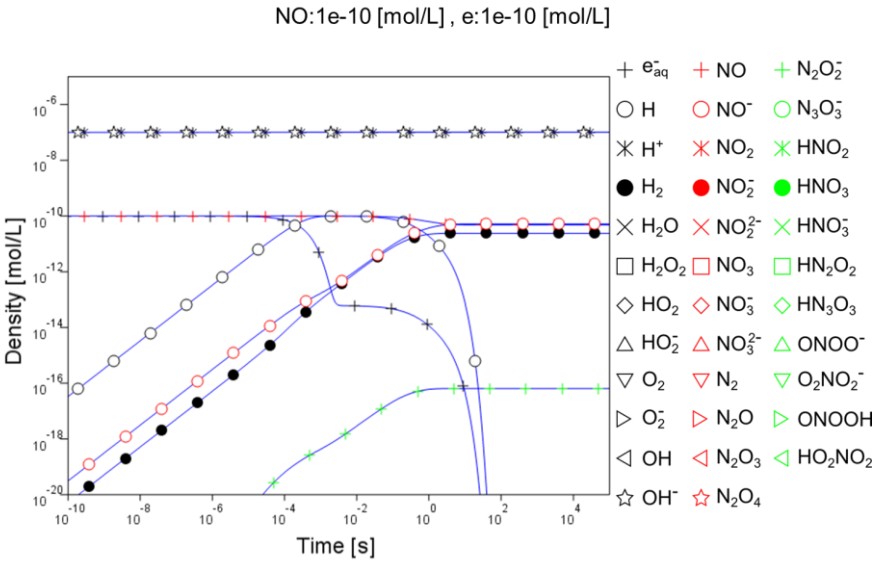

Figure 1: *Time evolution of densities of various species in pure water when NO of 10^{-10}mol/L and electrons of 10^{-10}mol/L are added at t = 0, obtained from global simulation.*

Non-thermal atmospheric argon plasma treatment as a novel approach to improving wound healing: Results of a first randomized placebo-controlled clinical trial on skin graft donor sites

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Background
Cold atmospheric plasma has already been shown to decrease the bacterial load on chronic wounds. However, until now it is not yet known if plasma treatment may also improve wound healing.

Objectives
To assess the impact of cold atmospheric argon plasma on the process of wound healing.

Methods
40 patients with skin graft donor sites on the upper leg were enrolled into our study. The wound sites were divided into two equally sized areas and randomly assigned to receive plasma treatment or argon gas only as a placebo mode for 2 min. Wound healing was evaluated independently by two blinded dermatologists, who compared the wound areas with regard to re-epithelialization, the amount of blood crusts, fibrin layers, and wound surroundings.

Results
From the 2nd treatment day onwards, wound areas treated with plasma (n=34) showed significantly more often improved wound healing than placebo-treated areas (day 1: $p=0.25$, day 2: $p=0.011$, day 3: $p<0.001$, day 4: $p<0.001$, day 5: $p=0.004$, day 6: $p=0.008$, day 7: $p=0.031$). Positive effects were observed in terms of improved epithelialization and fewer fibrin layers and blood crusts, whereas wound surroundings were always bland, independent of the type of treatment. Wound infection did not occur in any of the patients, and no relevant side effects were observed. Both types of treatment were well-tolerated.

Conclusions
Cold plasma treatment has shown to have positive effects on wound healing, but the mechanisms contributing to these clinically observed effects have to be further investigated.

Acknowledgment: We thank Ms Sabine Helmig for her excellent patient care.

Treatment of Diseases by Inhalation of Atmospheric Pressure Plasma Flow

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Recently, atmospheric-pressure plasmas are indispensable for sterilizing, disinfecting, decomposing hazardous materials and modifying material surfaces and new biomedical applications have also been found although the mechanisms of action remain unknown. Plasmas contain many neutral molecules, ions, and radicals and oxidative nitrogen compounds such as Nitric oxide (NO) are generated under atmospheric conditions. NO in mammals including humans is an important cellular signaling molecule for many physiological and pathological processes. Especially, NO inhalation is used to treat persistent pulmonary hypertension of the newborn, heart load reduction during open-heart surgery and primary pulmonary hypertension. We aimed to distinguish endogenous and exogenous NO in a porcine model pulmonary of hypertension to clarify the relationship between NO concentration in the bloodstream and hypote nsion.

A schematic diagram of the experimental setup is shown in Figure 1. The coaxial plasma source has a 1-mm-diameter tungsten wire inside a glass capillary, that is surrounded by a grounded tubular electrode. The AC/DC amplifier and multifunction synthesizer controlled by PC provides a high voltage for plasma generation. Plasma was generated under the following conditions: applied voltage, 8 kVpp; frequency, 3 kHz; helium (He) gas flow rate, 1 L/min. On the other hand, sphygmomanometry of a blood vessel proceeded using a device comprising a disposable force transducer, a bedside monitor for simultaneous electrocardiography and signal pressure measurements, and a pressure pack containing physiological saline to adjust the pressure in the catheter. We directly measured NO using a catheter-type NO sensor placed in the coronary sinus through an angiography catheter from the abdomen. The mini pig (weight: 10–15 kg) were initially sedated with ketamine and anesthetized with sevoflurane using an anesthesia device with a mechanical respirator.

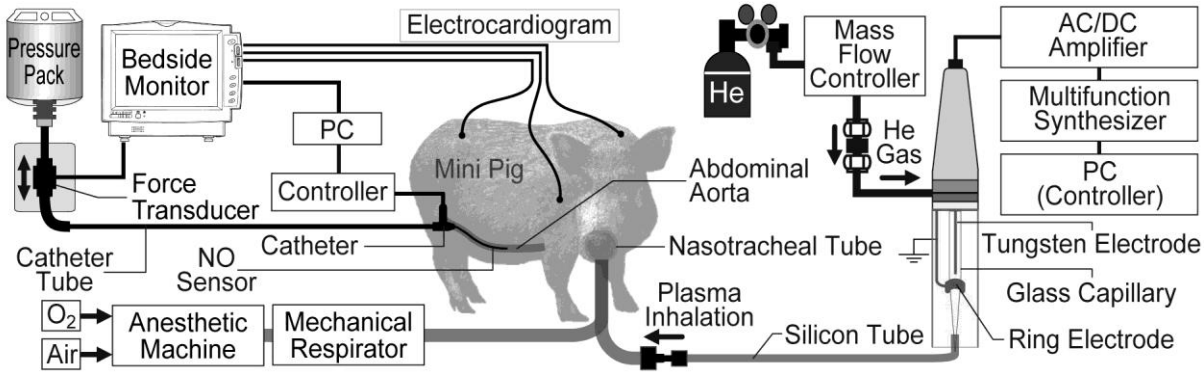

Figure 1: *Experimental setup*

According to the NO concentration in the abdominal aorta as a function of the duration of plasma inhalation, the NO concentration started to increase from 10 s, reached a maximum value at about 40 s and then gradually decreased. In addition, from the results of blood pressure in the abdominal aorta after inhaling He gas and plasma, blood pressure did not decrease while inhaling He, but decreased from 110/65 to 90/40 mm Hg after inhaling plasma. Blood pressure started to fall about 10 s from starting inhalation, and reached the nadir 40–50 s later. The results of the present study are very similar to those obtained by other groups that used a catheter-type NO sensor to determine the acute or chronic effects of angiotensin II on the bioavailability of NO in rabbits.

Low-temperature atmospheric plasma increases the expression of antiaging genes of skin cells without causing cellular damages

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Efforts to employ various types of plasma in the field of skin care have increased consistently because it can regulate many biochemical reactions that are normally unaffected by light-based therapy [1]. One method for skin rejuvenation adopted a high-temperature plasma generator to remove skin epithelial cells [2]. In this case, the catalyzing effects of the plasma were rarely used due to the high temperature. Hence, the benefits of the plasma were not magnified. Recently, many types of low-temperature plasma devices have been developed for medical applications but their detailed functions and working mechanisms are unclear [3].
The present study examined the effect of low-temperature microwave plasma on skin cells. Treatment with low-temperature plasma increased the expression of anti-aging genes in skin cells, including collagen, fibronectin and vascular endothelial growth factor A (VEGF-A). Furthermore, the plasma treatment did not cause cell death, but only induced slight cell growth arrest at the G2 phase. Although the cells treated with low-temperature plasma showed moderate growth arrest, there were no signs of thermal or genetic damage of skin cells. We also tested the possible role of the plasma on skin barrier function. The cells treated with plasma showed decreased expression of E-cadherin and lack of cell-to-cell interactions. The plasma treatment to mouse skin did not cause tissue damage, but increased penetration of hydrophilic substances into the skin. Overall, this low-temperature microwave plasma device could be useful for the wound healing and the absorption of drugs or cosmetics.

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The Exposure to the Non-Thermal Atmospheric Pressure Plasma Can Control the Proliferation of Mammalian Cells

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Recently, atmospheric pressure plasmas (APPs) have been utilized as a novel tool of medical applications such as wound healing and blood coagulation. APP abounds with electrons, various ions, radicals, and neutral atoms which cause specific interactions with cells [1]. However, its application to human cells has been mainly focused on cell death. In this study, a non-thermal APP which was generated by an atmospheric pressure dielectric barrier discharge was applied to three different human cell lines without heat generation. We observed that the exposure of APP to human adipose-derived stem cells (ASC) and the primary lung fibroblast IMR-90 cells induced increased cell proliferation in a specific condition. On the other hand, the same exposure of APP to HeLa cells dramatically decreased their viability. These observations suggest that different types of human cells differentially respond to the exposure of APP. The cleaved forms of caspase-3 and poly ADP-ribose polymerase (PARP) which is the markers for cell apoptosis were detected in APP-exposed HeLa cells, demonstrating that the decreased viability of HeLa cells is due to cell death. In the caspase-dependent apoptotic pathway, PARP can deplete cellular adenosine triphosphate (ATP) which leads to cell death [2]. When APP-exposed HeLa cells were treated with an extracellular ROS scavenger, sodium pyruvate, the viability was recovered in a concentration-dependent manner. These results suggest that APP specifically induces apoptosis in HeLa cells by generating extracellular ROS. Altogether, this study suggests that APP can be a useful method to control the proliferation of different types of human cells.

This work was supported by Korean Research Institute for Chemical Technology (KRICT) funded by Ministry of Knowledge Economy, Korea (SI-1105).

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The Effects of In-vivo Application of Nonequilibrium Atmospheric Plasmas on Corneal Wound Healing in New Zealand White Rabbits

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The application of atmospheric plasmas in medicine began about 20 years ago and has grown into the separate field of Applied Plasma Medicine, attracting great attention from researchers due to potential applications in dentistry, drug delivery, dermatology, cosmetics, wound healing, cellular modifications, scar formation control, cancer treatment etc. Recently we demonstrated efficiency of cold plasmas for ablation of mid-sized subcutaneous bladder cancer tumors on mice [1].

In this work we studied the effects of cold plasma therapy on corneal wound healing. Due to the transparent nature of the cornea, controlled wound healing is essential to ensuring ideal corneal clarity and optimum visual acuity following a corneal injury. A small group of 12 New Zealand white rabbits was used for these studies. The procedure included surgery, where epithelial defects to rabbits' corneas were induced, followed by immediate application of cold plasma, and then post-surgical monitoring of corneal clarity and epithelial healing time. Microscopic features of the treated corneas were compared with control corneas, which were not treated with cold plasma. Monitoring of the wound size at about 30 hours after the surgery indicated faster wound healing for the rabbits treated with plasmas compared with controls.

This research was supported by Medical Faculty Associates Collaborative Research Program of George Washington University.

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Plasma sterilization of pharmaceutical products: from basics to production

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The decontamination of thermolabile pharmaceutical products by a plasma process is of growing interest in research and application. Available methods like toxics (ethylene oxide) or electron beam sterilization have either issues in handling and security or produce toxic remnants, which need to be taken care of in additional process steps. Also challenging for some decontamination methods are biomolecules like prions and pyrogens. Plasma sterilization poses an alternative treatment with several advantages, especially for thermolabile pharmaceutical goods.

An industrial process was developed in close cooperation with groninger & co. gmbh. It was presented for the first time on the last ICPM. Its application is the outer decontamination of syringe containing tubs before they are filled in a clean room. The tubs mainly consist of polystyrene and are sealed by Tyvek® foil on top. The process runs at pressures below 10 Pa. Plasma generation is achieved by two opposite coils, mounted at the top and bottom of the chamber, with 3-5 kW available RF power at each coil.

To understand basic principles of plasma sterilisation a laboratory set-up double inductively coupled plasma reactor (DICP) [1] is used. In this reactor the influence of different sterilization agents can be investigated, such as UV/VUV radiation or reactive species. Since the DICP is similar in dimensions and power coupling to the industrial reactor, theses experiments can be performed under nearly the same conditions.

In order to gain knowledge about optimization parameters, several plasma diagnostic methods have been applied to both reactors. Langmuir probe measurements provide spatially resolved information on electron density and temperature, hence discharge homogeneity. In combination with calibrated optical emission spectroscopy also the UV/VUV surface irradiation and gas temperature are determined. Investigated parameter variations include duty cycle, power input per coil, pulsing frequency, pressure and gas composition. Additionally, microbiological tests have been performed to investigate sterilization efficiency.

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Low Temperature Non-Thermal Plasmas at Atmospheric Pressure: Diagnostics and Medical Applications

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This study was devoted to diagnostics of low temperature plasma non-thermal plasmas at atmospheric pressure and investigation of its bactericidal effect against bacteria in biofilms and within eukaryotic cells.

In our studies the low temperature plasmas were produced by microwave generator with frequency of 2.45 GHz at low power (~ 150 W) and at low temperatures of a gas (argon) flow (< 40 C) and by the ferroelectric bed reactor (ferroelectric packed-bed technology) that employs a high-voltage DC and AC power supplies in conjunction with a tubular reactor packed with high-dielectric ceramic pellets. Usually the pellets are held within the tube arrangement by two metal mesh electrodes. When external voltage is applied across the high dielectric layer, the pellets are polarised, and an intense electric field is formed around each pellet contact point. Many pulsed discharges take place around each contact point of the ferroelectric pellets, and the discharge energy can be controlled by changing the dielectric constant of the pellet, and by the voltage waveform.

Complex plasma diagnostic measurements under various regimes of work were carried out. It was performed with the use of method of optical imaging, optical emission spectroscopy, chemical gas analysis of plasma stream, and probe diagnostics of SHF radiation of the plasma torch (for microwave discharge). In the experiments the high resolution spectra of the OH around 308 nm, Ar and N_2 in the spectral range 320-850 nm were obtained. Calibration of the spectrometer was carried out by deuterium and halogen lamps. A gas composition of plasma flow was analyzed: the concentrations of nitric oxide NO, nitric dioxide NO_2 and ozone O_3 were measured. The results of the probe measurements of SHF radiation for plasma torch were also presented. At some regimes of microwave plasma torch the SHF radiation can exceed 10 mW. Estimations and measurements concerned with the use of below-cutoff waveguide for elimination of SHF radiation were obtained.

The model of immersed surface-associated biofilms was used to assess bactericidal effects of plasma treatment. Reduction in the concentration of live bacteria in biofilms treated with plasma for 5 min was demonstrated. The intracellular infection model with the pathogenic bacterium, Chlamydia trachomatis, was used to study the efficacy of microwave argon plasma against intracellular parasites. A 2 min plasma treatment of mouse cells infected with C. trachomatis reduced infectious bacteria by a factor of $2x10^6$. Plasma treatment diminished the number of viable host cells by about 20 %. When the samples were covered with MgF_2 glass to obstruct active particles and UV alone was applied, the bactericidal effect was reduced by $5x10^4$ fold compared to whole plasma.

Insight in the complex argon/humid air plasma chemistry, by means of numerical fluid modeling

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Since experimental diagnostics are expensive, time consuming and only a limited amount of information can be obtained, numerical simulations have proven to be very useful n various research fields, but are still not often performed for devicesused in biomedical applications. With thezero-dimensional (0D) fluid dynamics model GLOBALKIN [1], anextensive reaction chemistry set was developed. Several hundreds of reactions were taken from available literature to describe the kinetics between the included species, given inTable 1. In this way it is possible to identify the relevant species, but also the major formation and destruction pathways. It is important to mention that for a plasma jet device, these pathways will change drastically. Inside the device the chemistry is mainly a noble gas discharge with air impurities, followed by mixing Ar/humid air and finally an afterglow region where noble gas is only present in minute quantities.

In a second stage, once the different pathways are unraveled, it is possible to determine a reduced chemistry set for sophisticated two-dimensional (2D) fluid dynamics modeling. In this way a compromise is made between reaction set accuracy and calculation time. The advantage is that with the 2D fluidcode nonPDPSIM [2] much less assumptions have to be made than that there is associated with zero-dimensional modeling, furthermore additional information is obtained. The latter concerns e.g. self-consistent electric field, fluid dynamics (gas mixing), etc.

Table 1: *Included species for the argon/humid air chemistry set.*

Ground state particles	Excited states	Charged particles
Ar	Ar(^{4}S), Ar(^{4}P), Ar_2* ($a\ ^3\Sigma^+_u$)	e^-, Ar^+, Ar_2^+
N_2, N	N_2($A\ ^3\Sigma^+_u$), N_2 ($a'\ ^1\Sigma^-_u$), N(^{2}D)	N_2^+, N_4^+, N^+
O_2, O_3, O	O_2 ($a\ ^1\Delta_g$), O_2 ($b\ ^1\Sigma^+_g$), O(^{1}D)	O_2^+, O^+, O^-, O_2^-
NO, NO_2,N_2O,NO_3,N_2O_5		NO^+, NO_2^+,NO_2^-,NO_3^-
NH, HNO, HNO_2, H_2, H		H^+, H_2^+, H_3^+, H^-, ArH^+
H_2O, H_2O_2, HO_2, OH		H_2O^+, H_3O^+, $H_2O_2^-$, OH^+, OH^-

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Characterization and Simulation of Plasma Chemistry Produced by Surface Micro Discharge

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The surface micro-discharge (SMD) plasma source has been used to study the bactericidal effect of cold atmospheric pressure plasma [1-2]. To understand the responsible mechanisms and agents, the key is to derive chemistry of delivered plasma to microorganisms.
The plasma source consists of a mesh electrode, a Teflon board with a thickness of 0.5 mm and an Aluminum foil. The plasma is produced in ambient air by applying a high voltage (HV) signal between the mesh and foil. The pick-to-pick value of HV signal is about 10 kV with various frequency between 1-10 kHz.
In this contribution, we present a zero-dimensional model of plasma chemistry produced by SMD source. The chemistry of nitrogen and oxygen is included in the model and the role of other species presented in ambient air is neglected. The major creation and loss terms for different species are determined by tracing the reactions from the very beginning till equilibrium. Dependency of species densities on some of the plasma parameters are studied. Electrical characterization and optical emission spectroscopy is used to validate the results of simulation. However, density of dominant species are measured by mass spectrometer.

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How to tailor low temperature plasma at atmospheric pressure for a given biomedical application?

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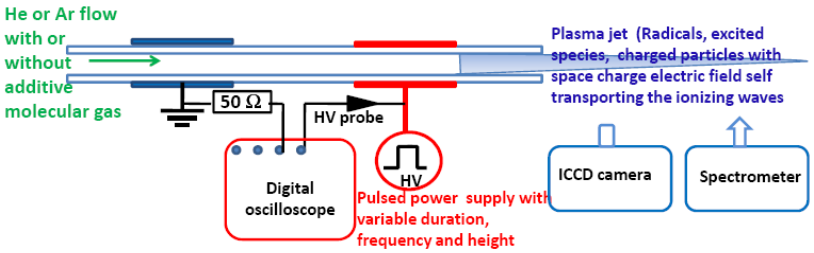
How to really tailor a low temperature plasma jet that produces many atomic and molecular active species at atmospheric pressure (radicals, excited species, charged particles, photons) and even electric field ? Is it possible to selectively control specific active species identified as efficient in plasma medicine field for a given disease associated for instance to wound healing, blood coagulation, oncology, dermatology, etc.? How to quantify and tune the production rates of such specific active species? These are some of the questions that can be addressed nowadays to the plasma community researchers involved in biomedical fields. The answers, which are already the subject of research in progress, depends on the plasma device, its geometric design (electrode configuration, reactor size), its driven power (DC, RF, pulsed, MW) and the carrier gas (composition, flow rate) (see e.g. ref 1 and the refs given therein). To contribute to the answers of such questions, we focus on a typical plasma jet device using helium gas flowing a quartz tube surrounded by two thin electrodes powered by a mono-polar pulsed high voltage supply (see figure hereafter [2]).



The operating parameters that can be investigated are the reactor geometry (inter-electrode space, wide of electrodes, nature of the dielectric), power supply (pulse duration, frequency and height), carrier gas flow and composition (He or Ar with a small admixture of O_2 or N_2 or Air). The investigation tools that we used are the classical ones needed for experimental characterizations (emission, absorption and laser spectroscopy, ICCD imagery, electric measurements) complemented by experimentally validated models of the electro-hydrodynamics and kinetics processes and phenomena for the ionizing waves dynamics, the hydrodynamics of the carrier gas flow and the chemical kinetics of reaction occurring along the plasma jet. The importance of the validated basic data needed to feed such complex models is also emphasized. Our discussion will be illustrated during the conference by some results on the evolution along the tube axis (versus certain operating parameters) of the electric field magnitude and the production rates of some active species (atomic oxygen, metastable, charged particles) with comparisons to a second device plasma jet device [3].

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Preliminary study of the argon gas flow from an atmospheric plasma jet applicator

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A project to study the atmospheric plasma jet produced from a small applicator has been started through a collaboration between Quantemol Ltd. and Creo Medical Ltd. The aim of the project is to obtain a detailed understanding of the spatial and energy distributions of ions and plasma species leaving the applicator and to understand their role in any anti-microbial effect.

To initiate this project, flow simulations of the bulk neutral gas (argon) has been undertaken. This step was taken to help guide the design of the applicator itself and to optimize the spread of the gas flow. These simulations were achieved by using the gerris flow solver [1] which implements a finite element/volume method [2]. To enhance computational time axial symmetric simulations were produced, with the symmetry axis running along the center of the pin inside the applicator. The simulation domain is shown in figure 1, where the left hand side is defined as an inflow using parameters supplied by Creo Medical Ltd. and the upper and right hand sides are defined as outflow boundaries. The resulting scaled argon concentration is shown in figure 1, where red equates to unity and blue to zero. These simulations have also been extended to look at the interaction between multiple jets and different applicator shapes.

Using these simulations we have been able to outline possible changes which can be made to the applicator design. Further simulation results (i.e. three dimensional flows) will also be reported at the conference.

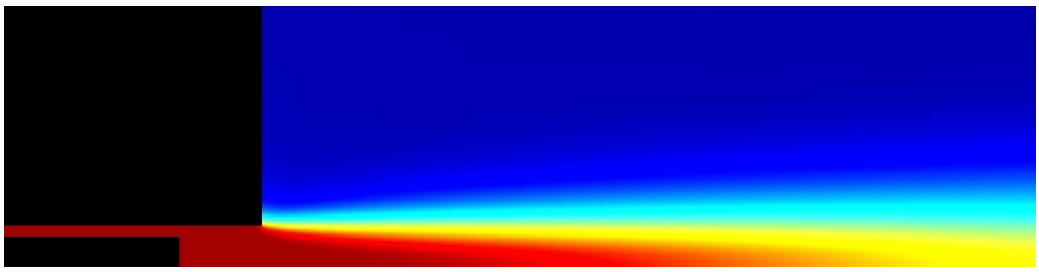

Figure 1: *Image of the argon concentration flowing from the end of the plasma jet applicator*

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Measurement of active species concentrations in nitrogen and argon/nitrogen flowing afterglows at reduced pressure.

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Nitrogen containing post-discharges at reduced pressure (1-20 Torr) are today widely used in numerous surface treatments such as steel nitriding, modification of wettability or of adhesion properties. In the late afterglow region, the flowing gas is free from agressive charged species but still contains large amounts of reactive species (atoms, metastable and vibrationally excited molecules) flowing at room temperature. For these reasons, flowing afterglows are particularly well suited to the cold sterilization of the medical instrumentation.

During the last 15 years, the antibacterial capabilities of nitrogen/oxygen [1-3] and of pure nitrogen flowing afterglows [4-5] have been studied. In this last case, a 6 log reduction of an initial bacterial concentration (i.e. a sterilization) was obtained either at room temperature with a high microwave power (P_{MW} = 300 W) injected in the discharge or for an operating temperature of 60°C with a lower injected microwave power (100 W).

For both cases, the concentration of the nitrogen atoms (which were proven to be the sterilization agent) was shown to be higher than 10^{21} m^{-3}. The present paper is devoted to the characterization of the late afterglow region of N_2 and Ar/N_2 flowing discharges. Results will be presented concerning the variations of the gas temperature and of the concentrations of the high lifetime species such as the nitrogen atoms, the vibrational levels of the molecular fundamental state $N_2(X, v)$ and the metastable species $N_2(A)$ with the operating parameters (pressure, microwave power, gas flow rate). For $N_2(A)$, the spectroscopic method recently developed by Pointu and al. [6] was adapted to the reduced pressure range, conducing to a maximum concentration of 2.6 10^{16} m^{-3}, in good agreement with the values already published in the litterature [7].

This work is supported by the ANR PLASMAVIV program.

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Transition from non-uniform to uniform discharge in nanosecond pulsed FE-DBD and linear corona non-equilibrium plasmas

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In recent years, atmospheric pressure non-equilibrium plasmas have been proven to be viable tools for decontamination and sterilization of surfaces and living tissues; current research is focused on exploring the feasibility of plasma aided medical therapies, such as blood coagulation, chronic wound remediation and cancer treatment.

Among the various requirements posed by medical applications there is the pressing need for securing uniform discharges, phenomena closely related to the effectiveness of the treatment. However, several plasma sources (e.g. dielectric barrier discharge, DBD) can generate a highly non-uniform discharge [1], especially when the treated substrate is topographically non-uniform. Indeed, the antimicrobial effectiveness of a nanosecond pulsed DBD has been recently demonstrated to be higher than that of a microsecond pulsed one; this result being ascribed to the higher discharge uniformity of the former [2].

In this work, time-resolved imaging was adopted to investigate the transition from non-uniform to uniform discharge in two different plasma sources operated at atmospheric pressure: a floating electrode dielectric barrier discharge (FE-DBD) [3] and a novel linear corona discharge. Both plasma sources have been supplied with high voltage generators capable of producing nanosecond pulses; for both, in order to emulate non-uniformities of biological tissues and sharpen the contrast between discharge regimes, a topographically non-uniform substrate has been used, acting as the floating electrode. An intensified CCD camera with nanosecond gating time has been adopted to visualize light emission from the discharge in synchronization with voltage and current waveforms measured by an oscilloscope.

The effects of voltage, frequency and gas composition on the transition between discharge regimes have been studied; a comparison between nanosecond pulsed discharges under different operative conditions is presented.

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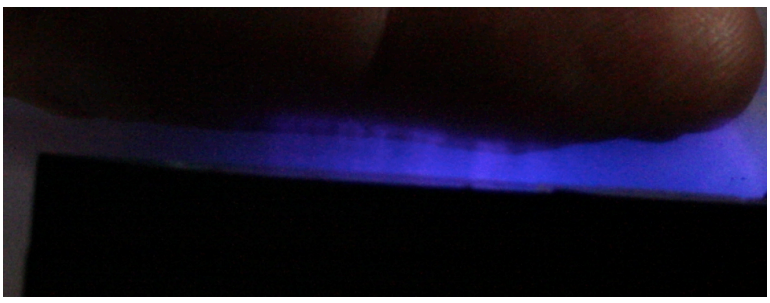

Effluent composition, thermal output and fluid-dynamics of a dual gas plasma needle device for biomedical applications: Part I

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The complexity of plasma interaction with biological material and the stiff requisites imposed by biomedical treatments put a premium on diagnostics as a mean to investigate process feasibility and to develop plasma sources tailored for specific applications. Consequently, large efforts have been dedicated to characterize plasma sources for biomedical applications and to identify the most suitable diagnostic techniques [1-2].

In this work a set of diagnostic methods have been used to investigate plasma behavior (gas temperature, heat flux, effluent composition and fluid-dynamics) in a novel multi-gas device developed by the authors and based on the plasma needle concept [3].

The presence of emitting reactive species in the effluent was analyzed at different axial positions, downstream the needle, by means of time-resolved optical emission spectroscopy (OES) in the ultraviolet, visible and near-infrared region. Emission spectra were collected using a 500mm spectrometer synchronized with an iCCD camera, while voltage and current signals of the plasma source are recorded by means of an oscilloscope. Time-resolved recordings of selected spectral regions have been carried out using a photomultiplier tube (PMT) coupled with an oscilloscope.

A wide set of measurements has been performed changing the operating conditions of the plasma source and for different geometrical and flow combinations of primary gas (argon, helium) and secondary gas (air, nitrogen, oxygen).

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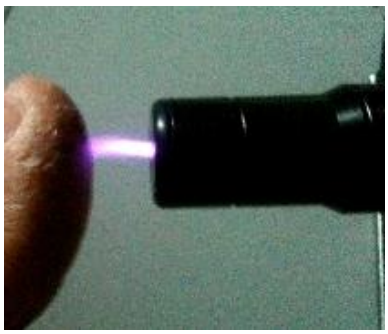

Effluent composition, thermal output and fluid-dynamics of a dual gas plasma needle device for biomedical applications: Part II

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The complexity of plasma interaction with biological material and the stiff requisites imposed by biomedical treatments put a premium on diagnostics as a mean to investigate process feasibility and to develop plasma sources tailored for specific applications. Consequently, large efforts have been dedicated to characterize plasma sources for biomedical applications and to identify the most suitable diagnostic techniques [1-2].

In this work a set of diagnostic methods have been used to investigate plasma behavior (gas temperature, heat flux, effluent composition and fluid-dynamics) in a novel multi-gas device developed by the authors and based on the plasma needle concept [3].

Axial temperature and heat flux profiles of the afterglow region were obtained by means of a highly accurate optical fiber temperature sensor; while Schlieren imaging was adopted to investigate the fluid-dynamics of the afterglow region; flow fluctuations generated by the effluent when impinging on substrates of different geometries (plain substrate, Petri dishes, etc.) were investigated by means of a fast CCD camera (up to 200,000 fps) coupled with Schlieren technique and subsequent statistical elaboration of high-speed recordings. Results from Schlieren imaging and fluid-dynamic modeling are compared.

A wide set of measurements has been performed changing the operating conditions of the plasma source and for different geometrical and flow combinations of primary gas (argon, helium) and secondary gas (air, nitrogen, oxygen).

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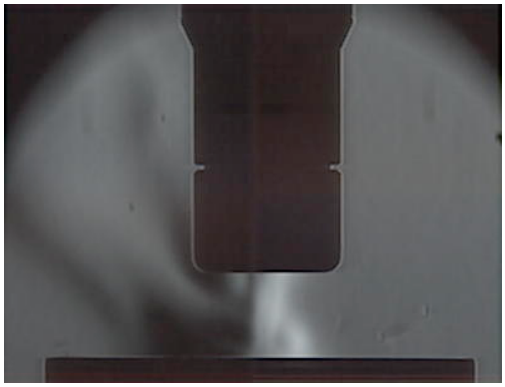

Chemical pathways governing the production of Reactive Oxygen Species (ROS) in atmospheric pressure He+O_2+H_2O plasmas

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It is well-known that atmospheric-pressure plasmas can be engineered to produce reactive oxygen species (ROS) and reactive nitrogen species (RNS) known to play important roles in biological systems. Here we concentrate on the generation of ROS, and in particular on the chemical pathways that govern the generation and loss of ROS in atmospheric pressure rf (13.56MHZ) plasmas sustained in helium with admixtures of O_2 and H_2O.

Both O_2 and H_2O are good precursors of ROS [1,2] and they can be combined to create cocktails of ROS of different compositions [3]. Due to the presence of O_2 and H_2O, these plasmas tend to be electronegative and display interesting dynamics, particularly when created in small gaps [4]. From a practical point of view, it is important to understand the chemical pathways leading to the production of the biologically relevant ROS, as this will provide guidelines for the optimization of the plasma sources for a particular application.

By means of 1-dimensional fluid simulations (61 species, 878 reactions), the key ROS and their generation and loss mechanisms are identified for admixtures containing 0-1% oxygen and 0-0.3% water content. Although most ROS can be generated in a wide range of oxygen and water concentrations, the chemical pathways leading to their generation change significantly as a function of the feed gas composition.

It is found that for a given oxygen concentration, the presence of water in the feed gas decreases the net production of oxygen-derived ROS (ozone, singlet oxygen and atomic oxygen), while for a given water concentration, the presence of oxygen enhances the net production of water-derived ROS (hydrogen peroxide, hydroxyl radicals and hydroperoxyl radicals). As a result oxygen rich mixtures tend to produce larger quantities of ROS whereas water rich mixtures produce cocktails with higher oxidation potential due to the presence of hydroxyl radicals.

The shift on the main chemical pathways governing the production of ROS implies that care must be taken when selecting reduced chemical sets to study these plasmas and has important implications in the reproducibility of plasma treatments performed in uncontrolled environments and/or samples.

This work was supported by the UK Engineering Physical Science Research Council.

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Development of microdicharges in silicon operating in DC for medical applications

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Potential applications of microdischarges are numerous and include local treatments, lab on chip, sterilization… One of the most important technological and scientific issues of these new microdevices remains the elaboration of the microreactors. They have to be robust enough to sustain power densities as high as few hundreds of kilowatts per cubic centimeter.
Arrays of microreactors built from silicon wafers in clean room facilities have been proposed and developed recently [1]. They consist of Micro Hollow Cathode Discharges (MHCD) operating in parallel in DC. One of the remarkable properties of these MHCDs relies on the fact that they can operate in DC, in a stable regime at atmospheric pressure, without evolving to an arc regime [2]. Operation in AC is also possible and offers some advantages in terms of homogeneity and life time [3,4].
We will present recent results obtained in DC excitation. Discharges were performed in helium and Argon. The microreactor geometry was investigated to achieve the best results in terms of life time and ignition. Although we were able to ignite up to 1024 microdischarges (100 µm diameter holes) (figure 1), we observed many spikes on the current waveform, which indicate that the microplasma is not so stable. We varied different parameters such as pressure and current. V-I curves were systematically acquired during the experiments. Optical characterizations were also carried out (imaging system and optical emission spectroscopy). Breakdown mechanisms in DC were investigated [5]. The life time of the device varies from few minutes to few hours in DC operation depending on the injected power. We have studied the damage mechanisms of our microdevices by using a Scanning Electron Microscope.

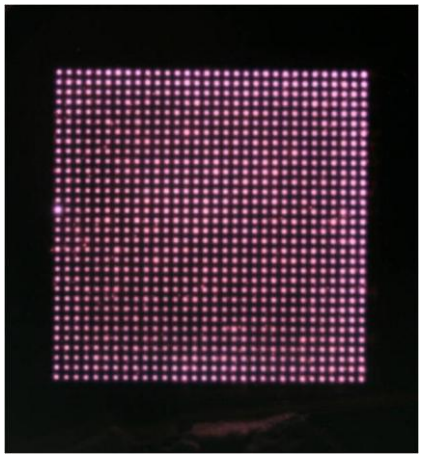

Figure 1 : Array of 1024 MHCDs of 100 µm diameter operating in Helium at 350 Torr

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Space and time resolved characterization of *in-air* Pulsed Atmospheric Plasma Streams for biomedical applications

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Non-Thermal Atmospheric Pressure Plasma Jets (NT-APPJ) are powerful tools of great interest for the growing Plasma Medicine field. The Plasma Gun, developed in GREMI, producing Pulsed Atmospheric pressure Plasma Streams (PAPS) is used for *in vitro* and *in vivo* studies of the antitumor effect of plasmas. Besides numerous *in-capillary* studies concerning PAPS velocity, morphology and splitting-merging behaviour [1], recent achievements about *in vivo* antitumor activity of the Plasma Gun, led to focalize on plasma chemo-physical properties of the *in-air* expending jet.
PAPS generated with the Plasma Gun, under short time excitation (µs) from 100Hz to kHz in Helium at low flow rate (100 to 1000 ml per min) have unique features such as high ionization front velocity (108 cm.s-1) and long propagation in flexible dielectric capillaries (~m) prior to *in-air* expending jet. In this work, they have been studied in terms of their reactive species (RS) production.
Emission intensity as well as spatial distribution evolution of RS versus parameters such as gas flow, repetition rate and applied voltage amplitude, were under the scope of this work. To achieve optimization of the plasma delivery *in vitro* and *in vivo*, parametric studies had been performed using space and time resolved spectroscopy (OES) to follow the relative evolutions of reactive oxygen species such as OH*, NO* and nitrogen molecular species (N2* and N2 +*) considering their highly probable involvement in biological processes. Filtered imaging had been achieved focusing on species radiation in UV and Visible domains on those species and He*.
Fast imaging reveals spatial distribution of RS within the PAPS. NO* emission intensity per pulse was shown to increase with the repetition rate which was not the case for other species. We have shown that distance between dielectric capillary exit and targets, target coupling with the ground as well as gas flow rate clearly change RS production and spatial distribution opening ways to control RS delivery on the target.
This work gives an overview of the dynamics taking place during PAPS propagation in the *inair* expending jet resulting in the ability to optimize experimental parameters to suit desire treatment conditions.

This work was supported by ANR "PAMPA" and by Région Centre through the APR program "Plasmed". DR was financially supported by CNRS and Region Centre, MV by Germitec and VS by Conseil Général du Loiret.

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Dynamics of Pulsed Atmospheric pressure Plasma Streams generated by a Plasma Gun

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A specific plasma jet device named Plasma Gun has been developed in GREMI for biomedical applications. Recent studies using this device concerned plasma antitumoral effect and potential endoscopic treatments. The plasma gun is based on dielectric barrier discharge (DBD) flushed with rare gases at low flow rates (10-1000 sccm), and powered by a pulsed generator. It allows Pulsed Atmospheric- pressure Plasma Streams (PAPS) generation at high velocities (10^8 cm.s^{-1}). PAPS can propagate over long distances in dielectric capillaries (from few tens of cm to m). At the capillary outlet, a plasma plume is generated in ambient air, leading to production of reactive species which participate to the plasma treatment. In order to optimize plasma target exposition, it is necessary to better understand the mechanisms of generation and propagation of the PAPS in the dielectric capillary and at its outlet.
In this work, generation and propagation mechanisms were investigated mainly by means of ICCD imaging. Fast ionization wave (FIW) velocity measurements are obtained using a bunch of optical fibers, connected to a PMT, placed along the PAPS propagation path. Two propagation modes have been highlighted: Wall hugging PAPS (Wh-PAPS) and Homogeneous PAPS (H-PAPS). PAPS propagation is dependent on the voltage pulse shape and, to a lesser extent, on the pulse repetition rate and the gas flow rate. It appears that the photo-ionization plays a minor role, but the PAPS propagation mechanisms are clearly dependent on the plasma tail characteristics (namely impedance) [1] that connects the higher intensity zone (corresponding to the FIW front) to the DBD reactor. Indeed, the PAPS propagation is sustained by the high electric field induced by the ionization front. Even at long distances, if the local electric field is high enough in the vicinity of the FIW, a secondary PAPS can be generated in another capillary close to the initial one through the dielectric wall.
A recent study based on a numerical model developed by Z. Xiong and M.J. Kushner at the University of Michigan, has investigated mechanisms of PAPS propagation, splitting and mixing. It has been shown that the PAPS splitting is symmetric, in terms of electron density n_e and electron temperature T_e [2] as can be *a priori* estimated from experiments performed in T-branched capillary. Even if there are still discrepancies between calculated and measured velocities, both simulation and experimental data emphasize the key role of the plasma tail between FIW and initial discharge volume on the final characteristics of the plasma created at the capillary outlet.
This work was supported by ANR ”PAMPA” and by Région Centre through the APR program “Plasmed”. V.S. was financially supported by Conseil Général du Loiret.

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Cold plasma type reactors and sources suitable for medical applications

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The cold plasma reactors are more often used in the last decades for diverse activities such: the pollutant treatment from air or from solutions, for plastic or metallic surfaces treatment, for improving the combustion or for treatments with biological applications. The requirements imposed to these multiple applications of the cold plasma are very different, so we have proposed to realize an appreciation of the these requirements to be able to identify the "preferences" for each type of the cold plasma reactor from the multitude of the constructive variants which can be founded in the specialized laboratories today.
Thereby, for surfaces treatment or for implant materials treatment to ensure the bio-compatibility with the human tissue, there can be used reactors with Corona discharges, DBD or especially GlidArc. The last ones are benefitting by the flexibility of operation offered by the command device with auxiliary electrodes, because the useful action of the electrical discharge is depending on its power, eventually on the specific energy on the volume unit of the blown gas (J/l).

In the case of treatments on the living tissues (human tissue), targeting the blood coagulation or wound healing, in the first is placed the problem of how the avoid the electrocution - which means that the plasma power supply parameters have to be conveniently chosen, especially the working frequency, but also the constructive type of the reactor, being preferred the one which work with a floating potential electrode. Also very important is that the action on the living tissues cells (which fortunately has been proved that is selective) should not be very intense; the power/surface unit (W/cm^2) should have reasonable values. So, it can result the possibility to enounce characteristics and typical constructive variants for cold plasma type reactors for different medical applications which made the subject of some interesting researches in our days.

In the second part of this paper are presented two power supplies used to produce cold plasma discharges, one suitable for GlidArc discharges and one for DBD discharges. The first one is based on a microcontroller and can provide trains of pulses with the frequency of 100 Hz, pulses which can have variable width and phase. The second one, used for DBD discharges, can provide control pulses with variable frequency (between 10 and 20 kHz) used to command a high voltage ferrite core transformer. To be able to compare the two discharges, we have treated distilled water using a GlidArc reactor and a DBD reactor measuring the pH modifications in time. Also we have done spectroscopic analysis of the plasma produced by the DBD torch using an iCCD camera and we have determined the rotational temperature of the plasma using a method based on the comparison between experimental and theoretical rotational structure of the molecular emission spectra of the OH band at 306.357 nm, by identification of the optical apparatus function.

Extraction of Penicillin G by polypropylene fibers treated with nitrogen plasma

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This work has been devoted to improve penicillin G (PNG) extraction using polypropylene fibers treated by nitrogen plasma and loaded with cationic surfactant Trioctylmethylammonium chlorid (TOMA-Cl).

In the first part, the adsorption of surfactant was studied on two types of fibers: virgin polypropylene fibers (FPPV) and treated polypropylene fibers (FPPT). It was found that the adsorption on FPPT is better than with that of FPPV. This is due to the difference between the physico-chemical properties of the two surfaces; the first has been plasma-treated. Therefore some new polar functionalities have been attached. The fiber becomes more hydrophilic and thus promotes electrostatic interaction between the surface and the surfactant molecules, contrariwise to the FPPV surface is hydrophobic, so the only possible interactions between the surface of fibers and the surfactant molecules are hydrophobic - hydrophobic type.

In the second part, by using the two types of fiber for the extraction of PNG, we found that the extraction is enhanced with the loaded and plasma-treated FPPT than FPPV. The effect of initial concentration of PNG, temperature and pH was also studied.

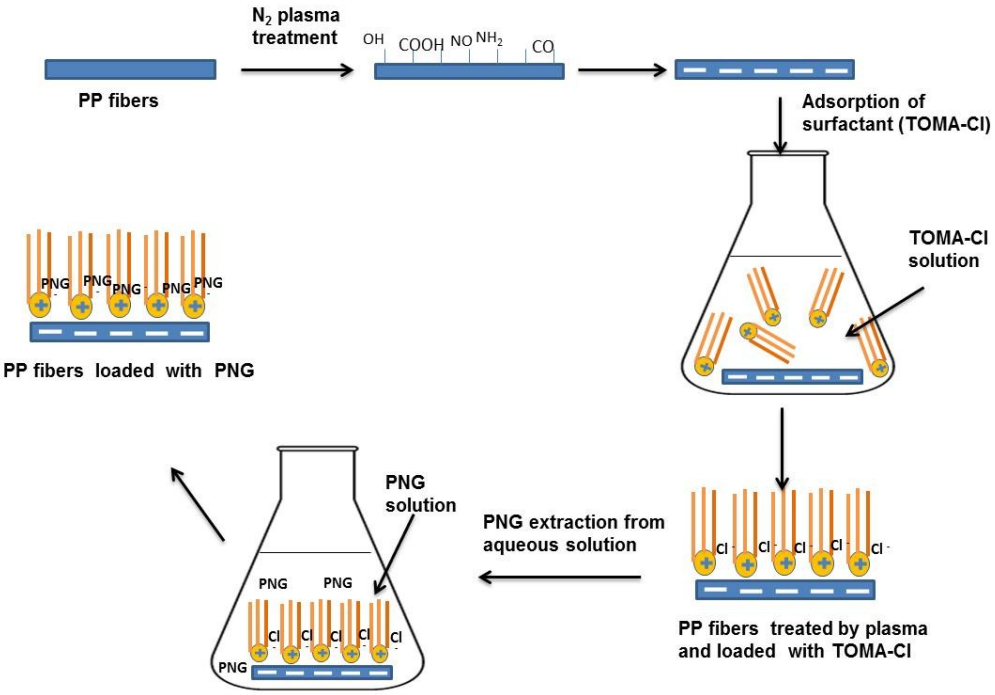

Figure 1: *Outlined plan of experiment work*

Radial control of cell colonization inside 3D scaffolds by means of plasma processes

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The production of 3D porous biodegradable scaffolds with proper porosity, pore size, shape chemical composition and mechanical integrity, able to act as temporary backbone for the regeneration or repair of a living tissue, represents a paramount challenge for scientists working on tissue engineering applications [1].
The optimization of surface properties of scaffolds is a critical aspect, as they influence the interactions between the cells and the material. Very often, scaffold's chemical composition is not fully cell compatible, e.g., polymer hydrophobicity. On top of that, cell adhesion in the core regions is often hampered by the tortuosity of the 3D porous polymer structure, leading to limited and heterogeneous scaffold's cell colonization.
By modifying both chemical surface properties and morphological parameters of the scaffolds, a better control over cell adhesion mechanism can be achieved.
Plasma processes can be used to create chemical gradients inside the scaffolds, enabling homogeneous cell colonization [2]. Nonetheless, the 3D architecture of the structures can represent by itself a great barrier to the penetration of the plasma species throughout the scaffold pores, promoting uniform treatment of the 3D structures. Thus, a fully interconnected porous scaffold is required in order to guarantee improved plasma penetration and consequent cell ingress inside the scaffold core regions.
In this study, poly(ε-caprolactone) (PCL) scaffolds, produced by means of conventional and additive manufacturing techniques were treated using low pressure plasma depositions and treatments with the aim of creating chemical gradients throughout the 3D scaffold thickness.
Scaffolds were treated in a stainless steel parallel-plate plasma reactor, with low pressure plasma depositions, fed with C_2H_4/N_2 mixtures, followed by H_2 post treatment, or plasma treatments with O_2/H_2 mixtures. In the first case, nitrogen-rich hydrocarbon films were deposited creating chemical gradients inside the porous structures; while with the second process, hydroxyl groups were grafted on the PCL scaffolds. Chemical (XPS), Wettability (WCA absorption kinetics), morphological (SEM) and mechanical (compression tests) characterizations were performed on scaffolds, before and after plasma modifications. *In vitro* biological analyses were performed on both plasma treated and untreated scaffolds using Saos2 osteoblast cells. Quantitative (MTT assay) and qualitative (actin staining) results clearly highlighted the influence of plasma processes on the behavior of osteoblast cells. In particular, chemically modified scaffolds with amines or hydroxyl groups, revealed better cell proliferation respect to the pristine material.
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Atmospheric Plasma Jet SiOx-Thin-Film Deposition on Enamel

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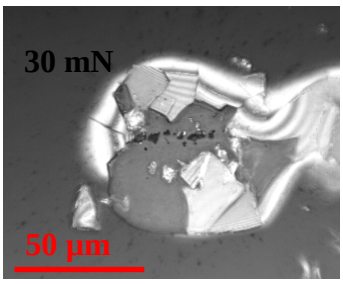
Physical plasmas have found manifold applications in industry and in medicine in recent years [1]. In medicine plasmas were successfully applied for instance for surface cleaning, sterilisation and disinfection [2-3]. Modifications of tooth surfaces are generally interesting for improvements in restorative dentistry. State of the art inhibition of pit and fissure caries is practiced by sealing using resin [4] after cleansing and phosphoric acid etching of the fissures.

The present study is focused on protection of tooth surfaces by deposition of flexible quartz (SiOx) thin films by a cold atmospheric plasma jet. Polished and etched enamel slices from the vestibular face of bovine incisor crowns were used. Etching was carried out by means of 37.5 % phosphoric acid gel for 30 s. Plasma jet treatment of these surfaces has been performed: working distance of 4 mm, scan velocity of 1 mm/s, helium flow 1,200 sccm, oxygen flow 15 sccm, helium flow with hexamethyldisiloxane (HMDSO) as precursor for Si 5 sccm, nitrogen flow 1200 sccm, average microwave power 2.6 W, single pulse power 150 W, pulse width 5 µs. The deposited films were analyzed by SEM and Talystep stylus roughness and step height measurements. Further wear resistance tests have been performed using a 10 µm diameter spherical stainless steel indenter with normal forces from 10 to 70 mN, 10 cycles 50 µm travel distance back and forth within 10 s. Deposited SiOx film thickness was measured from 383 nm to 393 nm. Films on polished enamel were destroyed in the wear test with a normal force of 30 mN. The indenter broke through the layer at half of the wear distance, shown in Fig. 1a. The acid pre-etching improves the layer adhesion and stability. The film was stable in the wear test.

a) 

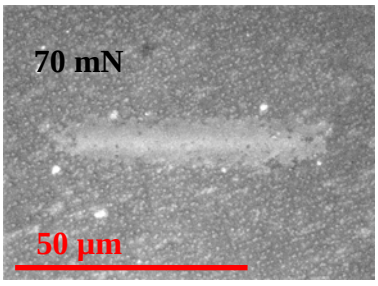
b) 

Figure 1: Results of wear tests a) destroyed SiOx-layer on polished enamel, b) wear resistant SiOx layer skid mark on phosphorus acid pre-etched enamel

SiOx plasma deposition might enable interesting new way for caries prevention. Next we will perform corrosion tests of the SiOx film.

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Cold plasma technology for fast immobilization of enzymes

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Over the years, the immobilization of enzyme molecules onto solid support gave rise to a wide range of analytical or industrial applications. Today, following the fast evolution of microfluidics and nanotechnology, the elaboration of efficient enzyme immobilization processes is becoming of great interest for the development of new and original analytical tools or microreactors. The immobilization of biologically active species constitutes therefore a crucial step in the fabrication of bio/chemical microelectromechanical systems (BioMEMS) for which the potential application fields may concern biological and medical analysis, environmental investigations or clinical diagnosis.

Recently, cold plasma polymerization of 1,1,3,3,tetramethyldisiloxane (TMDSO) has been successfully used for the simple fabrication of microchannels [1]. In the context of BioMEMS manufacturing, we present a fast, innovative, and biocompatible method for the rapid fabrication of bioactive coatings using this plasma polymerized 1,1,3,3,tetramethyldisiloxane (ppTMDS) as carrier matrix. Using β-galactosidase as enzyme, we aim to develop a one-step immobilization procedure in order to fabricate a bio-functionnal layer where the enzymes are expected to be entrapped into the polymer matrix while preserving their native structure and their activity. Following one remote afterglow plasma enhanced chemical vapor depositions (RPECVD) experiment and several washing sequences of the sample, enzyme activity and stability was determined through studying the enzymatic hydrolysis of *ortho*-nitrophenyl-β-galactoside (*o*-NPG) in *ortho*-nitrophenol (*o*-NP) by spectrophotometry. Furthermore, different deposited coatings were analyzed by imaging techniques (SEM, AFM) in order to obtain information about the surface, before and after exposition to activity tests for different coating thicknesses. Finally, we investigated the diffusional limitation after several activity assays and also as function of the thickness of the coatings.

The results greatly reveal the feasibility of this non-conventional immobilization procedure: a single step technology allows fast immobilization of enzyme while retaining their bioactivity after several assays. Further investigations and optimizations of the technological process will certainly enable the development of new biofunctional coatings for specific applications.

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Comparison of various deposition strategies to prepare non-fouling plasma polymer films by atmospheric-pressure surface DBD

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PEO has been widely considered for use in biomedical applications as protein resistant and nonimmunogenic material. This study investigates three different geometries to prepare PEO-like plasma polymer thin films by surface DBD at atmospheric pressure using di(ethylene) glycol vinyl ether carried by Ar flow as working gas. We used recently developed reactor based on the amplitude-modulated AC surface dielectric barrier discharge (SDBD) as source of active species for the monomer fragmentation. In this work, various properties of plasma polymers prepared using three different deposition strategies will be presented and discussed.

The three deposition geometries that were inspected:

1) Substrates were placed in parallel with respect to the SDBD at a fixed distance.
2) Substrates were placed perpendicularly to the jet created by the SDBD effluents.
3) Substrates were used to create a rectangular tunnel (1.5 × 1 mm, 20 cm long) and the plasma polymers were deposited on the tunnel's inner surfaces by SDBD effluents injected by the jet.

Results related to non-fouling PEO-like plasma polymers were obtained by FTIR and XPS. The deposited films show the PEO-like character in all three cases. The main differences in deposition rates and film qualities will be discussed in connection with variations of the parameters of the SDBD (power, duty cycle, working gas flow rate and composition).

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The quartz crystal microbalance with dissipation unit (QCM-D) as a tool for the evaluation of surface biocompatibility

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Recently, a promising new approach for the identification of biomaterials' surfaces hemocompatibility using quartz crystal microbalance with dissipation unit (QCM-D) has been reported [1], [2]. Previous adsorption studies showed that surfaces with high affinity to albumin and low affinity to fibrinogen feature improved hemocompatibility [3], [4].
In this work, QCM-D was used for monitoring the adsorption of fibrinogen onto modified model PET surfaces. Figure 1 shows areal mass (ng/cm^2) of adsorbed fibrinogen onto PET modified surfaces. Surfaces have been subjected to several pre-treatments including low-temperature oxygen and nitrogen RF plasma treatment and pre-adsorbtion of polysaccharides exhibiting anticoagulant properties such as heparin and dextrane sulphate. Adsorption of fibrinogen onto treated PET surfaces compared to untreated surface of PET was found to be significantly reduced for all applied treatment methods. The use of non-equilibrium gaseous plasma opens new possibilities for improvement of biocompatibility of PET surfaces.

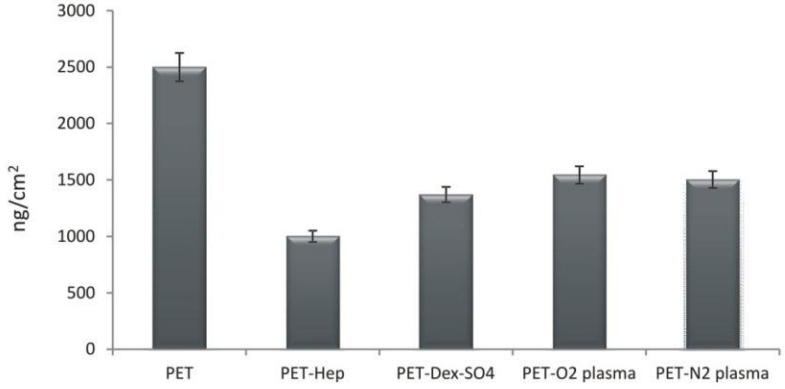

Figure 1: *Fibrinogen adsorption (mass/area at third overtone) onto non-modified (PET), polysaccharide coated (heparin, dextran sulphate) PET surfaces and plasma treated PET surfaces*

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Non-sticking antibacterial protection ofbiomedical devices

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DLC is known to be non-toxic and safe for biological applications [1]. With positive reviews from the FDA, it has become the most desirable material for coating biomedical devices and tools like stents, heart valves, surgical instruments, etc [2]. Another important characteristic relevant to bomedical applications is its non-sticking effect which was found to be better for r H-free DLC compared to its hydrogenated counterpart [3].Apart from its high potential to preventadherence of live tissue cells on surgical instruments during surgery, microbial adhesion is also lowered. For further improving the outcome of surgery, antimicrobial metals like Ag, Cu, Au, etc can be incorporated into the DLC using plasma-based deposition systems.

We present here the bio-performance of H-free DLC-Ag-Cu complex coatings obtained by an original plasma-based deposition technology developed in our group. The main assets of the coating technology are: ability to obtain homogeneous fine mixture of compoundsat a nanometric level, high adherence on the substrate (including stainless steel), ability to coat temperature sensitive materials like plastics and textiles, scalability for larger production.

Our films were found to be hard, with about 42 GPa hardness and very smooth (about 2 nm roughness). The anti-bacterial efficacy of these coatings against various bacteria as well as their non-sticking effect on living cells was assessed and compared to bare stainless steel.A time dependence observation of bacterial count on our complex surfaces has shown total innactivation of all bacteria studied within five minutes after surface contact.Such coatings will have a major impact in preventing stickingof living cells and bacteria on medical instruments.

Figure 1:*The plasma source and thin films deposited on stainless steel*

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Plasma processing of 3D scaffolds to address specific cell response suitable for long term implants and tissue engineering

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Tissue engineering emerged in the early 1990s to address limitations of organ transplantation and synthetic tissue replacements, focusing on coupling cells and a biocompatible matrix known as a scaffold [1]. Tissue engineering approaches are mainly based on the use of 3D biocompatible, bioerodable scaffolds, together with cells, to reconstitute a specific tissue *in vitro* that might be utilized for the replacement of diseased tissue *in vivo*. An ideal tissue-engineering scaffold should be porous and should possess an appropriate surface chemistry to induce desired cellular activities and to guide 3D tissue regeneration. Despite their adequate tailorable mechanical properties, synthetic polymers do not contain chemical features that are able to stimulate specific cell behavior, resulting in poor ingrowth of cells. This limitation could seriously compromise the future *in vivo* application of these scaffolds. For these reasons, plasma modification of 3D scaffolds could be an important approach to overcome these drawbacks. In order to overcome the problem of correct cell ingrowth, a plasma coating on the external surface of polycaprolactone (PCL) scaffolds (pore size ranging from 150μm to 300μm, [2]) was deposited. The chemical composition of the coating contains ether groups (e.g. Polyethylene oxide-like, PEO-like[3]) which are able to discourage cell adhesion. In this way cells are addressed to colonize the internal part of the scaffolds. FTIR and XPS analyses showed that the plasma deposited films, recognized by the presence of ether groups in the coatings, covered only the external surface of the materials. Cell viability tests showed that plasma processing was an effective approach to enhance cell adhesion. A combination of PEO-like coating deposition with an O_2 plasma treatment inside the scaffold core has also been used to improve the performances of 3D PCL scaffolds. In addition, in this work, an innovative strategy that combines cold plasma sputtering of hydroxyapatite and plasma treatments to produce calcium phosphate (CaPs) coatings on polymeric scaffolds useful for long term implants and tissue engineering [4] was experienced. Oxygen plasma treatments were used also in this approach, in combination with plasma sputtering, to produce an inner surface suitable to stimulate cell-adhesion. Biological tests show that cell clustering, spreadness and actin stress fibers were more evident on scaffolds treated with oxygen and oxygen plus CaPs with respect to the control (untreated PCL) and PCL coated with CaPs alone.

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Aging of PEO-like plasma polymer films prepared by atmospheric and low pressure discharges

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Poly(ethylene oxide) (PEO) has been widely considered for use in biomedical applications as protein resistant and nonimmunogenic material. Plasma polymers based on PEO chemistry have been retaining interest among the plasma deposition community for about the last twenty years.

In this study, the comparison of aging of PEO-like plasma polymers prepared by two different methods was investigated. In particular, polymers prepared either by plasma-assisted thermal evaporation of conventional PEO [1] or by atmospheric pressure amplitude-modulated AC surface DBD in argon with the di(ethylene) glycol vinyl ether monomer vapors [2, 3] were studied.The ability to maintain the PEO character in air was analyzed over 2 years period of storage.

The chemical changes were analyzed by FTIR and XPS. Surface topography was scrutinized by Atomic Force Microscope. It was found that the plasma polymer films prepared by the low pressure method are more stable when stored in air. The content of the ether groups in such films decreases from 80 % to 71 % whereas atmospheric pressure films degrade more significantly with concentration of ethers decreasing from 67 % to 45 %.

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Fibroblasts attachment on carbon nanowalls surface functionalized by reactive plasma

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The cellular and tissue responses are strongly affected by a variety of factors including the surface properties of the material (surface energy, topography, presence of specific functional groups) and the cell type. For example, the attachment and spreading of fibroblast cells are in general correlated with the hydrophilicity of the surface or with the presence of functional groups on the surface, including amine groups.
Plasma functionalization methods are well known for their ability to create functional groups on a specific surface to enable the interaction with cells. In this contribution we report on the biological response of fibroblasts to carbon nanowalls (CNWs) as-deposited or functionalized by reactive plasma. Layers of CNWs were synthesized on silicon substrates by radio-frequency plasma jet assisted chemical vapor deposition, from acetylene in presence of hydrogen [1]. After the deposition process, the surface of CNWs was functionalized by plasma generated in argon admixed with nitrogen. In addition to continuous CNWs films, patterned samples were prepared by deposition and functionalization through a metallic grid with regular openings of 700 μm arranged in hexagonal geometry. The surface of CNWs films, prior to and after plasma functionalization, was evaluated by Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR) and contact angle measurements. These techniques indicate that plasma treatment led to a slight modification of morphology, but insertion of new functional groups, and a strong change of surface wettability from hydrophobic to superhydrofilic.
Furthermore, connective tissue fibroblasts-like cells L929 were used to evaluate *in vitro* the attachment ability on the untreated and plasma treated carbon nanowalls carpets. The number of attached cells was determined by optical imaging, while morphological changes of the fibroblasts attached were monitored using SEM. The cells viability was assessed by MTT tests, after 24 h of incubation. The results indicate that the as-grown CNWs layers inhibit the cell adhesion and induce the modification of cell morphology [2]. Contrary, on the plasma treated surfaces the promotion of adhesion and attachment of fibroblasts was obtained. Moreover, on functionalized patterned samples, a preferentially growth of fibroblasts at the border between carbon nanowalls and silicon areas was observed. These results show that carbon nanowalls may be modified specifically through the methods of plasma technology in order to improve their biological interface.

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Plasma based deposition of patterned organic fluorine-free (super)hydrophobic-(super)hydrophilic surfaces

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One important aspect of biomedical research is related to the controlled production of so-called biomaterials. Plasma technology is known to be an important tool for the production and surface modification of synthetic polymers used for the control of bio-interfacial interactions [1]. Either as thin films, as nanoparticles or nanocomposites: plasma produced or processed materials have found an increasing number of applications in biomedical research An important factor for many applications is the wettability of the surfaces. The control of the hydrophobicity or hydrophilicity is for example crucial for the production of antifouling coatings, for the design of microfluidic elements or for newly developed "lab on a chip" applications. The wettability of a surface commonly depends on two factors, the surface chemistry and the surface roughness or to use a more general term the surface topography [2].

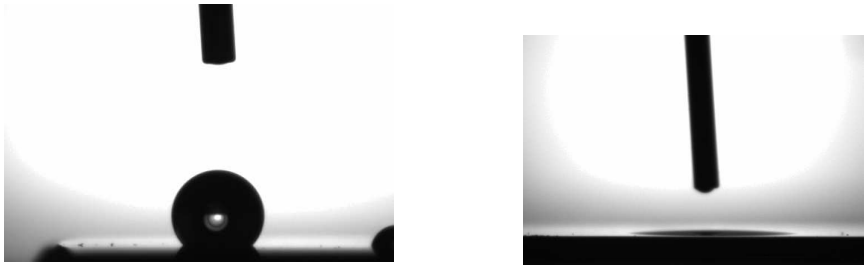

Figure 1*: Water droplets on plasma polymerized surfaces. a) shows a surface containing a high amount of nanoparticles. (b) shows the same surface after post process plasma treatment.*

In this contribution we will focus on the production of carbonaceous coatings deposited by means of a capacitively coupled low temperature plasma. The experiments show that the hydrophobicity can be significantly enhanced due to the controlled deposition of (plasmapolymerized) nanoparticles (figure 1a). The post treatment of such surfaces with a (e.g.) nitrogen plasma leads to an opposite effect: to surfaces with a strong hydrophilic character (figure 1b). Depending on the treatment time and the plasma parameters it is possible to gradually change the contact angle. The combination of both methods allows a simple production of materials with alternating patterns of (super)hydrophobic and (super)hydrophilic surfaces as they can be used for example in new lab on chip applications [3]

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Plasma polymer deposition on scaffolds of poly(D,L)lactic acid. Effect on the adhesion and the proliferation of fibroblasts, osteoblasts and keratinocytes

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Polylactic acid (PLA) is a biodegradable aliphatic polymer existing both as P(L)LA and P(D,L)LA. PLA undergoes scission in the body to lactic acid with L-lactic acid as a natural intermediate in carbohydrates metabolism[1]. Although PLA good biocompatibility, its surface presents low wettability and low surface energy that affect cell adhesion and proliferation[2]. In this paper an interesting atmospheric pressure plasma treatment is described to modify poly(D,L) lactic acid scaffolds surface properties, in order to enhance protein adsorption and consequently its ability to induce cell adhesion and proliferation[3]. The physico-chemical properties of the resulting surfaces are investigated by water contact angle (WCA), FTIR-ATR spectroscopy, X-Ray photoelectron spectroscopy (XPS) for C1s, O1s and N1s. The adsorption of proteins from bovine serum was then studied as a function of gas plasma treatment. Also cell adhesion and cell proliferation on plasma modified PLA sample were assessed. The first macroscopic effect observed after cold plasma treatments was a change in wettability of the PLA scaffolds. The FTIR-ATR analysis showed the presence of the ester groups such as OH and NH_2 functional groups. The XPS spectra confirmed on the one hand the incorporation of additional carbonyl, carboxyl or hydroxyl functional groups when acrylic acid was employed as precursor, on the other hand the incorporation of amino, amido and imino functional groups occurred when was employed 1,2-diaminopropane.
PLA-COOH was able to adsorb an higher protein concentration compared to normal PLA, while surprisingly also PLA-NH_2 increased the quantity of adsorbed protein. Murine fibroblasts (3T3), murine pre-osteoblasts (MC-3T3) and human keratinocyte (HaCaT) were able to adhere on PLA and plasma modified PLA, but MC-3T3 showed a higher affinity for PLA compared to the other cell types and this affinity was even higher onto PLA-COOH where pre-osteoblast spreaded, while this effect was less important onto PLA-NH_2. After 48 hours proliferation of adherent cells was assessed both observing cells stained with acridine orange and using the TOX-8 assay that scores cell number as a measure of mitochondrial activity: it was evident a good cell proliferation for both MC-3T3 and HaCaT cells. Moreover HaCaT cells formed an almost confluent cell layer onto PLA-COOH. As expected 3T3 fibroblast proliferated very slowly onto every surface.

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Plasma preparation of titanium surfaces for stents

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Thrombosis and restenosis are the most common problems during insertion of biocompatible implants like titanium stents into human blood, due to aggregation of platelets on their surfaces. Because of this reason, we studied the response of blood platelets to a plasma treated titanium surface. The aim was to design a functionalized surface which would repel blood platelets or prevent their adhesion. Therefore, we functionalized surfaces with low-temperature inductively coupled oxygen plasma treatment, which in the first stage cleaned the surface of titanium, and in the second promoted incorporation of oxygen functional groups as well as the growth of a titanium dioxide film. In this paper we show that oxygen atoms or oxygen containing groups play an important role in the repulsion of platelets and their deactivation. At the same time, increased surface temperature of samples either through sequential thermal deactivation in heat oven at 150 ^{o}C or heating the surface with ion bombardment during the treatment, lowers the oxygen content and the surface repulsion for platelets.

Nanostructured antibacterial coating of endoscopes by using atmospheric plasma sources

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Medical instruments are used frequently for the diagnosis and therapy of medical disorders. Contaminated surfaces of medical products have been shown to contribute to nosocomial outbreaks. The most frequently identified pathogens are staphylococci, including methicillinresistant Staphylococcus aureus (MRSA). Presently more than 20% of nosocomial *Staphylococcus aureus* isolates in Germany are MRSA [1]. In other countries, e.g. Japan, France or the United States, the proportion of MRSA exceeds 50% with increasing infections. In other reports the mortality rate of patients with device-associated infections varies from 35% to 45% [2].

For that reason the surface research for developing antibacterial materials using chemical or physical modification of the solid surface has been intensified in the last years. In this paper a novel concept was realized in order to prevent the colonization of MRSA and other pathogen bacteria on the surface of medical devices (endoscopes): Plasma based nanostructured coating technique was demonstrated in biopsy channel (lumen) of endoscopes. The coating with good biodegradable nanoparticles reduces the colonisation of multiresistant *Staphylococcus aureus* and other pathogen bacteria.

The nanoparticles can be removed using standard cleaning procedures between 60-80 $_{o}$C. Thus, all adhering microorganisms and other contaminants in the endoscope channel can be removed. At the end of the cleaning procedure and treatment the coated endoscopes are in their original state with sterile surface and they are completely free of possible contaminations. To increase the integration potential of the foreseen process the work is focused on non-thermal atmospheric plasma source: “Plasmoscope”- a special plastic tube, which includes a helical electrode structure developed at INP. The work is supported by the BMBF under the contract acronyms and numbers: Endoplas 13N9324 and Nanogiene 13N11357.

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Bio-physical Analysis of yeast responses to non-thermal plasma at atmospheric pressure

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The study for application of plasma technology to eukaryotic microbes (yeasts, fungi, protozoa, etc.) has been recently reported in increasing number of studies [1][2]. In spite of an enormous number of studies on application of plasma, mechanisms for plasma action have been rarely investigated [3]. Understanding mechanisms of plasma action is essential for developing more efficient plasma technology. In this study, we analyzed responses of an eukaryotic microbe (yeast) to plasma using physical & biological methods as a first step for elucidating mechanisms of plasma action. Non-thermal Ar plasma at atmospheric pressure was used, and yeasts submerged in water or media (YPD) were treated with plasma. During plasma exposure, number of colony formation unit (CFU) was more dramatically decreased when yeasts were treated in DI (de-ionized) water than in YPD (Yeast-extract peptone dextrose) media. Yeast cells treated in DI water were shrunk more severely after plasma treatment. The amount of genomic DNA was decreased more rapidly in yeasts treated in DI water than in YPD media (longer than 1min.). Value of secondary electron emission coefficient (γ) measured by γ-FIB (Gamma Focused Ion Beam) was increased upon plasma exposure, particularly in yeasts treated in water, indicating a possibility of damage on cell surface molecules. pH was significantly decreased in water after plasma treatment but not in YPD media suggesting that pH decrease might be a reason for yeast inactivation by plasma. However, we have also found a possibility that some factors directly from plasma can affect yeast responses and further study is on going for identifying these factors.

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Plasma-Microbubble Generator for Water Purification

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Water purification technology by the plasma-microbubble (P-MB) generator has been developed. Atmospheric-pressure μ plasma (APμP) was generated by a pulsed corona discharge using a metal pipe electrode of 0.70 mm in diameter [1] with a peak-to-peak voltage of 6-7 kV and a pulse repetition frequency of 950 Hz. Radicals such as O*, OH, N* and O_3 generated by the air μ plasma were enclosed with microbubbles and these microbubbles were instantly ejected into a water. The most significant character of a microbubble less than 50 μm in diameter is the decrease in size and subsequent collapse under the water in contrast with an ordinary bubble of several mm in diameter, because of long stagnation and excellent gas dissolution due to Henry's law [2]. Therefore, the gas-liquid interface of the microbubbles is expected to act as the chemical reaction field. Figure 1 shows a compact P-MB generator developed in this study. The generator was made of an acrylic cylindrical pipe (15 mm in diameter and 10 cm in length). APμP was stably generated in the plasma chamber, in which a water did not flow backward from the liquid chamber. The photographs of Indigo Carmine solution (32 L with a concentration of 20 mg/L) treated by the air P-MBs are shown in Fig. 2. Decolorization was attained after the P-MB treatment of 34 h. On the other hand, no change was observed in the microbubble-treated dye solution with no plasma. To clarify what's happened in the treated water, the air P-MB treatment on deionized pure water was conducted and pH and UV absorbance were examined. The pH abruptly decreased to be 4-5 with increasing treatment time and UV absorption spectrum showed a strong peak at a wavelength around 210 nm which seems to originate from the H_2O_2 or NO_2^- [3]. From these results, it was confirmed that H_2O_2 and OH radical produced in the water-microbubble interface as well as O_3 contributed to decolorize the dye solution.

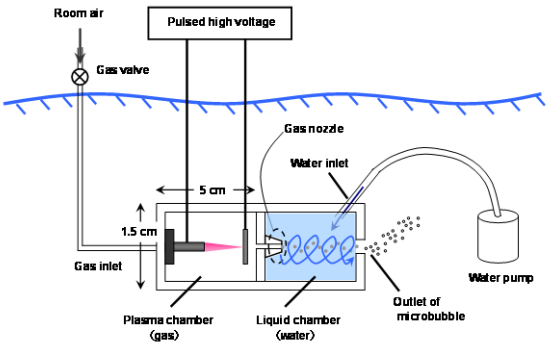

Figure 1 *Experimental setup of P-MS.*

Figure 2 *Indigo Carmine solution after the P-MB treatment.*

The P-MBs were also applied to sterilization of *E.*coli in an environmental foul water.

The authors would like to thank S. Ishikawa for technical support to develop the plasma-microbubble generator. This work was partly supported by a Grant-in-Aid for Scientific Research C from the Ministry of Education, Culture, Sport, Science and Technology of Japan.

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Study of the Disinfection Abilities of Water Treated by Atmospheric Pressure Cold Plasma

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A direct current atmospheric pressure cold plasma mcriojet (PMJ) was used to treat water for 5 minutes and 20 minutes, respectively. Argon with 2% oxygen and argon with 2% oxygen and 10% nitrogen were used as the operating gases. The plasma activated water (PAW) was subsequently applied to *Staphylococcus aureus (S. aureus)* suspensions for various time periods over a time span of 2 hours. The inactivation efficacies of the PAW generated by PMJ with the two gases are in the following order: $Ar/O_2(2\%) > Ar/O_2(2\%)/N_2(10\%)$. It was also observed that the antibacterial ability of the PAW increase with the plasma treatment time. PAW with 20 min plasma exposure is still bactericidal after 2 h with a reduced inactivation efficacy. In a separate series of experiments, we compared the PAW generated with the PMJ (1) suspended above the water surface and (2) submerged in water, where a better inactivation efficacy was found for the latter.

A scanning electron microscope (SEM) was used to evaluate the bacterial morphology before and after the PMJ treatment. Optical emission spectroscopy (OES), high performance liquid chromatography (HPLC), and atomic absorption spectrophotometry (AAS) were employed to identify and monitor the reactive species in the plasma-liquid system, such as H_2O_2, O_3, and NO_3^-/NO_2^- as well as Cu (Cu^+/Cu^{2+}). Possible disinfection pathways will be discussed at the conference.

Dental treatment using LF plasma jet with the reduced pH method -Disinfection of Dentin-

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In the dental treatment, the control of infectious microorganisms is very important, however, the methods for sterilization of infected dentine have not been established yet. In the present study, we evaluated the bactericidal effect of low frequency atmospheric pressure plasma jets with the reduced pH method [1] against oral pathogen including *Streptococcus mutans*, *Enterococcus faecalis* and *Candida albicans*, which are causes of dental caries and incurable root-canal infection. The results showed that the LF jet irradiation had bactericidal effects on oral pathogens in liquid with lower condition than pH4.5. As shown in Fig.1, after irradiation at pH 6.5, viable cells number was gradually reduced. On the other hands, no viable cells could be detected at pH 4.5 or 3.5, for 2 and 3 minute irradiation, and it was significantly different from the control ($p<0.001$). For *E. faecalis*, the D values at pH 3.5, 4.5 and 6.5 were calculated to be 0.30, 0.47 and 2.00, respectively [2].

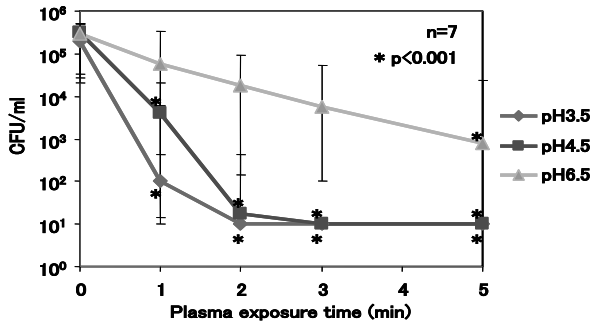

Fig. 1 CFU assay of *E. faecalis* in various pH solutions.

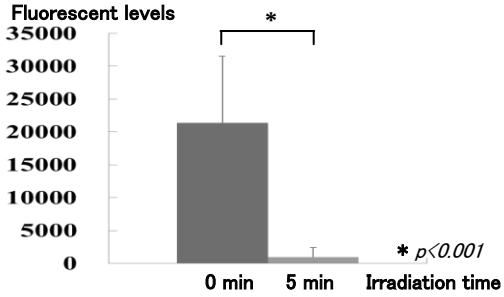

Fig 2. REDOX indicator assay after plasma irradiation on dentine infection models in the buffer of pH3.5

In the similar test using the infection model of hydroxyapatite pellets or dentine slice models, the significant bactericidal effect on *E.faecalis* was detected by metabolical REDOX indicator assay (Fig.2). With the infection model using human whole teeth, the enough bactericidal effect was gained with 2min irradiation. These results indicate that LF jets might be applied to the clinical dentistry.

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Investigation of the antiseptic efficacy on *in vitro* biofilms of tissue tolerable plasma combined with common antiseptic solutions

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Therapies of chronically infected wounds in human and veterinary medicine are often complicated because of biofilm enveloped microorganisms, drugs resistant microorganisms or antiseptics. Especially the biofilm protects the microbial colonization against a complete inactivation by chemical substances [1]. Therefore "cold" plasma under atmospheric conditions could be an alternative or supplement to conventional therapies if the plasma does not damage the tissue irreversible. Physical plasma has an unspecific effect against all bacteria according to current knowledge and was often tested against "planktonic" and sessile biofilm bacteria [2] [3]. In addition, some plasma sources tested on cell culture or on living tissue showed tissue tolerable properties or supported the reorganisation of skin [4] [5].
Plasma irritates and damages microbial membranes and induces changes in cell permeability. The hypothesis is that plasma promotes the penetration of antiseptics in deeper layers of biofilms even for short exposure time. Moreover, bacteria are stressed by plasma produced reactive species that sensitise bacteria against antiseptics, too. Thus, a synergistic effect of plasma and antiseptic was expected. That could open up a completely new strategy for chronically infected wound healing therapies.
For that study, the „PlasmaJet", *kinpen09*® (1.1 MHz, 2-6 kVpp) with the working gas argon was used [6]. The *kinpen09*® is a tissue tolerable plasma (TTP) source [4]. The used antiseptic solutions were Octenidine, Chlorhexidine and Polihexanide which are used for wound infections or biofilm treatment. The bacteria *Pseudomonas aeruginosa* und *Staphylococcus aureus* were used for cultured biofilms since they are often involved in chronic wound healing processes [7]. The biofilm was cultured in microtiter plates for 48 h. The TTP was applied before and after chemical antiseptic treatment to compare the chronological order with the treatment of TTP and antiseptic solutions alone.

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Inactivation of Aspergillus Fumigatus with Low-Temperature plasma

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In the last decades, plasma technology has made an important breakthrough in the treatment of cancer cells and destructive microorganism like bacteria [1]. In this case, we experimented the most common mold infection; Aspergillus Fumigatus, which is common in asthmatic, causing invasive infections in the lung [2].

For this purpose, we exposed our samples to a mesh enhanced dielectric barrier discharge plasma in atmospheric pressure with different exposure times (till 45 seconds). The sample plates were covered with 100µL of Aspergillus Fumigatus suspension with 10^3 CFU/mL concentration. In all cases the voltage amplitude was about 2kV, and the frequency was 16.5 kHz. Figure 1 provides an overview of samples that was exposed to the dielectric barrier discharge. After three days incubation, the treated area was measured.

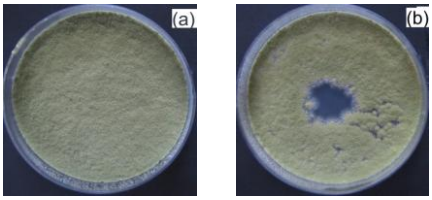

Figure 1: *A comparison of treated area ;(a) control , (b) treated for 25 seconds.*

Figure 2 illustrates the decontaminated area of Aspergillus Fumigatus samples by the DBD plasma as a function of exposure time. It is apparent from the diagram that as the exposure time increases the treated area grows.

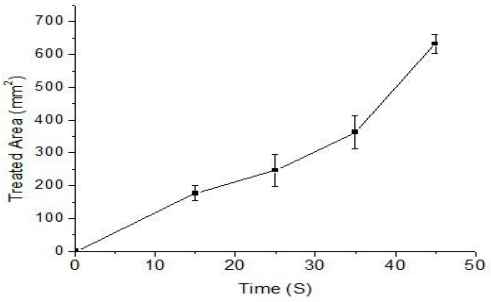

Figure 2: *Treated area of Aspergillus Fumigatus at different exposure times.*

In conclusion, we have studied the effect of DBD plasma on Aspergillus Fumigatus fungi. As it can be seen, by rising the exposure time, the treated area has increased.

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Effect of cold atmospheric plasma treatment on dental pulp in rat molars

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Cold atmospheric plasma treatment of hard tooth substances has shown in previous investigations to disinfect by killing of adherent bacteria [1], to efficiently clean Ti surfaces from biofilms [2] and to improve tooth-filling interaction by surface modification [3].
The aim of this study was the investigation of the influence of cold plasma treatment on the rat dental pulp in combination with adhesive filling therapy.
Occlusal cavities were prepared in first upper molars of 20 Sprague-Dawley rats (1 x 1.5 x 0.5 mm, remaining dentin: 0 – 0.3 mm, diamond bur ISO 008, 6.000 U/min, air/water spray cooling). In a split mouth design, one prepared rat molar and the adjacent unprepared molar were treated with cold atmospheric plasma (pulsed microwave 2.45 GHz, mean power 3 W, plasma jet with Gaussian profile: 8 mm length 1.5 mm FWHM, 2.0 slm He, treatment time 5 s per tooth, surface temperature: maximum 40 °C). The prepared cavities were filled with a self-conditioning adhesive and flow composite material. After 24 h and 28 d ten rats each were sacrificed and the upper molar segments were dissected. Teeth were demineralized by 10 % EDTA for 4 weeks and embedded in paraffin. Histological sections (6 µm) were prepared and stained with haematoxylin-eosin (HE) and chlorazetatesterase (CAE). The odontoblast layer, appearance of inflammatory cells, necrosis, pre- and secondary dentin formation were assessed in the histological sections.
Distinct inflammation was detected in pulps of teeth treated with plasma and filling as well as in pulps of teeth which were only filled after 24 h. After 28 d in both groups secondary dentin formation and reduced inflammation were observed. Pulps of teeth treated with cold plasma but not filled did not show any increased inflammation compared to untreated controls neither after 24 h nor after 28 d.
In this animal experiment the treatment of vital teeth with cold atmospheric plasma did not result in an increased inflammation of pulp.

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Interaction of pulsed electrical discharge produced at gas-liquid interface with bacteria in water

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Bactericidal effects of non-thermal plasma produced by various types of electrical discharges in gas or liquid phase received considerable interest during recent years due to their potential utilization in various biomedical applications. Chemical effects induced by the reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generally accepted to play the dominant role in the plasma interaction with living matter in the atmospheric air plasma systems. In underwater plasmas, physical processes, such as high electric field, UV radiation and shock waves, may significantly contribute to the biological effects in addition to the chemical effects that are largely attributed to OH radicals and H_2O_2. When the gas phase discharge is generated in close proximity to a liquid surface, chemical species produced by the discharge in the gas or at the gas-liquid interface can penetrate or dissolve into the liquid and initiate biocidal processes in water (such as OH radicals, O_3, H_2O_2, NO_3^-, NO_2^-). In addition to the production of ROS (such as OH radicals, O_3, H_2O_2), production of RNS produced by the discharge under atmospheric conditions with suitable nitrogen sources results into the formation of NO_2^- and NO_3^- in water and increased acidity of plasma treated water. There are also possible synergistic effects of the above mentioned processes, which lead, for example, to the formation of peroxynitrites in water [1-3].

In this paper we investigated chemical and biological effects induced in water by pulsed corona discharge produced at gas-liquid interface between a planar high voltage electrode made from reticulated vitreous carbon (RVC) and the water surface [4,5]. Formation of O_3, H_2O_2, NO_3^-, NO_2^- and $ONOO^-$ produced by the discharge was measured under various conditions and the bactericidal effects of the discharge were studied on inactivation of *Escherichia coli.* The mechanisms of bacterial inactivation and contribution of ROS and RNS was studied in dependence on the composition of the gas atmosphere (oxygen mixtures with nitrogen or with argon) and on the pH value of plasma treated water (controlled by buffers).

The work was supported by the Grant Agency of the Academy of Sciences of the Czech Republic (IAAX00430802), by the Czech Science Foundation (104/09/H080) and by the Financial support from specific university research (MSMT No. 21/2012).

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Convective and diffusive transport of oxidative species from a plasma jet

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Plasma jet systems based on dielectric barrier discharges are frequently investigated for skin and wound disinfection. TNO has focused on applicability of a proprietary ~300 mm wide linear plasma jet system based on surface discharges covering an elongated ceramic structure. The system allows disinfection of irregular shapes in a short period of time and is motivated by hand disinfection in hospitals, biopharmaceutical industries, sanitary rooms etc. The downstream gas from plasma jets in N2-O2 mixtures contains atomic oxygen and nitrogen, ions such as O2
– and various more stable compounds such as O3. Nature and concentration of those are difficult to determine and depend on flow dynamics, surface morphology and wetting. A plasma jet using Argon has been reported to create reactive species penetrating even in the follicular reservoir as shown in reference [1].
The ability of plasma produced oxidative species to diffuse to areas of skin where convective flow is limited has been studied. Bacterial contaminations (*S. Aureus* and *E. Coli*) have been filtered on membranes (47 mm diameter, 0.45 µm pores) and covered with a 180 µm thick cellulose Whatman filter having a particle retention typically below 4-12 µm. Figure 1 shows a schematic of the set up providing two parallel sheet shaped flows issuing from a 100 µm wide electrical discharge space and a 0.2-0.5 mm wide shielding gas jet. Treatment conditions have been varied by passing the substrate holder below the jets at controlled speed, distance, plasma power, plasma gas and shielding gas flow rates.

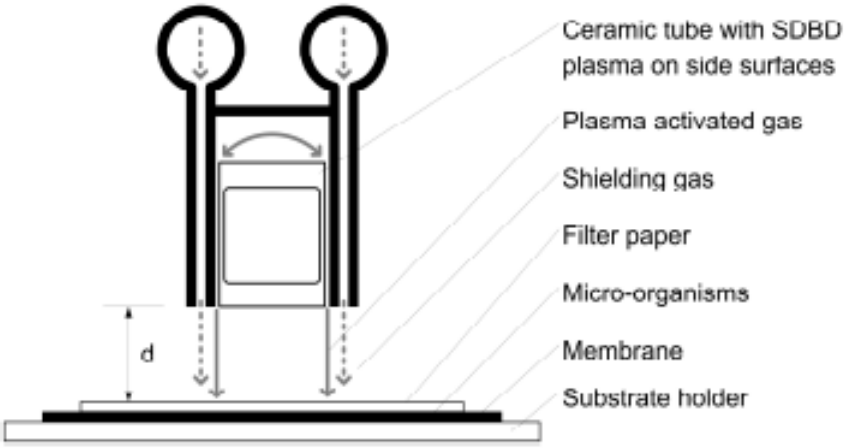

Figure 1: *Electrode system and substrate composition*

CFU count tests show the importance of filter humidity for achieving significant inactivation even at large distance up to 40 mm. Further the paper will describe visualization of methylene blue dye degradation (known as radical dependent) covering an artificial hand.

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On the Use of Plasma Sterilization for Planetary Protection: Investigation of the Destruction of Bacterial Spores from a Laboratory Strain and a Spacecraft Assembly Isolate by a Low-Pressure VHF-CCP

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The sterility of various objects is a major demand in many fields, e.g. medicine, pharmacology, food-industry, or even for planetary protection. Since the requirements for sterilization are quite similar in the case of medicine and planetary protection, it suggests itself to apply plasma sterilization also for planetary protection.

Planetary protection has the aim to preserve the ability to study other worlds as they exist in their natural states, to avoid contamination that would obscure ability to find life elsewhere – if it exists, and to protect Earth's biosphere, in case it does [1]. Therefore, spacecrafts need to be constructed and assembled under conditions as sterile as possible. Plasma sterilization can contribute to planetary protection, since it is a very effective tool, causing different types of stress for bacteria. Furthermore, it is a sensitive and material-friendly sterilization method that can be tuned to meet specified requirements.

For the investigation of the destruction of bacterial spores we have chosen *Bacillus subtilis* 168, which is not only often used as astrobiological model system, but also widely used for various industrial applications, e.g. sterilization. In addition, spores of the spacecraft assembly facility isolate *Bacillus pumilus* SAFR-032 were investigated. The spores were deposited aseptically onto the surface of stainless steel screws, to simulate a spore-contaminated spacecraft hardware component. The screws were exposed to H_2-plasma for different treatment times (15 s, 30 s, 45 s, 60 s). Additionally, evaporated liquids were applied to obtain a two-step process to enhance decontamination efficiency.

Several experimental conditions led to full spore inactivation. The results reveal that the spore survival depends on various factors, e.g. initial spore load and strain-specific sensitivity to H_2-plasma. Spores of *B. pumilus* SAFR-032 were significantly more resistant to H_2-plasma than the laboratory strain *B. subtilis* 168 [2].

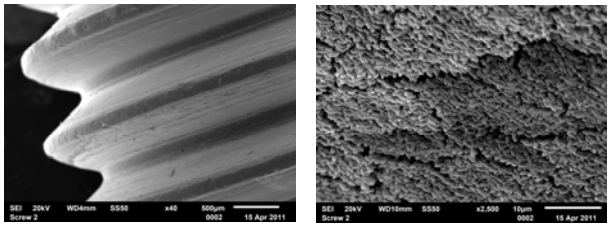

Figure 1: *SEM images of the contaminated screws*

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Plasma Synergy with Conventional Therapies for Cancer and Wound Sterilization

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Ambient gas plasmas are cytotoxic to cancer cells and bacteria at least in part because they create reactive oxygen and reactive nitrogen species (RONS). It is known that these reactive species can also play important roles in conventional therapies, including cancer chemotherapy and antibiotics. For example, anti-tumor synergy between NO-donating compounds and some redox-active chemotherapeutics has been documented [1]. We therefore explored the possibility that plasma-generated RONS could act synergistically against cancer and bacteria with conventional chemotherapeutic agents and antibiotics, respectively.

Treatment of MCF7 breast cancer cells by indirect dielectric barrier discharge and subsequent flow cytometry shows that cytotoxicity correlates with an increase in intracellular reactive oxygen species. Additionally, plasma treatment acidifies the culture medium, and creates nitrates, nitrites, hydrogen peroxide and/or ozone. These species are known to have anti-cancer activity. Plasma treatment of cancerous cells with and without mutant p53 is compared, as well as non-cancerous cells, both immortalized and non-immortalized.

Air plasma antibacterial effects include not only direct killing but also inactivation of pyrogenic bacterial compounds such as lipopolysaccharide (LPS) and lipid A. Since many antibiotics are known to act via ROS [2], we investigated plasma treatment combined with antibiotics to test for possible synergy in this application as well.

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Parametric investigation of a N_2 flowing post discharge source for decontamination of the inner surface of the small diameter tubes

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Non thermal plasma technologies have recently been receiving attention as an alternative technology for surface decontamination of thermally sensitive medical materials [1]. This work focuses on an atmospheric pressure nitrogen corona discharge in a point-to-point geometry [2]. The nitrogen post-discharge was flowing in a 650 mm quartz tube (8 mm inner diameter). Contaminated samples (*E. Coli* suspended in a 20% LB/distilled water solution) were deposited onto the inner surface of the tube, 100 mm (Sample up) and 630 mm (Sample down) away from the source. Bacteria exposure and spectroscopic measurements (for estimation of nitrogen atoms density) were made simultaneously with a 20 $L.min^{-1}$ flow for pure nitrogen and nitrogen with controlled traces of oxygen. Thermal effects were also estimated. Direct CFU counts were made for the two deposit locations for increasing exposure time. As presented in the survival curve (survivors vs. exposure time), for a 30min treatment time, a 6 log reduction of survivors was obtained for up samples whereas less than a 2 log reduction was observed for down samples. Correlation between active species density along the post-discharge and the decontamination efficiency will be discussed.

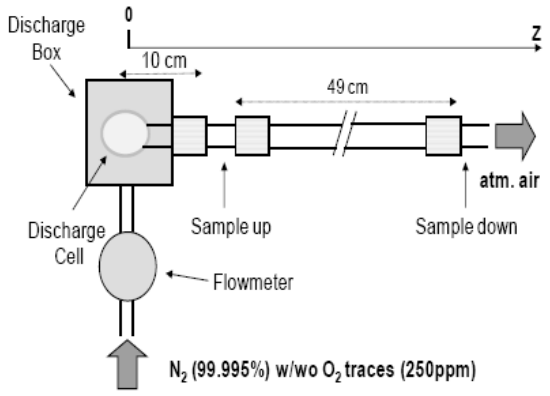

Figure 1: *Experimental Setup*

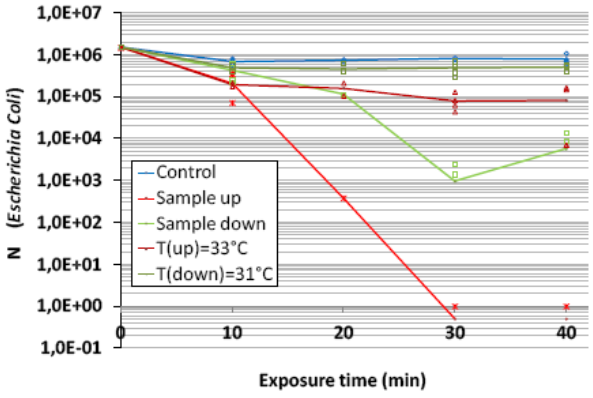

Figure 2: *Survival Curve*

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Comparison of bacteria inactivation using two distinct devices of low temperature plasma jets at atmospheric pressure

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In the field of biological decontamination and sterilization of for example the medical tools, non thermal cold plasmas are an interesting alternative to the classical devices based on thermal processes (autoclave or dry oven). The later devices are generally not very well adapted to the treatment of for instance sensitive instruments as endoscopes or catheters. As is known, the non thermal plasmas generated more particularly at atmospheric pressure using DBD or corona discharges driven under different power modes (DC, pulsed, RF, MW), can be efficient sources of active species, easy portable, able to do remote treatments of living tissues, adaptable to many surface configurations and generally do not need any pumping system for gas conditioning in comparison to the lower pressure plasma sources (see e.g. ref 1 and the refs given therein).

This contribution is first devoted to comparative electrical and spectroscopic characterizations of two different low temperature plasma jets. The first one is generated by a corona discharge directly activated in ambient air without using any rare gases [2] and the second plasma jet uses helium gas flowing inside a quartz tube surrounded by two thin electrodes powered by a mono-polar pulsed high voltage supply [3]. The aim of these experimental characterizations is to estimate the injected power in each plasma jet case and to analyse the produced active species.

Then, the second aim is to test the bactericide efficiency of these two specific low temperature plasma jets. The effects of the bacteria inactivation will be shown and analyzed for several exposure times to the two plasma jets with and without interposing a grounded mesh (to filtering the charged particles) between the plasma jet and the treated bacteria.

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Bactericidal properties of cometary discharge with inserted grid

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The ability of the DC cometary discharge [1], [2] with inserted grid to decontaminate or sterilize the human skin was observed. First, the guidance of the European Standard [3] was followed: the clean fingertips were artificially contaminated with the suspension of Gram-negative *Escherichia coli* bacteria and treated for various time intervals with the discharge as a disinfectant agent. We achieved 100 % decrease of *E.coli* bacteria number within 4 minutes, as related to the untreated control. However, the best achievement for treating the fingertips covered with the natural physiological microflora consisting mainly of Gram-positive *Staphylococcus epidermidis* was only the decrease of bacteria number to 21.1 % of the original value within 10 minutes. The conclusion is, that:
1. the discharge is able to inactivate Gram-negative bacteria;
2. this ability is substantially lower regarding the Gram-positive ones;
3. the European Standard does not respect this discrepancy.

Acknowledgment: This work was supported by grants MSM ČR 002162080, MSM ČR 6046137306, and SVV-2010-260506.

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ROS and RNS formed in water sprayed through transient spark in air and their bactericidal effects

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Recent studies show that the bactericidal effects of atmospheric pressure cold air plasmas in contact with water are dominantly due to reactive oxygen and nitrogen species (ROS, RNS). We investigated the chemical effects induced in water electro-sprayed through DC-driven positive transient spark discharge at a constant flow rate of 0.5 mL/min.

The chemical effects induced in the plasma treated water were measured by pH and conductivity probes, spectrophotometrically for peroxides (OO^{2-}), and by high resolution ion chromatography for nitrites (NO_2^-) and nitrates (NO_3^-). Aqueous solutions of various initial conductivities were used: deionized water (σ=1 μS/cm), NaH_2PO_4 solutions of σ=500-700 μS/cm mimicking tap water, and physiological saline solutions (σ=7 mS/cm). The initial pH was 5-7. After spraying the solutions through a positive transient spark, conductivity and acidity increased (pH dropped down to ~3).

Bacterial suspensions of *E. coli* (CCM3954) and other bacteria (*S. typhimurium, B. cereus*) were sprayed through the discharge. Up to 7 log reduction in the number of bacteria was obtained in water or in saline solution. Acidity itself is not the main bactericidal agent, as confirmed by the tests with the nitric acid solution of the same pH. Acid environment in synergy with plasma agents leads to the bacterial inactivation. To elucidate the pH effect on the plasma induced water chemistry we tested aqueous solutions buffered with dilute phosphate buffer (PB) that did not decrease their pH after plasma treatment. Non-acidic environment resulted in higher nitrites, slightly lower peroxides and nitrates, and strongly reduced bactericidal effect (about 1 log bacteria reduction). Fig. 1 shows bactericidal effect (log reduction) and NO_2- concentrations as functions of pH after treatment.

Air plasma water treatment produces nitrites, nitrates and peroxides. It seems that the acidified nitrites are the dominant bactericidal agents in water treated by air plasma.

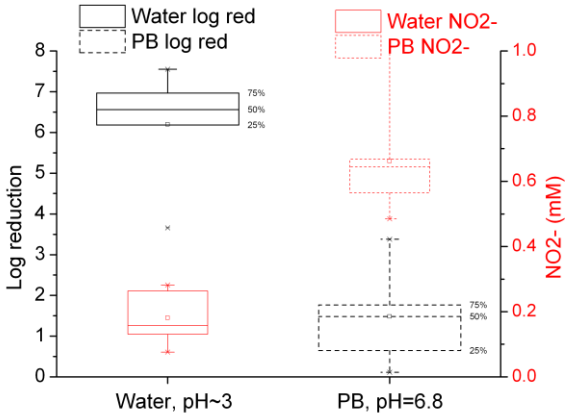

Figure 1: *Bacteria log reduction and nitrite concentration in water and phosphate buffered solutions depending on pH after plasma treatment.*

Supported by VEGA 1/0668/11, Slovak Research and Development Agency APVV SK-CZ-0179-09 and SK-FR-0038-09, and the Grant Agency of AS CR (project No. IAAX00430802).

Determination of Plasma Parameters of a Low-Pressure VHF-CCP used for Sterilization

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Sterilization and decontamination of medical instruments and implants made out of new high performance synthetic materials are a key issue in modern medicine. However, established sterilization and decontamination processes are not practical due to the high temperature, toxic agents or radiation used. In the past years the utilization of low-temperature plasmas for sterilization and decontamination purposes has become a promising alternative. It has already been demonstrated that a small and cost-effective VHF-CCP is capable of sterilizing germs and spores and to decontaminate proteins. To further explore the sterilization and decontamination processes several plasma parameters and fluxes of the VHF-CCP were determined.

The discharge was a H_2 plasma driven between 68 MHz to 72 MHz at 10 Pa with a power increased in steps of 100 W from 100 W to 400 W. The measurements were done with and without medical devices in the discharge chamber to investigate the influence onto the parameters. The plasma parameters were determined by Optical Emission Spectroscopy (OES) providing the gas temperature T_g, electron temperature T_e and electron density n_e. With these determined results, the neutral gas flux onto the surface j_g, the Debye length λ_d, plasma frequency ω_p as well as the dissociation, excitation and ionization rates could be calculated. The investigation of spectral lines offered the determination of species.

In order to further explore the sterilization mechanisms, more biological experiments with low pressure plasmas will have to be undertaken, which factor the already gained findings and allow them to interpret more comprehensively.

An innovative method of cold plasma for sterilization of medical devices

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In the medical field, the most commonly used methods for sterilization of medical devices are steam sterilization (autoclave), ionizing radiation and ethylene oxide. The evolution of medical techniques and technologies and the emergence of new materials have led to great advances in medicine. However, the sterilization of some new devices presents some difficulties linked to their vulnerability to sterilizing agents. Therefore, a new sterilization process has been studied for over 10 years: cold plasma sterilization. The idea is to expose a sample to plasma created in a chamber at atmospheric pressure or in vacuum condition. Many studies have demonstrated the efficacy of plasma on microorganisms inactivation [1, 2, 3]. But, these methods do not permit to ensure the preservation of the sterility during and after treatment. Moreover packaging and storage methods of sterile equipment represent an important part of the process leading to obtain sterile conditions according to European norms (EN ISO 11607, EN 868).

Taking these facts into consideration, we have developed a process which allows the conservation of the sterile state by creating a plasma treatment directly inside the transport pouch. A sample packed in a sealed pouch is subjected to vacuum. Then a gaseous mixture is injected into the pouch through a filter. The plasma is generated only in the bag and not in the chamber. This method is protected by a patent (PTC/FR2011/052199).Various gaseous mixtures have been tested and applied to *Pseudomonas aeruginosa* suspensions or biofilms on hydroxyapatite coated titanium (TA6V).

Figure 1: *Generated plasma inside the packaging bag*

Our results reveal that *Pseudomonas aeruginosa* suspensions and biofilms are affected by this sterilization method. We observed a 106 Log decrease of the viability of the bacteria in suspensions which highlights the capacity of this technology to be efficient according to European Norms. The optimization of the parameters used could increase the efficiency on bacterial biofilms.

Acknowledgments: we thank Franck SZYNAL for his technical support

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Plasma treatment for agriculture applications

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Nonthermal plasmas are emerging and promising methods for sterilization. They have achieved great progress in recent years. It has been found that many kinds of plasmas can kill vegetative forms, spores and fungi, efficiently.
Influence of ozone in air and soil on seeds' and plants' development was broadly investigated by numerous researchers. Results highly depended on the kind of plant and were sometimes contradictory. Seedlings and seeds of chinese cabbage (Brassica pekinensis), Garland chrysanthemum (Chrysanthemum coronarium), muskmelon (Cucumis melo), tomato (Solanum lycopersicum) and spinach (Spinacia oleracea) were placed in separated containers with pre-ozonized soil and with non-ozonized soil, respectively. Soil ozonation influenced plants' growth in various ways. There was 24% of growth inhibition after 79 days in the case of cabbage due to decreasing pH of soil and parallel elimination of microorganisms useful for soil enrichment.
Results of young seedlings of chinese cabbage (A) and crown daisy (B) growth after the 20 minutes of soil decontamination with $100gO_3/m^3$ are depicted in Figure 1.
Improvement of growth was observed in the case of melons, tomatoes and plants', for which environmental stress has a beneficial influence on growth and fruit formation.

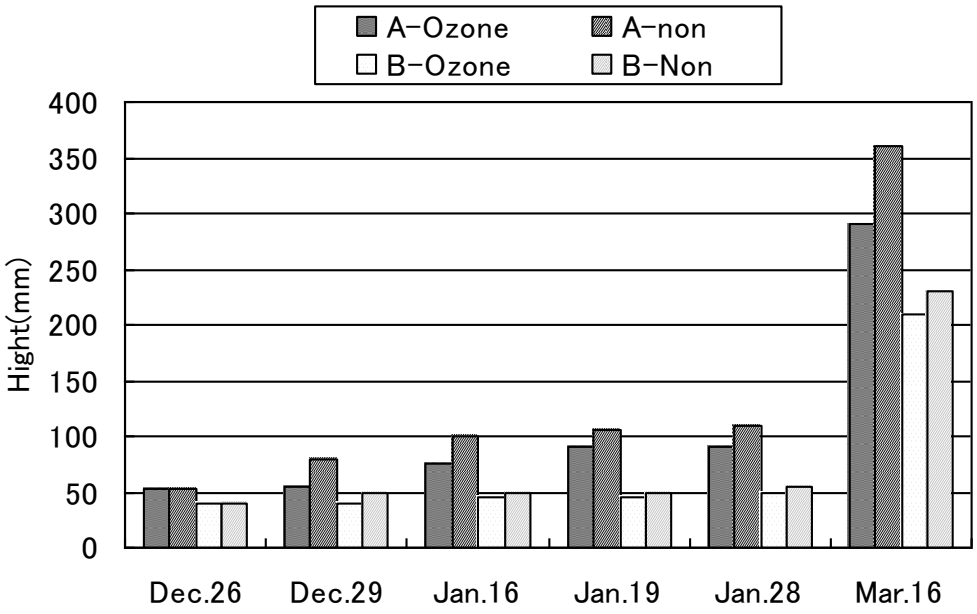

Figure 1. *Chinese cabbage (A) and crown daisy (B) growth experiment.*

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Poster - Session 2

Molecular dynamics simulation of plasma-bacteria cell wall interaction

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Low-temperature, non-thermal atmospheric plasmas have important applications in medical fields, including sterilization of surfaces and diagnostic devices, as well as for therapeutic techniques, e.g. wound healing and treatment of cancer and skin diseases. As a result, there is currently an increasing interest in plasmas for medical applications.

However, modeling of both the plasma itself and the interaction of plasma with living organisms such as bacteria is very limited until now [1]. Nevertheless, plasma simulations can be useful to obtain information about the density distribution of charged particles, molecules and radicals, their reaction rates, etc., which is difficult to obtain experimentally due to possible plasma disturbance with the measuring tool.

Simulating the interaction of the plasma with the surface of living organisms (e.g. grampositive or gram-negative bacteria) is also very difficult. If a proper interatomic potential can be constructed for describing all relevant interatomic interactions, molecular dynamics simulations can provide atomic scale insight in these interactions.

In the present work, we investigate the interaction of plasma species such as OH, NO, NO2, H2O2, O3 and O atoms with bacterial peptidoglycan (PG) by means of molecular dynamics (MD) simulations. In an MD simulation, the trajectory of all atoms in the system is followed by integrating the equations of motion. Forces on the atoms are derived from the Reactive Force Field (ReaxFF) potential [2]. In this work, we assume the gram-positive bacterium *Staphylococcus aureus* murein as the PG structure, which is typically 20-30 nm thick [3] and serves as a protective barrier in bacteria. Its chemical structure can be found in [4, 5]. Our results demonstrate that among the above mentioned species, only OH radicals and especially O atoms break C-C and C-N bonds, which subsequently leads to a destruction of the bacterial cell wall. This study is important for understanding the chemical damaging of the bacterial PG on the atomic scale.

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Modified Scaffolds in Radial Flow Bioreactor for 3D Mammalian Cell Culture

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The bioreactors are used to generate volumetric cell cultures, to conserve the bulk properties of the tissues and the morphology, on biomaterials used like scaffolds for a great proliferation as the 2D cultures [1].

We used 3D scaffolds of PLLA, modified in the surface with polypyrrole (PPy) by plasma polymerization, to growth hepatic cells (Hep G2) in a radial flow biorreactor (RFB), this modified scaffolds increase the cellular proliferation and protein production, and shown that we got a RFB for mammalian cell culture. The PPy plasma polymerization has demonstrated that increase the cellular adhesion without modified the properties of the target scaffolds and it can be biocompatible, using for cell cultures and implants in rats [2].

We design the RFB, to use a lot of modified materials by plasma polymerization and different mammalian cells, using the microscopy we obtained the images of the material and the cells, and then show the system is a good bioreactor for the 3D proliferation of hepatic cells in volumetric scaffolds modified in the surface and show that the protein secreted by the cells in the RFB increased using the modified scaffolds than the target scaffolds [3]. These experiments concluded that we obtained a three-dimensional hepatic cell culture on covered scaffolds of a thin film of polypyrrole (PPy) morphological and physiological viable.

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Cell-Adhesion and Bone Integration on Different Plasma Coatings

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The secondary implant stability is of essential importance for the outcome of cementless joint replacement. Therefore, the implants have to be integrated in the surrounding bone stock. Bone cell on-growth can be supported by modifications of implant surfaces and can contribute a fast integration of the implant into the bone stock. Different plasma coatings on surfaces of endoprosthetic implant are in development to improve their osseous integration. In order to analyse the effects of plasma coatings measurement of the adhesive strength of bone cells on implant surfaces is of high interest and a preclinical possibility to prove surface properties [1]. Furthermore, the in-vitro data have to be validated with appropriate in-vivo models.

Thereby, plasma polymerised allylamine (PPAAm) [2] and plasma polymerised ethylenediamine (PPEDA) [3] coatings were deposited on TiAl6V4 disks samples. For determination of cell adhesion polished disks were used and shear stress of the bone cells, as the parameter for the adhesive strength, was determined after 24 h cell cultivation on uncoated, PPAAm and PPEDA coated samples. For the animal model uncoated, PPAAm and PPEDA coated rough TiAl6V4 samples were implanted in the tibiae of Sprague Dawley rats. After six weeks bone integration of the different samples was analysed via micro-computer-tomography and histology.

Bone cells showed a better early adhesion on PPAAm and PPEDA coated implant surfaces. After 24 h shear stress of MG 63 cells was significant higher on PPAAm ($p \leq 0.05$) and PPEDA ($p \leq 0.05$) coated samples compared to uncoated samples. Between both plasma coatings no significant difference ($p = 0.31$) was observed. After six weeks in the animal model the uncoated and plasma coated samples showed bony integration, whereas no significant difference in the bone-implant contact area between all three surface were found.

The initial better in-vitro adhesion of bone cells on plasma coated implant surfaces showed no influence on bone integration after six weeks in-vivo. However, the better adhesion of bone cells on plasma coated implant surface could be advantageous, concerning the „race for the endoprosthetic surfaces“ against bacterias in order to enable prevention of implant related infections.

This work was supported by the BMBF program Campus PlasmaMed (sub-project PlasmaImp 13N9775, 13N11188).

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In-vitro investigations of different non-thermal atmospheric pressure plasma sources on human keratinocytes

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Physical plasma, the fourth state of matter, is characterized by a mixture of ions, electrons, radicals, electric and magnetic fields and UV-light. Some of these ingredients are already known to cause manifold reactions in mammalian cells. To ensure a safe application of non-thermal atmospheric pressure plasma in medicine the interactions between physical plasma and human cells have to be clarified.

Subject of this study was to investigate the influence of three different non-thermal atmospheric pressure plasma sources on human keratinocytes (HaCaT cells). Adherent HaCaT cells were treated with a surface dielectric barrier discharge (1), a volume dielectric barrier discharge (2) and a plasma jet (3). In order to achieve biological effects different treatment times were necessary. With volume DBD and plasma jet HaCaT cells were treated up to 2 min, surface DBD allowed treatment times from 1-20 min.
To investigate the influence on viability number of adherent cells was counted 24 h after plasma exposition. DNA damage, detected by alkaline single cell gel electrophoresis (Comet assay), was measured subsequently and 24 h after incubation with physical plasma. Further the influence of DBD plasma on the cell cycle was analyzed using flow cytometry (24 h).

24 h after plasma treatment a dose-dependent decrease of number of recovered adherent cells was observed, independently from the plasma source.
Immediately after plasma treatment a dose-dependent increase of DNA damage (parameter: tail intensity by Comet assay) was caused by all plasma treatment regimens, which was diminished after 24 h. With few exceptions (e.g. 60 s treatment volume DBD, 20 min surface DBD) values of tail intensity decreased to level of control cells. An additional change of cell culture medium subsequently after plasma treatment resulted in a higher percentage of recovered cells and in lower DNA damage.
24 h after plasma treatment HaCaT cells showed different distribution pattern of cell cycle stages. All three plasma sources induced a significant raise of the number of HaCaT keratinocytes in G2/M- phase at the expense of G1 phase. An increase of cells in the phase of Sub G1, which is an indicator for apoptosis, was not observed.

In conclusion non-thermal atmospheric pressure plasma caused a dose- and time-dependent influence on viability and DNA of HaCaT cells. An immediate cell culture medium exchange attenuated described effects. The procedure of the cell cycle is also affected by plasma treatment. There are no basic differences in the effects of the three different plasma sources used. Further studies should clarify which plasma component or which combination of components is responsible for described effects.

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Differential Sensitivity of Lymphocyte Subpopulations to surface DBD plasma

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Non-thermal atmospheric-pressure plasmas can be used for several applications in medicine. Plasma treatment can be applied to living tissues and cells, e.g. to induce apoptosis and growth arrest in tumour cells or to improve wound healing. However, detailed investigations of plasma-cell interactions are strongly needed. It is not yet clear whether plasmas will be useful in stimulating immune cells to change their behaviour or function. Therefore, it is still in question as to whether plasma can influence mononuclear cells (MNC) to become more sensitive against tumour cells or to generate regulatory cells important in autoimmune diseases.

This study focused on the influence of non-thermal atmospheric pressure plasma on cell surface molecules of rat spleen mononuclear cells (MNC) as one important step to gain insight into plasma-immune cells interactions. Such findings might lead to plasma applications in immunology or cancer treatment. Rat MNC isolated from the spleen were treated for 10 to 60s with plasma by surface dielectric barrier discharge (DBD) at atmospheric pressure in air or argon. Lymphocyte subpopulations and expression of L-selectin, ICAM-1 and LFA-1α expression on T-cells were analyzed by flow cytometry 1 to 48 h after plasma treatment. Further, apoptosis was analysed by annexin V and propidium iodide (PI) staining.

MNC are very sensitive to DBD/air plasma. 24h after a 60s treatment cycle all MNC were dead as shown by the annexin V/PI staining. Already 1 h after a 20s DBD/air treatment about 10% of early apoptotic MNC were detected. Plasma changed the ratio of T- and B-cells in favour of B-cells. Of the T-cells the helper T-cells were reduced while cytotoxic T-cells were less affected. L-selectin expressing T-cells were significantly reduced already 1 h after plasma treatment and that of ICAM-1$_+$ and LFA-1α$_+$T-cells only after 4 h. These effects were time dependent and less dramatic when using DBD/argon plasma.

In conclusion, different lymphocyte subpopulations are selectively sensitive to the effects of plasma. The effects are dependent on the duration of treatment as well as on the time after plasma treatment. By treating MNC with plasma, adhesion between cells can change, thus affecting cellular functions such as migration and proliferation. Due to the reduction of Lselectin, homing of lymphocytes can also be altered. On the other hand, the threshold for activation of MNC is possibly reduced by plasma treatment due to an increase in LFA-1α. Whether these changes can be used to generate regulatory T-cells, to sensitize immune cells against tumour cells or to modify homing of lymphocytes remains to be clarified.

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DBD plasma treatment of HaCaT keratinocytes: reactive oxygen species rather than ozone increase integrin expression

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Non-thermal atmospheric-pressure plasma has been shown to influence as first target the cell membrane with its embedded proteins. Such cell surface molecules as integrins, cadherins or the epidermal growth factor receptor (EGFR) are of importance in wound healing and also for development of cancer metastasis. Cold plasma comprises of electrons, positive or negative ions, free radicals [e.g. reactive oxygen species (ROS), ozone] and other excited atoms and molecules. Further, plasma has an optical emission in the UV-region, especially UVB. Each of these components can affect the cells during treatment.

This study focused on measurement of apoptosis, induction of intracellular ROS and cell surface molecules on human HaCaT keratinocytes. Adherent HaCaT keratinocytes were treated with plasma by a surface dielectric barrier discharge in air and argon (1 to 5 min) or with ozone (5 min). Ozone was generated by an Ozonisator and monitored by FT-IR (100, 400, 900 and 1800 ppm). Intracellular ROS, apoptosis (annexin V/propidium iodide staining) and cell surface molecules (α_2-, α_3-, α_4-, α_6-, α_V-, β_1-integrin, E-cadherin, EGFR) were analyzed by flow cytometry 24 h after treatment.

Besides a reduction of cell viability significant intracellular changes were observed. DBD/air plasma for 5 min caused an increased expression of α_2- and β_1-integrin whereas E-cadherin and EGFR expression was not influenced. The effects of DBD/argon plasma were less pronounced. Apoptosis was only increased by DBD/air plasma (5 min) although the proportion of apoptotic cells was rather low. Intracellular ROS detected by the fluorescent dye CM-H_2DC-FDA increased from 6.6 ± 1.1% (untreated control cells) to 18.0 ± 3.2% (5 min DBD/air) and 10.9 ± 1.3% (5 min DBD/argon). A concentration of about 100 ppm ozone was measured above the solid phase during a 5 min DBD/air treatment cycle which had no influence on integrin, E-cadherin or EGFR expression. 1800 ppm ozone caused an increase of α_2- and β_1-integrin whereas all other molecules measured were not affected.

Taken together, the extent of effects depended on the nature of plasma (air vs. argon) and the exposure time of cells to the plasma. Short (≤ 1 min) treatment cycles did neither change cell surface protein expression nor induced apoptosis or intracellular ROS. The effects of plasma on cell membrane proteins observed are rather attributed to induction of intracellular ROS than to generation of ozone.

* Work supported by the German Federal Ministry of Education and Research within the joint research project “Campus PlasmaMed” (grant no. 13N11182)

Differential Effect of Non-Thermal Atmospheric-Pressure Plasma on Angiogenesis

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The formation of new blood vessels is an essential feature of tissue remodeling as observed in e.g. wound healing or solid tumor development. For improving wound healing it should be promoted, whereas in treating tumors angiogenesis should be inhibited. Therefore, it is very important to know whether and how plasma influences angiogenesis.

This study focused on the effects of plasma generated by the kINPen 09 on angiogenesis using two different models: HET-CAM assay and rat aortic ring (AOR) test. In both models kINPen 09 treated medium (30 to 300s) was applied. ImageJ was used to analyze vessel area and fractal dimension after treating the CAM from embryonic day 11 to 13. Aortic rings were prepared from either LEW.1W or WOK.W rats. They were embedded in matrigel and treated daily for 4 days starting at day 4 after embedding. We developed a semi quantitative method to quantify production of microvessels from aortic rings.

In both models natural and spontaneous vessel formation was detected. In the HET-CAM assay vessel area and fractal dimension were significantly enhanced at embryonic day 14 by the 120s-plasma treated medium compared to untreated controls. There was no effect of plasma (60 or 120s) on vessel growth of aortic rings prepared from LEW.1W rats. The angiogenic activity of rings from WOK.W rats was significantly ($p<0.05$) inhibited by plasma (120s). Dexamethason was able to completely inhibit vessel sprouting from aortic rings of both rat strains.

In conclusion, the angiogenic response to plasma was found to be differentially influenced. It depended not only on the model used (HET-CEM vs. AOR) but also on the rat strain in the aortic ring test (LEW.1W vs. WOK.W). It will now be of importance to define the different molecular events during the angiogenic response to make plasma applicable for the different demands.

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The Effects of Air and Nitrogen Plasma Jets on Living Cancer Cells

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The atmospheric-pressure plasma has been proposed as a novel therapeutics for anticancer treatment. Recently, G. J. Kim et al. and K. Kim et al. reported that atmospheric-pressure air plasma jet and nitrogen plasma jet induce apoptosis of cancer cells via generating DNA damages[1][2]. And S. Kalghatgi et al. reported that the air plasma induce apoptosis via generation of ROS[3]. In this paper, we report the effect of ROS scavenger and evaluate the effects of nitrogen plasma jet and air plasma jet on living cancer cells.

In order to ascertain whether the ROS generated by plasma jets are implicated in plasma-mediated apoptosis, we treated HeLa cells with antioxidant scavengers and exposed the cells to plasma jet for 5 minutes. The results of cell analysis by FACs are shown in Fig. 1, which illustrates that the removal of ROS impedes plasma-induced cell death and that ROS mediate the plasma-induced apoptosis. Furthermore, the blocking effect of carboxy-PTIO (scavenger of NO) and sodium pyruvate(scavenger of H_2O_2) in the cells treated with air plasma is stronger than in the cells treated with nitrogen plasma. In consideration of the fact that the effectiveness of ROS scavenger depends on the gas generating the plasma, we form a hypothesis that the composition of ROS in the plasma differs depending on the gas generating the plasma jet. The hypothesis is supported by OES of plasma jet. The dominant peaks of air plasma spectra are different from the peaks of nitrogen plasma spectra. The different peaks in spectra prove that the air plasma and the nitrogen plasma generate different compositions of ROS as by-products of plasma.

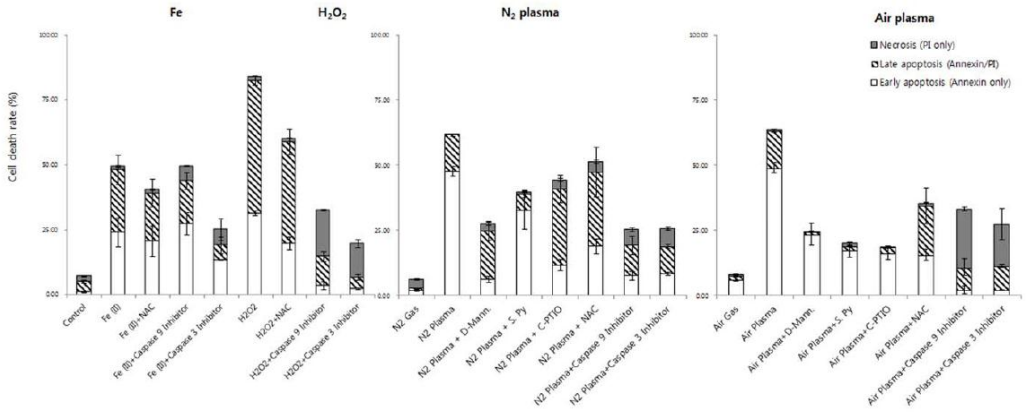

Figure 1: *The results of cell death rate after plasma jet treatment for 5min.*

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Dose dependent killing of leukemia cells by low temperature atmospheric pressure plasma

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The use of non-thermal plasma in medicine has become an increasingly important topic for physicists, biologists and medical personnel alike. A multi-disciplinary approach was undertaken in order to combine the plasma plume characterizations such as the reactive species that are generated with biological effects. The focus of this work was to determine the activity of low temperature atmospheric pressure plasma (LTAPP) towards CCRF-CEM human T-cell leukemia cells. Leukemia is one of the most prevalent cancers in children and requires safe and effective treatment. Even though some drug therapies have increased the short term patient survivability, long term morbidity still remains high [1]. In order to provide a safe and effective treatment regime, our work studies the effect of LTAPP on cell culture lines of leukemia. The plasma pencil, which utilizes high voltage pulses, was used to generate LTAPP [2]. The effect of LTAPP on the progression of cancerous cells was studied using a tissue culture cell line (ATCC CCL-119). The leukemia cells were grown in RPMI- 1640 supplemented with 10% Fetal Bovine Serum. The cells were grown at 37°C and 5% CO2-humidified environment in tissue culture flasks (T-75 mm2). The cells were grown to a cell density of ~1x106 cells per ml. Subsequently, 106 cells were seeded in each well of a 24- well culture plate. The cells were then exposed to varying doses of LTAPP and incubated at 37°C and 5% CO2-humidified environment for up to 3 days. Cell viability was determined at 12h, 36h, and 60h post plasma exposure treatment by a variety of methods, such as trypan blue exclusion assay. Cell morphology, DNA damage, and media changes were monitored to determine the specific attributes that resulted in cell death. The outcome of this study revealed that the effect of plasma exposure was not immediate, but had a delayed effect and increasing the time of plasma exposure resulted in increased CCRF-CEM leukemia cell death. Overall, our study facilitates the development of LTAPP as a therapeutic against cancer cells and provides a model template for future investigations involving LTAPP and diseased mammalian cells.

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Effects of nitrous acid and nitric acid in an air-water plasma system on Hela cell viability

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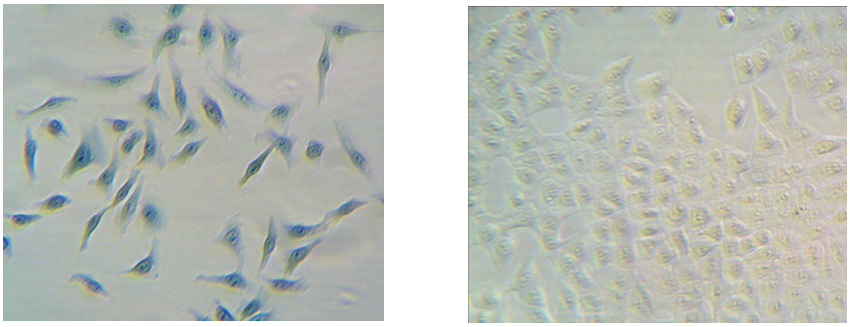
A air-water plasma system generates many chemical species such as ozone, nitrogen oxides and reactive species in air. Those species dissolve and react in water and produce stable chemical species such as hydrogen peroxide, nitrous acid and nitric acid which are transported due to their long life span [1]. Authors have been clarified the effect of hydrogen peroxide on Hela cell viability in an air-water plasma system. Similar trends in biological reactions of Hela cells were obtained in both plasma treated and hydrogen peroxide added culture media with respect to cell survival ratio, morphological damage process, ROS production in cells, response to catalase treatment, and comprehensive gene expression. Those results proved that hydrogen peroxide is the main inactivation factor of Hela cell viability [2]. However, effects of nitrous acid and nitric acid are also important in the case of greater concentration. In the present reasearch, we aimed at clarifiying the effect of those species on Hela cell viability using culture media supplemented with nitrous and nitric acids at a concentration up to 10 mM. Figure 1 shows trypan-blue staining of the cells cultured with nitrous acid (a) and nitric acid (b). The cells died with 10 mM nitrous acid (positive staining), whereas they survived with 10 mM nitric acid (negative staining). Cell responses for oxidation stresses were also investigated in this research.



(a) (b)

Figure 1: *Trypan-blue staining images of Hela cells incubated for 48 hours with 10 mM nitrous acid (a) or 10 mM nitric acid (b) added culture medium.*

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Plasmid DNA degradation induced by a plasma microjet

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Figure 1 : *Picture of a plasma jet interacting with plasmid DNA solution placed in an Ependorf tube.*

Recently, interest in plasma micro-jet at atmospheric pressure has increased due to their interesting advantages as their low temperature and the creation of reactive species. Various applications are planed and particularly in the biomedical field [1] (odontology, dermatology, cancer research,…). In order to understand what it is the real impact of this plasma on biological structures we exposed this plasma jet on plasmid DNA.

Our discharge consists of concentric tubular electrodes separated by a dielectric cylindrical structure. The device is made of a dielectric tube with an inner diameter of about 1 mm. A grounded electrode is wrapped around the external side of the dielectric, while a high voltage electrode is glued inside the tube. Pure helium is flowing through the inner electrode at a flow rate in the range 500–1000 cm^3/min. High voltage pulses (3-6 kV) are applied between the electrodes at a repetition rate frequency of 20 kHz. This jet is set up vertically with the gas flowing downwards for interacting with plasmid DNA solutions put inside microwells or Ependorf tubes (figure 1). In each micro well there was 200 µL of buffer solution with a DNA concentration of 20mg/L.

Different buffer solutions have been used in order to identify their influence on the DNA degradation. It will be shown that the nature of the buffer solution did not change the nature of DNA damages. The only difference was the treatment times required to get a given amount of damages. Moreover, it will be shown that these damages resulted from a direct interaction of DNA with the plasmajet without participation of by-products from the buffer solution. Analysis of the damages through specific enzymes (Fpg, Nth and Ape1) revealed that most of the damages were direct single and double strand breaks, while the oxidation of the amino-acid bases was of minor importance.

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Selective killing of melanoma cells with Air plasma and anti-HER2 antibody conjugated gold nanoparticle

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Plasma is effective in killing cancer cells. However, it can't distinguish cancer cells from normal cells. To solve this problem, an antibody against overexpressed protein in cancer cells and gold nanoparticles (GNP) which is harmless against the human body are used in this therapy [1]. In many cancer cells, some kinds of proteins have known to be overexpressed. These proteins are regarded as an attractive target for cancer therapy [2,3]. GNP can be used by conjugating with cancer specific antibody to achieve the selectivity in cancer treatment [4-6]. HER2 is a protein frequently overexpressed in melanoma cells. Thus, we made anti-HER2 antibody conjugated GNP (HER2-GNP) for targeting HER2 protein. After we added HER2-GNP into both G361 melanoma and HaCaT normal cells, plasma treatment resulted in significantly high death rate of G361 cells, compared with HaCaT cells. The death rate of G361 cells treated with HER2-GNP and plasma is over three times higher than that of HaCaT cells, which was slightly affected by plasma and HER2-GNP. Many vacuoles were observed in G361 cells. Furthermore, the destruction of HER2 consequently inactivated the phosphorylation of HER2, FAK and paxillin linked with HER2 protein. Therefore, this study suggests that plasma treatment with HER2-GNP can kill cancer cells effectively more than normal cells.

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Impact of cold atmospheric pressure plasma on human skin cell-lines

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In physics, plasma is known as the fourth state of matter and denotes a partially or fully ionized gas. Recently, cold atmospheric pressure plasma sources gain attention as a possible tool for biomedical applications, emitting UV radiation and creating reactive oxygen and nitrogen species (ROS, RNS). It is well known that prokaryotes die during a plasma treatment whereas eukaryotes are able to protect themselves and survive the same duration of treatment. Therefore plasma is very interesting for chronic wound healing. The aim of this work is to study plasma-based activation of skin cells.
The human keratinocyte cell line HaCaT was treated with the atmospheric pressure plasma jet kINPen 09. The effect of plasma on proliferation and cell death was analyzed with Alamar Blue Assay and Annexin V and Caspase 3 via flow cytometry.
Furthermore Realtime-Polymerase Chain Reaction (RT-PCR) was done with 84 genes (extracellular matrix and cell adhesion genes, inflammatory cytokines and chemokines, growth factors and signal transduction genes) which are related to wound healing. This RT-PCR showed the up or down regulation of different genes like the stimulating substances VEGF or IL6 after plasma treatment [Fig. 1].
These results underline the huge potential of plasma for wound healing and give first insights in the underlying mechanisms on the cellular level.

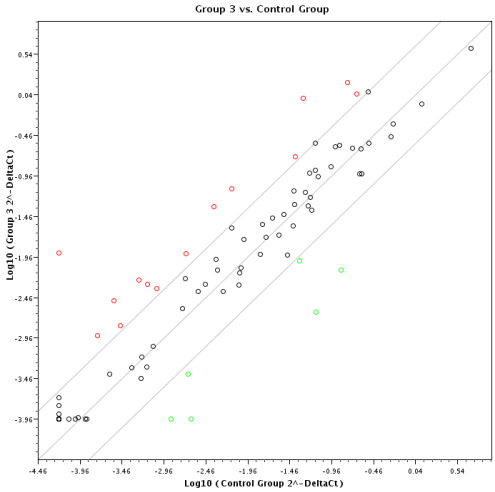

Figure 1: *Analysis of 84 genes with RT-PCR. The red dots shows up-regulated genes and the down-regulated genes are visualized with green dots. The black dots at the defined range are not changed after plasma treatment.*

Cellular growth on rough surfaces after removing of biofilms by brush and/or atmospheric pressure plasma

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In dental implantology the use of titanium (Ti) implants with rough surfaces are common. The development of peri-implantitis in consequence of bacterial deposits is a problem of great importance and approximately one quarter of patients with a dental implant develop peri-implant lesions ten years after installation [1]. It is necessary to re-establish the surface characteristics to create the preconditions for bone regeneration [2]. Atmospheric pressure argon/oxygen plasma is able to establish a hydrophilic and therefore cell-adhesive surface [3].

We used rough Ti surfaces (SLA, diameter 5 mm, Straumann, Switzerland) and cultured a saliva biofilm on it. Beside the untreated biofilm surface (BIO), an autoclaved (AUTO), with brush treated (BR), with argon/1%O_2 plasma treated (PL) and a brush + argon /1%O_2 plasma treated (BR+PL) surface was investigated. Subsequently human osteoblastic cells (MG-63) were seeded (22.700 cells/cm²) onto the specimens and cultivated for 60 min and 24 h in DMEM at 37°C and 5% CO_2. Cell area as well as morphology of cells was investigated by using scanning electron microscopy (SEM).

Preliminary results indicated that cells on BIO (354 ± 97 µm²), AUTO (401 ± 127 µm²) and BR (329 ± 116 µm²) are significantly smaller after 60 min of cultivation in comparison to PL (525 ± 160 µm²) and BR+PL (668 ± 181 µm²). Interestingly the combined treatment with brush and plasma (BR+PL) resulted in even larger cells compared to PL ($P < 0,05$). In relation to the cell area MG-63 on plasma treated specimens showed a more spread appearance. Our investigations revealed that plasma treatment not only inhibits the bacterial deposits. On PL as well on BR+PL the biofilm was removed.

Obviously removing the biofilm with brush and subsequent plasma treatment have synergistic effects. Earlier investigations showed improved cell reaction in consequence of plasma treatment on pure Ti without bacterial deposits. The AUTO surface indicates that it is of great importance for growth of cells if there are bacterial remnants - indeed destroyed - on the surface.

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On anomalous asymmetric rf current of the plasma mediated electrosurgery device

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A study was conducted on the rf current flowing through the treated biological tissue during the plasma mediated electrosurgery at various experimental conditions (voltages, gases and tissue treatment speeds). An interesting result was found: in spite of installation of the blocking capacitor in the device, the asymmetric rf current which induces the dc current flowing the tissue and can cause the muscular stimulation during medical operation were observed after the symmetric current. The origin of this asymmetric current is the different value of the secondary electron emission coefficient of each material, between the electrode and the tissue. The physics revealed in this presentation is expected to provide an insight for the safety window of the plasma mediated electrosurgery device during the plasma surgical operation.

Apoptosis Induction on Fibroblast Cells by Atmospheric Pressure Plasma Treatment Using Nanosecond Pulsed Power Generator

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Apoptosis induction by atmospheric pressure plasma treatment is a promising technique for medical application such as tumor treatment, minimally invasive surgery and alternative to chemotherapy [1-2]. The control methodology and the mechanisms of the apoptosis induction using plasma treatment is still not clear due to the complex behavior in plasma-biological interaction. The target of our research therefore is the understanding of the mechanism of interaction between plasma and living organisms. The influence of atmospheric pressure plasma treatment on murine fibroblast cell lines (NIH3T3) is researched as a first step.

The short pulsed high voltages with 6-nanoseconds of pulse width are ignited by the inductive energy storage type pulsed power generator using semiconductor opening switches [3], and applied to the plasma needle type electrode with gas flow control in order to generate non-thermal plasma and prevent the electric field effects such as membrane charge.

In figure 1, the cell lines decrease with the duration of the plasma treatment on pure O_2 gaseous. On the other hand, He plasma enlarge number of the cells with the treatment duration and gain of 47% with 10 seconds treatment. A part of cell lines with O_2 plasma shrinkages and represents apoptosis treatment using TUNEL kit in figure 2. The apoptosis concentrically appear with centering just below the plasma electrode. These results indicate the plasma treatment included the electrically-charged particles, radicals and UV, works on the cell lines.

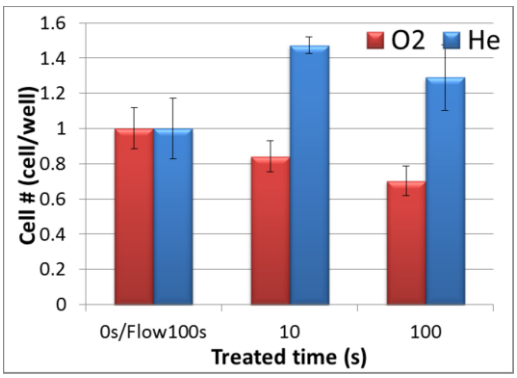

Figure 1: *living cells treated by O_2/He plasma.*

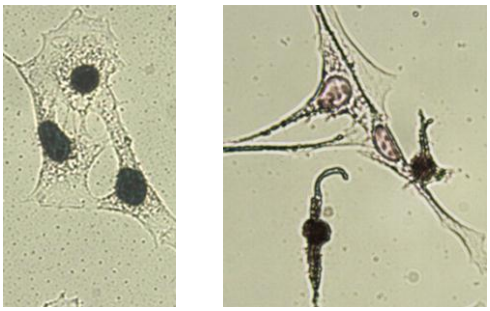

Figure 2: *Apoptosis detection using TUNEL kit (left: positive control, right: O_2 plasma).*

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Impact of non-thermal atmospheric pressure plasma on T lymphocytes and monocytes

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Atmospheric pressure plasma consists of partially ionized gas and contains a range of reactive species like free radicals and exited atoms. Since non-thermal plasma exhibits temperatures below thermal cell damage there are numerous applications in medicine, e.g. sterilization, blood coagulation and wound care [1]. Recently, it has been shown that plasma treatment can have lethal effects on bacteria, whereas eukaryotic cells can be promoted to grow and proliferate [2]. Therefore, non-thermal plasma treatment has the potential as a promising tool in wound healing. The aim of this study was to investigate the impact of non-thermal atmospheric-pressure plasma on human immune cells, in particular on Jurkat cells (T lymphocyte cell line) and THP-1 cells (monocyte cell line).

Proliferation and apoptosis of Jurkat and THP-1 cells after exposure to argon plasma from the plasma jet (kINPen 09) was assessed by Alamar Blue and flow cytometry measurements. In contrast to THP-1 cells, Jurkat cells displayed growth retardation at comparable doses. Furthermore, percentage of Annexin V positive and Caspase 3 positive cells increased in a dose dependent manner although THP-1 cells were less sensitive to apoptosis induction. In addition, a phosphorylation assay (Luminex Technology) showed that argon plasma treatment activated apoptotic signaling proteins like p38-MAPK (p38 mitogen-activated protein kinases) and JNK (c-Jun N-terminal kinases) in both cell types, whereas higher signals were detected in Jurkat cells at comparable plasma doses. Moreover, plasma exposed Jurkat cells showed a slight phosphorylation of the proliferative signaling molecules ERK1 (extracellular signal-regulated kinases) and MEK1 (MAPK/ERK kinase1). Interestingly, the heat shock protein 27 (Hsp27), which is known to inhibit apoptosis, was additionally activated in plasma treated THP-1 cells, indicating a possible mechanism how THP-1 cells may escape from programmed cell death. More detailed analysis of cell signaling cascades and associated gene activities of both cell types are planned for the future.

Dependent on treatment time and investigated cells, non-thermal atmospheric pressure argon plasma has stimulating or apoptotic effects on immune cells.

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Study of protein aggregation and enzymatic activity after exposure to dielectric barrier plasma jet in helium

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Molecular mechanisms of action in living organisms exposed to the direct action of plasmas are not clearly understood. Efforts have been made to understand the effects of plasma on DNA molecules, leading to advanced knowledge on plasma induced DNA damage [1,2]. However structural effects of plasma on other biological macromolecules as well very important for living organisms, i.e. proteins, are not reported in the literature, excepting proteins destruction by high power plasmas at low or atmospheric pressure [3]. For low power, non thermal plasma sources, operating at atmospheric pressure it is interesting to study the possible structural effects on proteins architecture. This can affect the functional properties of proteins, making this study a subject of interest for plasma medicine.
We report here results on protein structure and function after exposure to a dielectric barrier plasma jet in helium. As model proteins we used the bovine serum albumin (BSA) and pepsin. The plasma is generated in helium using the principle of dielectric barrier discharge. The protein powder was exposed 1 min to the action of plasma jet, in a microtiter plate. Various parameters of plasma (e.g. amplitude and width of driving voltage pulse, frequency) can be modified in order to understand the relationship between plasma properties and the structural effects on proteins. Spectroscopic studies were carried out on plasma modified proteins to probe structural modifications during plasma actions and to study theirs functional properties.
From all used methods for protein structure determination, we obtained the same result: a fraction of protein powder is destroyed during plasma exposure, a fraction remains unaltered and third fraction suffers structural modifications. In the case of plasma modified pepsin, we have found differences in the thermal denaturation temperature in comparison with native molecules. This is related to possible unfolding events induced during plasma exposure. This was verified also by extrinsic fluorescence spectroscopy studies using 1,8-ANS marker for hydrophobic proteins domains.
Plasma modified protein aggregation and adsorption on standard polymer surfaces was investigated using Rayleigh scattering in UV range and quartz crystal microbalance (QCM). Differences were found for the modified proteins aggregation kinetics as function of plsma treatment conditions. QCM adsorption of proteins on polystyrene coated electrodes showed that plasma treated proteins injection gives a lower vibration frequency than native proteins, corresponding to a lower adsorbed mass. Enzymatic activity of pepsin tested with BSA as substrate, shows a lower capacity of plasma treated enzyme to digest its substrate.
Acknowledgments: this work was supported by CNCSIS-UEFISCSU, project number PN II-RU PD 297/2010-2012.

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Ex vivo survival of human lymphocytes after plasma treatment

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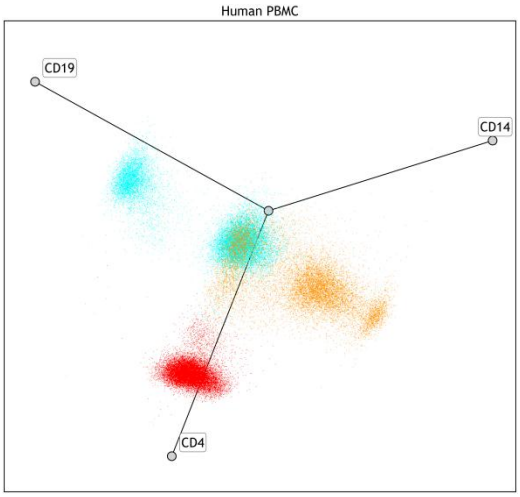
Non-thermal plasma research is becoming an increasingly recognized field in medicine and biology. As different plasma-sources approach final development stages worldwide, e.g. for dermal or dental applications [1], there is still a need for basic plasma-cell interaction studies. While some cell types as fibroblasts or keratinocytes have been under investigation for years [2], plasma-reaction of different immune cell populations have received only little attention until now. These cells are widely distributed throughout various body tissues and are likely to be influenced by plasma during treatment of skin diseases, non-healing wounds, during tooth implantation or even plasma coagulation in surgery [3, 4].

In this work, T and B lymphocytes were gathered from blood donations and treated *ex vivo* with a non-thermal plasma argon jet (kinpen 09®) either directly (treatment of cell suspension) or indirectly (cells were incubated in plasma treated medium). Cell viability was investigated thereafter via a resazurin-based plate reader assay and flow cytometric analysis of annexin V binding. Furthermore, the proliferative potential of lymphocytes before or after plasma application was assessed using ^{3}H-thymidine or CFSE assay. IC_{50} values were calculated for the plasma-setup used and populations were compared with each other.

Figure 1: *Flow cytometric characterization of isolated human PBMC.*



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Modulation of B10R mouse macrophage signalling pathways using non-thermal atmospheric pressure plasma treatments

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In recent years, there has been growing interest in developing non-thermal atmospheric pressure plasma (NAPP) devices for biomedical applications. Studies investigating interactions between cells and NAPPs have uncovered many potential applications, including sterilization of living tissues, apoptosis of cancer cells, blood coagulation and wound healing[1]. The latter was proposed given the capacity of NAPP devices to produce nitric oxide (NO). In the process of wound healing, NO is abundantly produced by inflammatory cells, especially macrophages, during inflammation[2]. In this stage, the main role of NO is as a cytotoxic agent as part of the non-specific immune response against pathogens such as bacteria, virus, parasites and fungi. Furthermore, NO also acts as a signalling molecule which mediates important events during wound healing, including cell proliferation, collagen formation and gene expression[3]. In order to further understand the effects of NAPP during wound healing, the interactions between cells present in the wound system and NO-producing NAPP must be explored.

In this study, the effect of a NAPP device on B10R mouse macrophage cells is investigated. The NAPP device produces a miniature atmospheric pressure plasma jet, with helium as the main plasma gas, driven by a pulsed radio-frequency power supply. Reactive species generation in the plasma is characterized using optical emission spectroscopy, demonstrating effective production of NO, hydroxyl radicals, atomic oxygen and other excited species. Effective transport of NO from the jet to the cells is demonstrated, and the post-treatment viability of cells following treatment is assessed. The treatment is shown to induce endogenous NO generation by the cells. Signalling-protein activation by phosphorylation following the plasma treatment is also shown to occur. Proteins tested for activation include protein kinase C isoforms, tyrosine kinases and p38 mitogen-activated protein kinases.

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Common versus noble- *Bacillus subtilis* differentially responds to air and argon gas plasma

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The study of low temperature gas plasmas is not only a physicist´s specific topic anymore. Especially since low temperature plasma is not only applied for decontamination and sterilization but also in the medical field in terms of wound and skin treatment.
In an initial study, the interaction between growing *Bacillus subtilis* and argon plasma was already investigated by using a growth chamber system suitable for low temperature gas plasma treatment of bacteria in liquid medium [1]. The gained results of this initial study are the basis of this now presented follow up investigation. Here, a second kind of plasma treatment- namely air plasma was applied [2]. With combined proteomic and transcriptomic analyses we are now able to investigate the plasma specific stress response of *B. subtilis* cells toward not only argon but also air plasma.
Besides an overlap of cellular responses due to both- argon and air plasma treatment (DNA damage and oxidative stress), a variety of gas dependent cellular responses such as growth retardation and morphological changes were observed. Only argon plasma treatments lead to a phosphate starvation response whereas air plasma induced the tryptophan operon implying damage by photo oxidation. Biological findings such as oxidative stress responses were supported by the detection of reactive plasma species by OES and FTIR measurements.

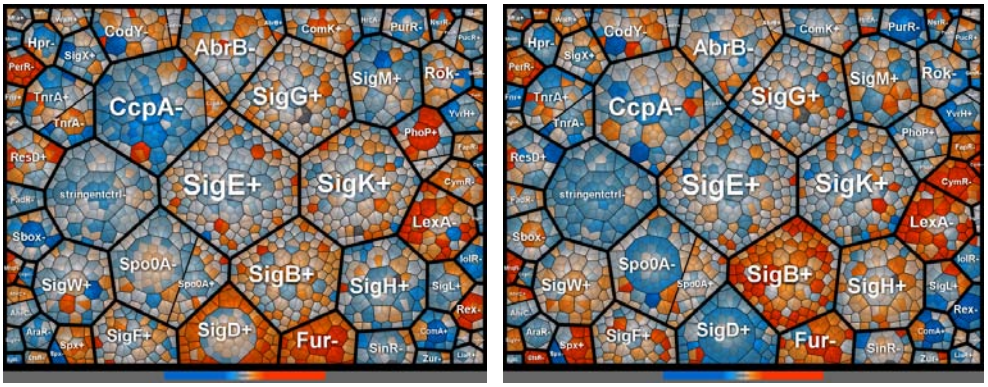

Figure 1: *(left) Gene expression of argon plasma and (right) air plasma treated B. subtilis compared with untreated cells. Treemap design is based on hierarchically structured regulatory data (black borders: regulon/thin black borders within the regulons: operon/smallest cells: gene). To visualize differences in expression level compared with the average, level colour coding was applied as following: blue—decreased level, yellow—same level as average, orange—increased level.*

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HIV-1 infected macrophages under cold atmospheric plasma jet treatment.

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It was recently shown cold atmospheric plasma jets can be widely used for decontamination and sterilization, and appeared to be quite effective in virus deactivation. In this work we report on the interaction of the cold atmospheric plasma (CAP) jet with human blood cells infected with HIV-1 for therapeutic applications in treatment of HIV patients.

The studies were carried out on normal and infected macrophages, isolated from human blood. Macrophages, cells of innate immune system, constantly circulating for tissue surveillance, express the receptor CD4 and CCR5 and are targets for HIV-1 virus. HIV-1 ADA (macrophage tropic virus) and HIV-1 LAI (T-cell tropic virus) pseudotyped with VSV-G envelope were used for macrophage infection. We used CAP jet with helium flow only and with addition of oxygen (~2%) in the case of cells/virus treatment. The impact of CAP jet on cells was evaluated through viability studies using MTT assays; the reverse transcriptase was used to estimate the rates of infection in the cells.

It was found that CAP does not affect the viability of the human macrophages. However, CAP can reduce the rates of the HIV-1 infection in the infected macrophages. Thus, the CAP jet can have a potential application for anti-HIV therapeutic approaches.

In vivo treatment of cells with plasmas in liquids

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Recently the application of plasmas for medical purposes has become a reality. Most of the plasmas currently used are created in flowing gas, usually helium or argon, at atmospheric pressure. Here however the discharge is created in an isotonic saline solution and response of MDAMB-231, a human breast cancer cell line, to plasma production within that solution is investigated. The results are compared to the effects of X-ray irradiation on the cells.

Plasma discharges operated in liquid environment are usually operated in non-conducting liquids with applied voltages of over several kV. Here the use of conductive liquid means that applied voltages of a few 100 V is sufficient and the plasma is created in a relatively welldefined vapourised region. Devices of this type are now being widely used as electrosurgical scalpels. Here however we use an asymmetric coaxial electrode assembly with small powered electrode with surface area of about 1mm2, driven at 325 V with a duty cycle of about 1 to 1000 and pulsed at ~1 Hz [2,3] to study the response of MDAMB-231 cells, to plasma production within DMEM growth media with added FBS and glucose, in which the cells are held for another hour post treatment before the medium is replaced.
We find evidence of both, decreased cell viability and DNA damage within the cells. Measurements indicate that the behaviour of both the cell survival rate and the strand breakages as function of the time of exposure to plasma follow the same functional relationship as that when they are exposed to low doses of 160 kVp X-rays.

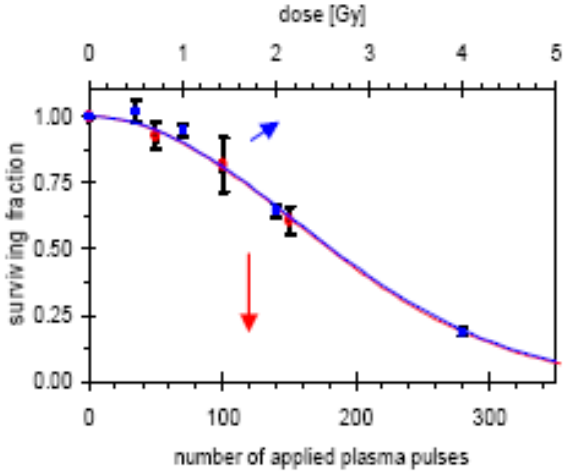

Figure 1. The surviving cell fraction following low dose plasma and X-ray exposure,

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Cell Proliferation Enhanced by Atmospheric-Pressure Plasma Application for Cells of Interest in Orthopedics

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It has been known that chemically reactive species generated by atmospheric-pressure plasmas (APPs) can enhance cell proliferation [1,2]. In this study, we have examined effects APP application on growth of mesenchymal stem cells and other cells that are of interest in orthopedics, using low-temperature low-frequency APP jets with He [2]. The cells examined here are rat bone marrow cells (Rat-BMC), rat adipose derived stem cells (Rat-ADSC), mouse Schwann cells, mouse osteoblastic cell line (MC3T3-E1), mouse embryonic mesenchymal cell line (C3H-10T1/2), mouse myoblast cell line (C2C12), mouse embryonic fibroblast cell line (NIH 3T3), human synoviocytes (HS) derived from a synovial membrane, and human osteosarcoma cells (HOS). In the experiments, three conditions were tested. In the 1st condition, low-temperature APPs were directly injected into a culture medium [Dulbecco's Modified Eagle Medium (DMEM) with fetal bovine serum (FBS)] containing cells and the cells were cultures in the same medium for a few days. In the 2nd condition, immediately after the medium containing cells was exposed to plasmas, the plasma-exposed medium was discarded and replaced with a fresh medium of the same kind and the cells were cultured for a few days in the new medium. In the 3rd conditions, plasma jets were injected into the same medium without cells, and then the cells were cultured in the plasma treated medium for a few days. In each case, cell proliferation (or cell death in the case of overexposure of the plasmas) was observed, which indicates that the presence of either chemically reactive species dissolved in the medium or solutes modified by such chemically reactive species affects cell viability. The level of free radical generation in the medium was examined by dROMs tests [3] and correlation between cell proliferation and oxidative stress were observed.

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Atmospheric-pressure plasma application on intra-cellular biochemistry

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Recently, atmospheric-pressure plasma (APP) becomes one of the most promising biomedical tools. APP shows a remarkable performance in various applications from sterilization to tissue recovery or surgery. The APP bio-medical application, called bio-plasma works through the cell membranes and causes various effects on cell organs. In this presentation, non-thermal plasma effects on intra-cellular molecules were investigated. Electrons, ions, and radicals were generated by radio frequency power, and the particles were inserted into the cells by electroporation. The plasma effects in the cells are presented by changing the plasma characteristics and the cell species.

Plasma Treatment of Skin Lipids

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In our current studies we analyze the influence of dielectric barrier discharge plasma at atmospheric pressure on the skin barrier, in particular the lipids of the stratum corneum, the outermost layer of the epidermis. The lipids of the stratum corneum are very important for the skin barrier function. In patients with ichthyosis or atopic dermatitis, the composition of the lipids is changed and leads to a modified and itchy skin. As the lipid layer is the uppermost layer of the skin it interacts primarily with the plasma.

The study tends to investigate how far the composition of the lipids can be influenced by plasma and whether a positive effect may be achieved in the treatment of ichthyotic or dermatitis skin.

The following diagnostic methods are used: optical emission spectroscopy (OES), X-ray photoelectron spectroscopy (XPS) and electrical measurements.

The lipid samples were obtained from the forearms of several people by a stripping test using cyanoacrylate adhesive. Subsequently the samples were analyzed with regard to the composition before and after a plasma treatment. In a further step the plasma treatment of the lipid samples was characterized in terms of power, homogeneity and temperature.

Before the plasma treatment was accomplished, the composition of skin lipids does not differ in males and females nor in subjects of different ages. After plasma treatment, however, the stoichiometric lipid composition was significantly affected. The total amount of carbon was reduced whereas oxygen as well as nitrogen increased. These alterations could be attributed to changed chemical bonds as we found a reduction in C-C bonds and an increase in C-O, C=O, C-N, and N-C-O bonds.

Differential Apoptosis Effects of DBD Plasma on Normal and Cancer Cells

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The non-thermal plasma has attracted medical researchers, since they showed higher apoptosis and DNA damageratein cancer cells and normal cells but molecular mechanism is unclear [1][2]. Recent progress in cold plasma jet has selectively eliminates cancer cells without damaging normal cells [3]. Therefore, this research proposes a comparison of dielectric barrier discharge (DBD) plasma effect on three kinds of normal cells lines and cancer cells lines, respectively. We measured the cell number, the mitochondrial activity (MTS assay), the amount of hydrogen peroxide (H_2O_2) and the mRNA expression level of apoptosis-related genes including p53, H2AX, caspase8, ataxia telangiectasia-mutated (ATM). The results show that the cell number, mitochondrial activities and amounts of H_2O_2 of cancer cells decreased more than normal cells after the plasma exposure except MCF7. In case of apoptosis-related genes, ATM and caspase8 were highly expressed in all cells, but p53 and H2AX were reduced or increased according to the cell types. The MRC5 and MCF7 are found to show lowered expression level of p53 and H2AX, which demonstrated almost similar growth rate, mitochondrial activity and H_2O_2 quantity. In addition, we found that DBD plasma exposure on cell suspension in media and media only have illustrated no difference in mitochondria activity, H_2O_2 quantity, and cell number. Thus, we can confirm that the DBD plasma generally induces higher apoptosis in cancer cells. The related molecular mechanisms such as NADPH oxidase will be investigated further.

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Air DBD plasma selective effects on different cell lines

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Cold plasmas at low/atmospheric pressure are widely utilized since almost 50 years to modify the surface of biomaterials with the aim of driving the interactions of proteins, cells, and biological tissues with materials in devices used in the biomedical field.
Furthermore, in the last ten years the use of plasma processes to directly treat living tissues was successful in different therapeutic field such as sterilization and decontamination of wounds, wound healing, blood coagulation and treatment of cancer[1,2].Such interactions can involve lethal or positive effects on living cell. In order to understand the mechanisms underlying the different interaction between plasma and eukaryotic cells, *in vitro* experiments with cell lines represent a very powerful tool.
In this work the selective effects of different doses of DBD (Dieletric Barrier Discharge) air plasma on different cell lines, an immortal one, SAOS-2 osteoblastoma, and a primary one, NHDF fibroblasts, was investigated.
In the home made plasma source discharges were operated in air and in pulsed mode, with cells positioned on the bottom of a Petri dish which works as dieletric of the ground DBD electrode.
Process with different number of pulse, respectively 1,3,9 and 27 pulse it was performed.
At 24h and 72h, after plasma exposure, cell proliferation and cell morphology on the different Petri dish was compared with that of control (untreated) cells by means of biological tests.
Atmospheric plasma discharges applied on the two selected cell lines have shown an effect strongly dependent on cell type. We observed a stimulating plasma effect for NHDF cells at low number of pulses which probably means that low doses of plasma generated species, e.g. oxygen and nitrogen reactive species, may induce positive effects in growth, proliferation and behaviour on this particular cell line. On the other side, an inhibition of cell adhesion and growth on the Saos 2 osteoblastoma cell line, directly dependent on the plasma doses, was clear.
Moreover, a gene expression study on SAOS-2 cells has shown an over expression of the heat shock proteins gene HSP 70 A when cells were exposed to a high dose of plasma.
The obtained results demonstrate that by properly tuning the dose of exposure of cells to air plasma it could be possible to stimulate in different cell types selective effects on cell growth, that would in turn be useful in several branches of Medicine as treatment of cancer and tissue regeneration.

Acknowledgements :S. Cosmai and D. Benedetti are gratefully acknowledged for their technical assistance in lab.

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Cold Plasma Selectivity and Application for Cancer Therapy

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Plasma is an ionized gas that is typically generated in high-temperature laboratory conditions. Recent progress in atmospheric plasmas led to the creation of cold plasmas with ion temperature close to room temperature. We have demonstrated the efficacy of cold plasma in a pre-clinical model of various cancer types (long, bladder, and skin) [1]. Both *in-vitro* and *invivo* studies revealed that cold plasmas selectively kill cancer cells. We showed that: (a) cold plasma application selectively eradicates cancer cells in vitro without damaging normal cells. For instance a strong selective effect was observed; the resulting 60–70% of SW900 cancer cells were detached from the plate in the zone treated with plasma, whereas no detachment was observed in the treated zone for the normal NHBE cells under the same treatment conditions. (b) Significantly reduced tumor size *in vivo*. Cold plasma treatment led to tumor ablation with neighbouring tumors unaffected. These experiments were performed on more than 10 mice with the same outcome. We found that tumors of about 5mm in diameter were ablated after 2 min of single time plasma treatment. The two best known cold plasma effects, plasma-induced apoptosis and the decrease of cell migration velocity can have important implications in cancer treatment by localizing the affected area of the tissue and by decreasing metastasic development. In addition, cold plasma treatment has affected the cell cycle of cancer cells. In particular, cold plasma induces a 2-fold increase in cells at the G2/Mcheckpoint in both papilloma and carcinoma cells at ~24 hours after treatment, while normal epithelial cells (WTK) did not show significant differences. It was shown that reactive oxygen species metabolism and oxidative stress responsive genes are deregulated. We investigated the production of reactive oxygen species (ROS) with cold plasma treatment as a potential mechanism for the tumor ablation observed.

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Determination of the effect of an Argon plasma coagulation (APC) discharge application on biological tissue

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Argon plasma coagulation (APC) is a surgical technology to treat biological tissue with an atmospheric-pressure discharge.
The degree and the size of tissue damage caused by APC depends on the power setting of the high frequency generator, the application time, and the distance between the APC probe and treated tissue [1].
Despite broad area of possible applications, the APC discharge is now not classified and characterized. The mechanism of tissue damage is not established.
To characterize the APC discharge, optical emission spectroscopy (OES), current-voltage measurements, and microphotography are applied. Microscopy is used to characterize the tissue damages by an application of APC discharge on porcine kidney at room temperature.
The APC discharge is ignited by application of high frequency generator (ERBE VIO 300 D, ERBE Elektromedizin GmbH, Tübingen) and the APC 2 unit (ERBE Elektromedizin GmbH, Tübingen). A flexible APC probe with an outer diameter of 2.3 mm, an inner diameter of 1.5 mm, and a length of 2.2 m (ERBE Elektromedizin GmbH) is used.
The distance between the APC probe and the tissue surface is 2 mm.
A combination of OES, current-voltage measurements, and microphotography show that a spark discharge is ignited in the positive phase of the applied high voltage. This transforms in a glow discharge in the negative voltage phase.
Microphotographic study demonstrates that the spark discharge, which produces thin plasma channel, is able to evoke carbonization of the tissue because of its high current density on the surface of the tissue.
To form a contrast, the glow discharge provides “soft” treatment and is able to coagulate the tissue surface with diffuse plasma.
The degree of tissue damages caused by APC operated in “spark” and “glow” modes is very different.
The observed plasma effects might provide new possibilities for future thermal tissue treatment in medicine.

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In Vivo Skin Test by Using a Portable Cold Atmospheric Plasma Device

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Cold atmospheric plasma (CAP) is regarded as a promising new technology for medical and hygiene applications [1][2]. Although worldwide groups have published extensive *ex vivo* and *in vitro* test results to address the biomedical efficacy of CAPs for different microorganisms, industrialization of this technology suffers extremely from the lack of *in vivo* data. In this contribution, we will present our recent *in vivo* test result on human skin by using a portable CAP device.

The portable device integrates a power supply unit and a plasma electrode into a cylindrical tube which has an outside diameter about 4 cm and a length about 15 cm. Plasma is produced by the surface micro-discharge principle and the high voltage pulsed signal for plasma generation is converted from DC output of rechargeable batteries by using a transformer and the supporting electronics.

The plasma treatments on the human skin were applied in two different ways: direct and indirect. For the direct treatment, the target skin touched the plasma electrode so that interaction between the skin and the plasma electrode may play an important role for alternating the plasma generation. For the indirect treatment, the skin was kept with approximately 1 cm away from the plasma electrode, therefore it did not affect the plasma production and reactive plasma species reached the skin mainly by diffusion. The bacterial flora of the human skin was sampled by the scrub-wash method using Teflon rings with inside diameter of 1.9 cm and Teflon sticks [3].

The result showed approximately 90% reduction of the bacterial load within 30 seconds plasma by the way of the direct treatment. The indirect treatment showed, however, lower reduction rate. The result is helpful to understand the plasma-skin interactions and can give advice to possible clinical studies of wound healing [3] and skin diseases [4] by using this portable CAP device.

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Effects of combined plasma jet and gemcitabine treatments on tumor proliferation of a murine orthotopic pancreatic carcinoma model

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Cancer of the exocrine pancreas is rarely curable and has an overall survival rate of less than 4%. While it is relatively rare for the time being, pancreatic cancer is also one of the most formidable and its incidence appears to increase significantly with number of cases of diabetes. Chemotherapy and radiotherapy treatments showed limited efficacy, development of new therapeutic strategies is then necessary.
Recent results were obtained on the treatment of glioblastoma [1] and colon carcinoma [2] using non thermal plasma (NTP). They led us to assess the antitumoral effect of NTP alone or in combination with gemcitabine a reference chemotherapeutic agent with radiosensitizing properties, on pancreatic cancer.
Experiments were carried out using the Plasma Gun developed in GREMI both *in vitro* on MIA PaCa2-luc cell lines (pancreatic cancer cells) and *in vivo* on orthotopically grafted tumor cells to induce a pancreatic carcinoma model in immunodeficient mice.
Plasma Gun showed an *in vitro* significant antitumor activity with an IC50 corresponding to 13s exposure duration. *In vivo* experiments were carried out using four mouse groups: one control group, one group treated only with gemcitabine (200 mg/kg), one group treated only using the Plasma Gun, and one group treated using a combination of gemcitabine (200 mg/kg) and Plasma Gun.
Our data showed a significant inhibition of tumor growth in NTP and/or gemcitabine treated mice, this from the 20th day post treatment. We demonstrated that plasma gun induced an inhibition of MIA PaCa2-luc cell proliferation *in vitro* and *in vivo* and that this effect is enhanced when combined with gemcitabine, a radiosensitive agent, this later being reported for the first time *in vivo*. Given these results, the possibility to use NTP in combination with a chemotherapeutical agent to increase its effects seems of very high interest for further developments in oncology involving cold plasmas, eventually delivered through an endoscopic approach. There is also a need for optimization of the sequence of chemotherapeutic agent administration and NTP exposition. This will be done in a forthcoming study.

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Antitumor activity of DBD and plasma Gun on colorectal tumors

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Local treatments of tumors are mainly based on surgical resection and/or treatment such as photodynamic therapy (PDT) or ionizing radiation (IR). Action mechanisms of IR and PDT are based on the generation of ROS in the vicinity of the cells. In this context, the use of Non Thermal Plasma (NTP) which can generate *in situ* ROS is currently under investigations. Different sources of plasma are available and allow different treatments. Plasma jet treatment allows a local treatment that is compatible with usual endoscopes for dysplasia or non resecable tumors while DBD allow a treatment of large tumor during surgical procedure. Previous studies have shown an *in vitro* antitumor activity of NTP on various cells lines and *in vivo* on subcutaneous xenografts. The aim of this work was to assess the antitumor potential non thermal plasma when applied directly on *in situ* orthotopic human colorectal carcinoma xenografts. For *in vivo* experiments, HCT116-Luc cells were xenografted in the caecum wall of nude mice and tumors were treated by a single NTP exposure 7 days post-induction (Plasma Gun or DBD – 10 min 2000Hz). Tumor growth was monitored by bioluminescence imaging once a week.

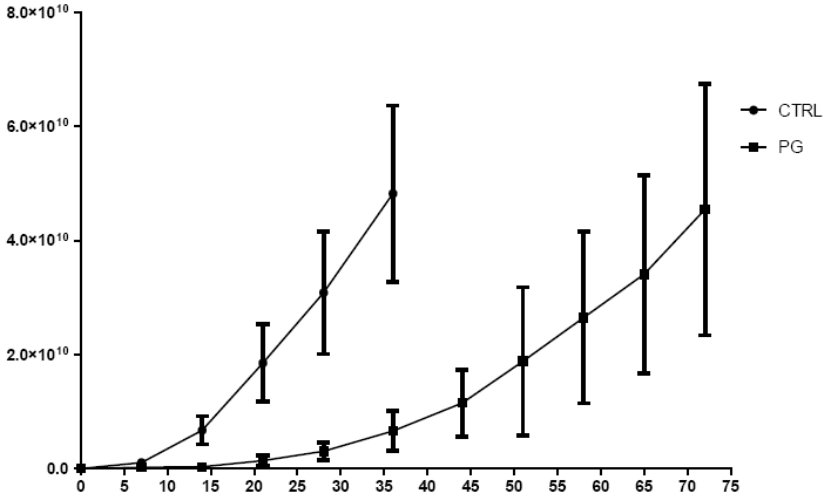

Figure 1: *Effect of plasma gun treatment on tumor growth in vivo*

A significant antitumor activity of plasma gun and DBD was previously showed on HCT-116 cell lines *in vitro* with a massive cell death induction. *In vivo*, localized treatments of tumor using plasma gun induced significant tumor stabilization of colorectal tumors with a BLI difference of 90% between treated and CTRL tumors at D22. This effect was confirmed by tumor weight measurements. Moreover, a significant metastasis decrease was observed. Very similar results were observed on this cell line using a DBD source. These results showed an increase of mice lifespan of 115% (figure 1). These results obtained using two different plasma sources showed the potential antitumor activity of NTP on orthotopic implanted tumors which are the most representative models of human neoplasms. Such results demonstrate the interest of NTP for treatment of dysplasia and non-resecable tumors.

An Atmospheric Pressure Plasma Brush

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Plasma jet devices generate plasmas in an open space (surrounding air) rather than in lieu of confined discharge gaps. Hence, they can be used for direct treatment and there is also no limitation on the size of the object. However, to the best of our knowledge, the dimensions of plasma jet nozzles that have been reported in the literature are mainly very small (sub-millimeter to several millimeters) making treatment of a large area difficult, only few large plasma jets are developed. One way to overcome this shortcoming is the use of plasma jet arrays. However, since individual plasma plumes generated by these plasma jet arrays are independent and not merged, it is relatively difficult to achieve uniform treatment effects.
In this paper, an atmospheric pressure room temperature plasma brush which can deliver uniform surface treatment effects is reported. The plasma structure which includes the negative glow, Faraday dark space, and positive column is clearly visible to the naked eyes. The width of the Faraday dark space diminishes with decreasing gap distance and this phenomenon is different from that observed from low pressure glow discharge plasmas. High-speed photographs taken at an exposure time of 2.5 ns show that the plasma propagates from the nozzle to the object in about 100 ns and 10 ns for gap distances of 6 mm and 2 mm, respectively and the results are consistent with electric measurements. The emission spectra reveal N2(B-A) bands in addition to those of O, N2+, N2 (C-B), and He, indicating that the plasma source is reactive and suitable for applications such as surface modification and materials processing.

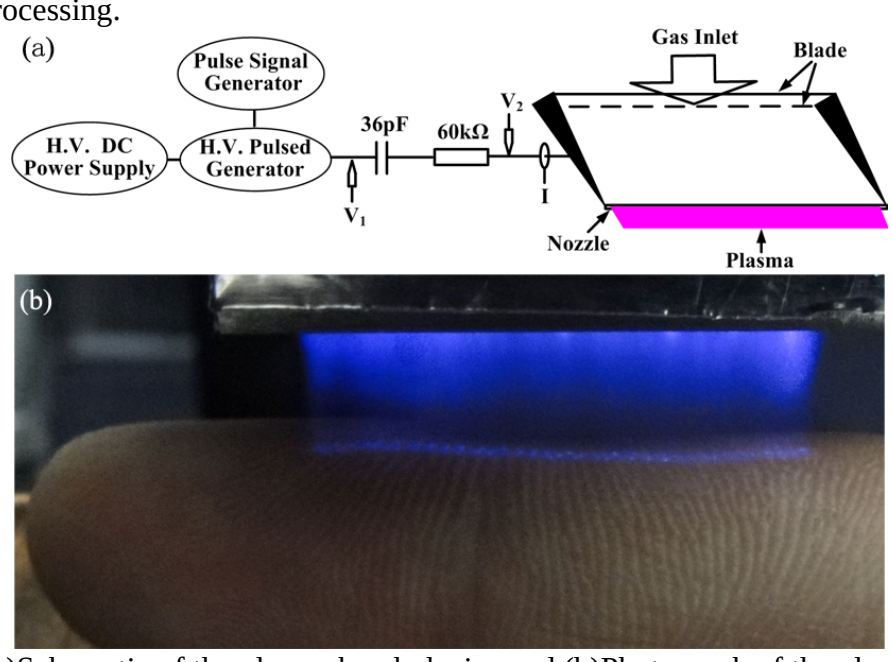

Fig. 1. (a)Schematic of the plasma brush device and (b)Photograph of the plasma brush

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DBD Cell Designs for Efficient UV/VUV Production suitable to Biomedical Treatment and Sterilization

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Dielectric barrier discharges (DBD) are the non-equilibrium glow discharge plasmas operated at/near atmospheric pressure [1-3]. The atmospheric pressure discharge is restricted to operate in the glow discharge regime by discharge current limitation through dielectric charging. The wide selection of electrode configurations, gases, materials and applied waveforms etc. make it a highly scalable and flexible discharge source. In many researches, DBD sources are found highly prominent for various Industrial applications [2]. These DBD sources are capable to efficiently produce UV/VUV radiations suitable to biomedical applications, air/water purification, discharge lamps and plasma displays etc. Hence it is always required to increase the discharge efficiency of DBD plasmas that provide UV/VUV radiation efficiently [4].

Our work includes the discharge efficiency improvement through DBD cell designs comprising co-planar electrode configuration. The discharge characteristics have been tested in a plasma display panel filled with Neon + Xenon (10%) gas mixture at 450 Torr pressure [4-6]. On discharge ignition, the xenon excimer formation results in the emission of VUV radiation of the wavelength 147 nm and 172 nm. The electrode designs are aimed to provide high intensity electric fields through optimization of discharge gap, cell capacitance etc. The electric field intensity has been obtained through 2-D computer simulation [5]. At discharge ignition, we have measured the light emission from discharge cells and simultaneously obtained the breakdown voltage and discharge delay time. The measured high luminous intensity, low breakdown voltage and low discharge delay time are direct signatures of high discharge efficiency and VUV production efficiency. A comparison of various electrode designs is presented. The optimized DBD cell designs with suitable gas mixtures can be successfully implemented portably as well as large scale DBD source formation for producing germicidal UV wavelength (240-280 nm) applicable to biomedical treatment and sterilization.

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Diagnostics of cold non-equilibrium atmospheric plasma jets

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Recently a great attention is attracted to the creation of the small size nonequilibrium atmospheric plasma jets and their interaction with living tissue. This facilitates the development of appropriate tools for their diagnostics. Traditional tools include photographing with fast ICCD cameras and optical emission spectroscopy. In this work we present our recent advances in development the diagnostic tools for cold non-equilibrium atmospheric plasmas.

A new method for temporally resolved measurements of absolute values of plasma density in the plasma column of small-size non-equilibrium atmospheric plasma jet utilizing Rayleigh microwave scattering was developed [1], [2]. The system utilizes irradiation of the plasma jet with the microwaves and following detection of the scattered signal using homodyne receiver. The system was calibrated using the dielectric scatterers with known physical properties. Calibrated system is able to measure absolute values of average plasma conductivity (density) and may be potentially applied for many types of atmospheric microplasmas such as for laser induced ionization of air, atmospheric inductively coupled plasma torches, rf microdischarges, and dielectric barrier discharges.

A simple method for the measurement of the electric potential of streamer associated with cold non-equilibrium atmospheric plasma jets was proposed [3]. The method utilizes external scatterer with certain DC potential applied to it, which is used in order to stop the streamer propagation. The proposed method allows to determine number of key streamer properties such as streamer head charge, electric field and conductivity/plasma density of the streamer column. Application of Rogowski coil for the measurements of the currents flowing in the streamer channel was also considered [1].

This research was supported in part by GWU Institute of Biomedical Engineering (GWIN), Princeton Plasma Physics Laboratory University Support program (sponsored by DOE) and by NIH National Center for Research Resources (NCRR). We thank Drs. Y. Raitses and A. Starikovskiy for valuable discussions.

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Measurements of streamer head potential in the cold nonequilibrium atmospheric plasmas

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The study of cold atmospheric plasmas has been significantly grown in recent years. The main reason for this extensive interest is cold nonequilibrium plasmas potential in the fields of bioengineering and medicine. The areas of possible application of cold plasmas include dentistry, drug delivery, dermatology, cosmetics, wound healing, cellular modifications, cancer treatment etc. The diagnostic tools that have been traditionally utilized for characterization of cold nonequilibrium atmospheric plasma jets include intensified charge-coupled device (ICCD) cameras, optical emission spectroscopy and electrical measurements of the discharge propertied. It was observed that streamer propagating along with gas flow is generated immediately after the breakdown. Recently a new method for temporally resolved measurements of absolute values of plasma density in the plasma column of small-size atmospheric plasma jet utilizing Rayleigh microwave scattering was proposed [1], [2].

This work presents a simple method for the characterization of streamers developing in cold atmospheric plasma jets [3]. The method is based upon stopping ("scattering") of streamer by means of external DC potential in order to determine the potential of the streamer head. The experimental evidence presented in this work does not support the model of the electrically insulated streamer head. On the contrary, it is shown that the electrode potential is transferred to the streamer head along the streamer column to which it is attached with no significant voltage drop. Based on the proposed method, we determine various streamer parameters such as head charge, electrical field in the head vicinity, average conductivity and plasma density of the streamer column.

This research was supported in part by GWU Institute of Biomedical Engineering (GWIN), Princeton Plasma Physics Laboratory University Support program (sponsored by DOE) and by NIH National Center for Research Resources (NCRR). We thank Drs. Y. Raitses and A. Starikovskiy for valuable discussions.

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Oxidizing species in late Ar-O_2-N_2 afterglow for bacterial treatment

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Afterglows are soft media which are known since the precursor works of Moreau *et al.* [1] as anti-bacterial media. Ar-N_2-O_2 afterglows were shown to be more efficient than Ar-O_2 post-discharges, mainly because of the UV emission produced by NO* emission [2]. If the role of oxygen atoms, NO molecules and photons has been carefully described, late Ar-N_2-O_2 afterglows still need to be characterized carefully. Theoretical approaches [3, 4] have been recently developed to gain understanding in reaction pathways leading to the synthesis or removal of active species. However, experimental data are to be produced to confirm the predicted behaviors.
Different tools are commonly used for the diagnostics of excited species. For ground state, metastable and radical species, one must resort to sophisticated techniques such as laser absorption, laser induced fluorescence, cavity ring down spectroscopy, Fourier transform infra-red spectroscopy, which are either poorly sensitive techniques or techniques dedicated for the study of specific species. Threshold ionization mass spectrometry technique allows us determine the nature and the densities of different active species.
In this work, we used the combination of optical emission spectroscopy and ionization threshold mass spectrometry to characterize an $Ar_{1-x}/(O_{2\ 1-y}/N_{2\ y})_x$ ($0 \leq x \leq 0.1$ and $0 \leq y \leq 1$) microwave afterglow (total flow rate: 200 sccm) from 1 to 10 mbar. It turns out that the determination of the concentration of oxygen-containing species is difficult to achieve, because of several artifact of measurements (dissociation of species on hot filament, filament oxidation, high control of impurity levels, etc.)
We could get reliable results on O, NO, N_2O, N, N_2, O_2 by mass spectrometry in a 5 mm inner diameter fused silica tube. For instance, for x=0.1 and y=0.5, an absorbed microwave power of 100 W and a flowing time in afterglow corresponding to ~5.2 ms, we determined at 5.6 mbar: [O]=1.85 × 10^{14} cm^{-3}, [NO]=1.77 × 10^{14} cm^{-3}, [N_2O]=3.04 × 10^{14} cm^{-3}, [N]=3.17 × 10^{12} cm^{-3}, [N_2]=5.72 × 10^{15} cm^{-3}, [O_2]=5.11 × 10^{15} cm^{-3} and [Ar]=1.06 × 10^{17} cm^{-3}. The gas temperature is 345 ± 27 K. By optical emission spectroscopy, we could follow the green emission of the NO_2* → NO_2 transition centered at 550 nm that immediately appears as soon as nitrogen is introduced in Ar-O_2 discharges. Finally, a first comparison with theoretical results could be made.

The authors wish to acknowledge Egide for financial support within the framework of a Balaton program.

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Spectral diagnostics of atmospheric-pressure argon plasma generated by a microwave plasma torch

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Deactivation of harmful bacteria can be performed by applying chemical or physical factors. Many types of high-pressure discharges are used for bacterial decontamination. In our work we present the results of experimental study of formation of atmospheric-pressure argon plasma stream generated by a microwave plasma torches. Plasma in the torches is produced with the use of 2.45 GHz microwave supply with a power varying from 50 to 150W. Spectral diagnostics of different plasma torches under various regimes of work was made. It was performed with the use of method of optical emission spectroscopy for elemental analysis of the plasma torch. Calibration of the spectrometer was carried out on deuterium and halogen lamps. In the experiments we obtained a high resolution rovibrational spectrum of the OH around 308 nm, Ar and N2 in the spectral range 320-850 nm. For diagnostics of intensities profile of spectral line we made a plasma torch with quartz windows, which allowed to measure them in the region of plasma generation. For argon line with the wavelength 811.5 nm, hydroxyl OH with the wavelength 308 nm and for nitrogen 420 nm we measured a profile distribution of intensity in the plasma torch in vertical section.

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Design and Performance Characteristics of a Novel Atmospheric Plasma Device for Biomedical Applications

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Due to the controllability of the physical and chemical parameters, low-temperature atmospheric plasmas find applications across an enormous range of health care procedures [1,2]. To address the most demanding challenges in medicine, it is necessary to develop plasma devices with tightly controlled physical parameters: plasma density and ions energy, intensity of irradiated light and UV, gas flow, operating gas temperature and charged species distributions. We report on designing and testing of a new device for low-temperature atmospheric plasma with adjustable plasma density providing a wide area of homogeneity of the main plasma parameters. The proposed discharge plasma source working at atmospheric pressure is basically similar to the device construction reported in [3]. However, the main distinctive feature of this device is an especial electrode placed on the outer wall of the chamber allowing us to adjust the power adsorbed by the plasma, and therefore change within certain limits the plasma temperature (30 - 55 C), ions density and the intensity of ultraviolet radiation. The performance of the proposed plasma reactor for deactivation of several types of Gram-negative and Gram-negative bacteria cultures was proven by experiments.

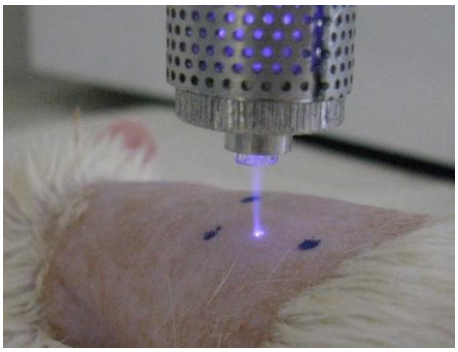

Figure 1: *A device photograph in action. The skin of living rat was exposed to plasma for 15 minutes without any signs of tissue damage or inflammation.*

For the designed plasma generator, we carried out a series of experiments exposed the healthy skin of living rats to study of the plasma effect with the same dose and exposure time as was used to inactivate bacteria. No signs of skin damage or inflammation were observed immediately as well as 2 hours after the plasma exposure (Figure 1). This work is supported by KAN200520804.

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Diagnostic and Design of Plasma Generated Reactive Species in Liquids to Investigate Cellular Effects of Plasma Treatment

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Only with the recent development of cold atmospheric pressure plasma sources plasmas are broadly studied for application in therapeutic medicine. These plasma sources generate highly reactive plasma components in ambient conditions and their gas temperature is below the destruction threshold of extremely sensitive surfaces such as biomaterials [1].
For an understanding of fundamental processes in plasma surface interaction, a control and detailed diagnostic of the reactive plasma components is vital.
In this work we present optical diagnostics on atmospheric pressure plasma jets combined with modeling yielding an understanding of fundamental processes such as air species diffusion into the jet effluent. Especially in treatment of physiological liquids in ambient air, atmospheric species play a key role in plasma liquid interaction (see Fig. 1). To gain control over the reactive components, their generation processes need to be controlled [2, 3]. The plasma jet is characterized by laser induced fluorescence spectroscopy, by absorption and emission spectroscopy and by flow simulations [4].

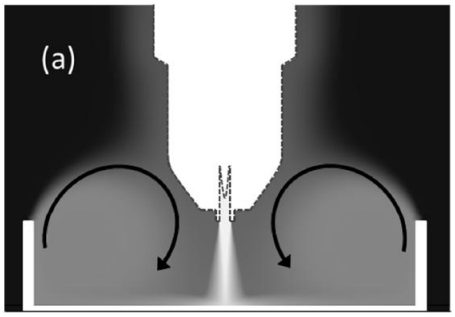

Fig 1: Flow Simulation of ambient species densities in plasma jet treatment in a petri dish [4]

With the gained knowledge, it is possible to tailor the reactive components and to influence plasma jet-liquid interaction. We show that reactive species generation within plasma treated liquid can be controlled and apply the findings to cells to investigate the effect of reactive oxygen and nitrogen species (RONS). The effects of plasma generated reactive oxygen species are compared to a combined reactive oxygen and nitrogen species composition.

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Atmospheric Pressure Plasma Jet for Non-Thermal Resistant Materials

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Temperature of plasma boundaries on surface undergoing treatment is crucial factor for classifying the device as a tissue tolerable plasma generator. Atmospheric pressure plasma jet (APPJ) is a kind of non thermal plasma operating at atmospheric pressure that can realize large area homogeneous glow discharge. Usually, high flow of substrate gas mixtures consisting of inert carrier gas such as helium and another reactive gas are used (1,2).
Group of Brisset proved that pathogens in aqueous targets can be inactivated by plasma techniques but the thermal factor was not responsible for the lethal effect on the targeted bacteria, (3).

Analysis of RF powered APPJ working parameters in dependence on gas flow rate and feedgas (air, oxygen, nitrogen, helium, argon, and their mixtures) was performed. It was possible to achieve temperatures below 40^{o}C compromising applied power and gas flow-rate. Photographs of the air plasma jet in different flow conditions are presented in table 1.

Table 1. *Photographs of the plasma jet generated in RF powered device with different flow of air. P=80 W, f=12,98 MHz.*

Gas flowrate, [m^3/h] / Temperature, [^{o}C]				
0,2 / 170	0,4 / 120	0,6 / 90	0,8 / 60	1 / 35
				

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Measurements of atomic nitrogen in an atmospheric-pressure plasma jet

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Atmospheric-pressure plasma jets (APPJ) are widely studied for multiple, novel applications in plasma medicine. To guarantee the safe and efficient use of these devices it is vital that a thorough understanding of the physics and chemistry of these plasmas is established. Reactive oxygen and nitrogen species (RONS) such as O, N, OH, NO are expected to play a crucial role in the applications of APPJs. However, so far they are only poorly understood, mainly because they are difficult to measure experimentally.

We present an experimental technique to directly measure atomic nitrogen, one of the important RONS in APPJs. Our two-photon absorption laser-induced fluorescence (TALIF) diagnostic uses 206.65 nm photons from a Nd:YAG-pumped tunable dye laser for excitation of ground-state N atoms. Fluorescence of 3 spectral lines in the range 742-746 nm is observed using a 10 nm FWHM interference filter and an intensified CCD camera. The ns-pulsed laser is sent through the plasma jet at a point 1 cm from the output of the plasma channel.

The plasma jet under study is a micro-scaled APPJ device designed for optimal access for optical diagnostics. It has been studied extensively in the past with for instance measurements of gas temperature, helium metastable, ozone, singlet delta oxygen and atomic oxygen densities. The plasma setup consists of 2 plane parallel stainless steel electrodes with quartz windows to enclose the discharge region along both sides, but allowing access with the 206.65 nm laser beam. The core plasma channel is typically 30 mm long and has a 1x1 mm cross-section. Helium gas at 1 slm with a molecular nitrogen admixture of up to a few percent are fed through the channel. The plasma is created by applying a 13.56 MHz voltage to the top electrode via an impedance matching network.

With our TALIF diagnostic we studied the influence of the nitrogen admixture concentration on the observed fluorescence intensity, and therefore the N atom density in the APPJ. In figure 1 the fluorescence intensity for different nitrogen admixtures is presented and it is clear that there is an optimum in the fluorescence intensity at about 0.2% nitrogen admixture.

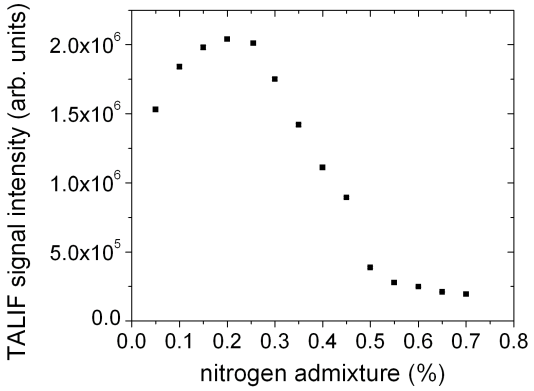

Figure 1: *TALIF signal as a function of the nitrogen concentration in the plasma jet feed gas.*

Temporal kinetics of light emission from plasma at the interface with animal tissues

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Direct exposure of tissues to atmospheric pressure plasmas is proposed in many clinical applications, in order to induce benefic effects, there were conventional medical methods failed. Examples are plasma induced wound healing, treatment of skin and other tissues, root canals decontamination, blood coagulation [1]. Taking this into account, besides determination of plasma properties, running free in air or in laboratory conditions, it is necessary to study the plasma properties at the interface with biological samples.
In figure 1 it is represented a simplified sketch of the experimental set-up used to study the plasma dynamics at the interface with biological samples. Plasma is generated in a cylindrical barrier discharge in helium. Temporal evolution, with respect to applied voltage pulse and discharge current, of total light emitted by plasma at the interface with the sample is monitored using a photomultiplier. Traces of photomultiplier voltage were stored and analyzed using statistical methods in order to confer high confidence to results. Using interference filters, dynamics of emitted light from selected species such as helium (706 nm), nitrogen molecular ion (391 nm) and oxygen (777 nm) was also studied function on parameters such as amplitude of the driving voltage pulse or its frequency. The influence of tissue type, characterized by its dielectric constant at studied frequencies, was studied using fresh samples of pork muscle, fat and skin.

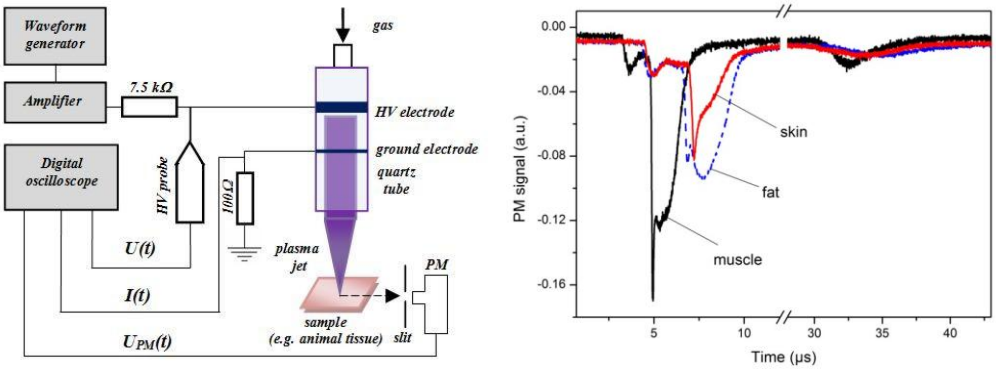

Figure 1: *Experimental set-up used for temporal dynamics studies and typical PM traces.*

The PM traces present two peaks from light pulses corresponding to primary and secondary discharges at the surface of the studied samples. Both, duration and area of these peaks, increase with the amplitude of the driving voltage pulse. Differences generated by driving frequency and tissue type are discussed for total light and selected excited plasma species.
Acknowledgments: this work was supported by CNCSIS-UEFISCSU, project number PN II-RU PD 297/2010-2012 and by grant POSDRU/88/1.5/S/47646.

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Ozone detection and production rate measurement by Mid-Infrared absorption spectroscopy in a plasma jet operating at atmospheric pressure

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It is already known that reactive species such as ozone have biological effects and have been used for sterilization of non-living objects [1]. In plasma medicine field, ozone is also investigated for healing wounds as an antibacterial agent. Therefore, we investigate the ozone production of a MHz radiofrequency plasma jet operating at atmospheric pressure in order to control the concentration of ozone in the effluent part. It is known that ozone molecules absorb infrared (IR) radiation [2]. By using very high resolution mid-infrared absorption spectroscopy in the *fingerprint* region (500-1500 cm^{-1}), we have been able to detect ozone tracks. The diagnostic is done by a tunable quantum cascade laser which generates a narrow infrared radiation. The absorption beam goes through a multipass cell in which the plasma jet is operated. Due to an additional air inlet, the inner atmosphere is adjusted to be similar to the application conditions in ambient air.

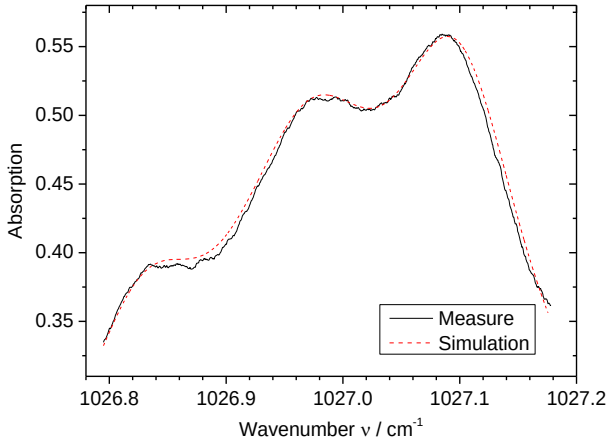

Figure 1: *Detection of ozone molecules. Absorption spectrum and simulation based on HITRAN database [2].*

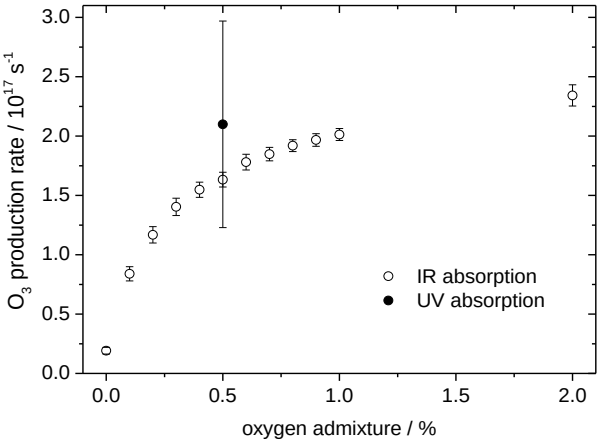

Figure 2: *Ozone production rate of the investigated plasma jet in dependence on oxygen admixture.*

Figure 1 shows an absorption spectrum compared *on-line* with a simulated spectrum yielding excellent results for the concentration measurement [3]. With the assumption that the ozone density is homogeneous within the cell and that ozone is not destroyed in the vessel, we can determine the production rate as shown on figure 2. This IR ozone measurement is compared with a UV absorption spectroscopy technique. It provides a higher accuracy of the absolute ozone concentration [3].

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Space resolved ozone detection in the effluent of a cold atmospheric pressure plasma jet

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Ozone is a biological active agent and long-living molecule. Therefore it plays an important role for decontamination processes and interaction with human cells. To know the ozone concentration is also important for risk management. The ozone concentration and production rate of an atmospheric-pressure plasma jet operated with argon and small admixtures of dry oxygen and wet argon were investigated by UV absorption measurements in the Hartley band. This technique determines high space resolved distributions of the ozone concentrations in the plasma effluent. For plasma medicine applications it is important to know how the ozone concentration in the plasma jet effluent is distributed.
To be sure that the absorption signal is only due to the ozone molecules the wavelength dependency of the line of sight optical depth τ was measured. Comparison of the results with literature shows a good agreement with the spectral absorption profile of ozone.
From the measurements of the optical depth result a high spatial resolution three dimensional map of the ozone densities, see figure 1. This study shows that the ozone density decreases rapidly with the distance in axial and radial direction from the nozzle.

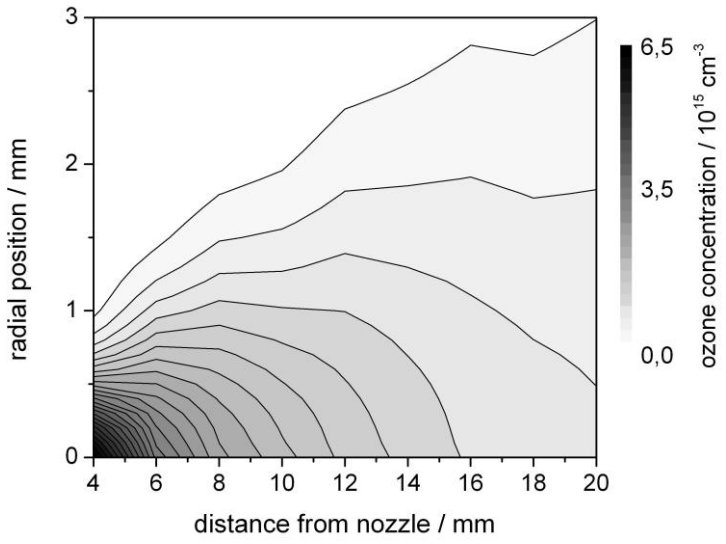

Figure 1: *Map of the space resolved ozone density distribution. The plasma jet nozzle is located at 0 mm distance from nozzle [1].*

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Plasma-Generated Reactive Species in physiological Solutions

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For plasma medicine the determination of reactive species in plasmas and in plasma-treated liquids is essential. Especially nitric oxide and free radicals play a fundamental role in mammalian systems. Here the focus is set on plasma-generated reactive species in physiological solutions such as cell culture medium, sodium chloride solution, and phosphate buffered saline (PBS). Because nitric oxide is rapidly oxidized to nitrate and/or nitrite by oxygen (eq. 1, 2), the measurement of nitrate and nitrite concentration as the end products of NO hold as an index for the integrated nitric oxide production. Nitrite and nitrate play a key role in plasma-treated liquids [1]. For this work a colorimetric assay was used for nitrate and nitrite concentrations measurements.

$$NO + O_2^- \rightarrow ONO_2^- \xrightarrow{H^+} NO_3^- + H^+ \qquad \text{(eq. 1)}$$

$$NO + O_2 \rightarrow N_2O_3^- \xrightarrow{2H_2O} NO_2^- + NO_3^- \qquad \text{(eq. 2)}$$

The detection of free radicals was performed via electron paramagnetic resonance (EPR) spectroscopy. Considering the short lifetime of radicals in solution, a chemical agent – a so-called spin trap – was added to plasma-treated liquids. Here, DMPO (5,5-Dimethyl-1-pyrroline-N-oxide) is used for measurement of $OH^{\bullet}$ and $H^{\bullet}$ radical. This spin trap forms with radicals (more or less) stable adducts which can be determined by EPR. Additionally the pH value and concentration of H_2O_2 was measured in parallel to each experiment. For these experiments two different plasma sources were used, an atmospheric pressure plasma jet (kinpen) and a pulsed discharge in liquids.
To create stable conditions for plasma treatment, the control of species, which can diffuse into the effluent of an atmospheric pressure plasma jet (humidity and air species), is necessary. A gas curtain was build and its effect on reactive species production in physiological solutions was investigated [2,3]. The gas curtain was used with varying ratios of nitrogen and oxygen as shielding gas.

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Biofilm removal from rough titanium surfaces with dental decontamination methods and/or atmospheric pressure plasma

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Peri-implantitis is a common problem in implant dentistry [1]. Biofilms located on the implant cause inflammation of the periimplant tissue and lead to bone destruction. Removal of biofilm from titanium surfaces is a precondition for a complete resolution of inflammation and re-osseointegration [2]. Atmospheric pressure argon/oxygen plasma could solve the decontamination problem as well as re-establishing surface characteristics, which are supportive for bone regeneration [3, 4].

We used microstructured hydroxylapatite coated titanium disks (grit-blasted + dual acid-etched, diameter 5 mm, Biomet 3i, USA) covered with a 30 day old ex-*vivo* plaque biofilm. Removal of biofilm was performed with cold atmospheric pressure argon/1%O_2 plasma (PL), brush (BR), CO_2-laser (LA), water spray (WA) or with a combination of BR+PL, LA+PL, WA+PL, respectively. An untreated (UN), plasma treated (UN+PL), and a biofilm covered (BIO) disk served as control. Treatment time was 120 s for a single procedure or 120 s + 60 s (PL) for combined treatment approaches. Biofilm removal was assessed with scanning electron microscopy (SEM) and x-ray photoelectron spectroscopy (XPS). The atomic percentage of elemental content of nitrogen (in at.%), obtained by XPS, served as marker of proteinaceous biofilm remnants.

As expected the highest elemental content of nitrogen was observed at the BIO control group (11.3± 0.5 at.%)). After 60 s plasma treatment, no nitrogen was detected on UN+PL surfaces, indicating complete removal of contamination. Compared to the BIO control group, a significant reduction of the nitrogen content was obtained after PL (3.8±1.2 at.%), BR+PL (1.2±0.2 at.%), LA+PL (4.1±0.9at.%), and WA+PL (2.9±3.7 at.%) treatment. Consequently, the nitrogen was reduced to a level reflective of pristine disks.

This study demonstrates the efficiency of an atmospheric pressure plasma as additional treatment option for biofilm removal. Plasma could be the first step to develop a simple, safe, and effective method to remove the biofilm without destroying the elaborate surface geometry and to promote re-osseointegration of peri-implantitis affected implants.

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Plasma needle treatment of Staphylococcus Aureus *(ATCC 25923)* biofilms

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New atmospheric pressure plasma sources opened a wide range of biomedical applications, such as sterilization of wounds and medical equipment, treatment of dental caries, faster coagulation of blood, etc. In this paper we will present results obtained in plasma treatment of formed and unformed (MRSA) biofilms. Plasma source used for these treatments was plasma needle that was previously used in treatments of planctonic samples containing bacteria [1]. Treatments were carried out on unformed biofilm for three different powers, two different flow rates of helium (0.5 and 1 slm) and several treatment times (10, 30, 60 and 120 s). The mean power was calculated and it did not exceed 2 W in all treatments (which in our experience does not heat the substrate by more than 6-7 degrees). Figure 1. shows comparison of absorbance after treated samples were allowed sufficient time to develop the fully formed biofilm. We can see that the longer exposure times and higher transmitted power to the plasma reduced biofilm production. Plasma treatment is more efficient on unformed than on formed biofilm. For presentation of results we used four categories of biofilm production: no biofilm, weak, medium and strong biofilm [2].

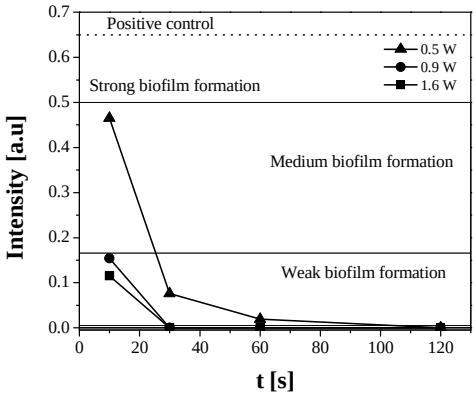

Figure 1: *Optical density apsorbances of biofilm formation after plasma treatment of the biofilm during formation for three different applied powers. The initial concentration of unformed biofilm was 10^6 CFU/ml and flow of working gas was 1 slm.*

This research has been supported by the MES Serbia, project III41011 and ON171037.

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Plasma models applied to polymer deposition and surface control for biological applications

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Thin films of polymers can be deposited using plasma enhanced chemical vapour deposition [1] and their surface chemistries greatly affect biological response. The deposition process depends on the type of precursor and gas mixture, the plasma coupling mode (capacitive or inductive), the plasma geometry (symmetric or asymmetric) and on the main operating parameters (i.e. frequency, gas pressure, gas flow, gas inlet position). The complexity of the precursors and the lack of basic reaction rates imply that a detailed understanding of the plasma bulk, plasma sheath and plasma-surface properties can not simply be derived. However a global model of these plasmas can often be developed by using argon cross sections to evaluate the dominant parameters and validate some of the experimental findings[2,3]. The latter are usually obtained using performant surface diagnostics with high spatial resolution.

Here we present some of the modeling studies found in the literature and summarize essential parameters in global models. As an example we develop a global model (in argon) for a low frequency capacitively coupled asymmetric glow discharge plasma polymerisation device used for one step multifunctional micropatterning of surfaces[4]. The plasma parameters estimated from the global model are discussed and compared to the chemical physical and topological properties of the surface. The latter result from a patterned live electrode which dominates the plasma process (here essentially a sheath process). The process produces multifunctional, selective surface chemistries capable of controlled protein adhesion, geometric confinement of cells and the spatial confinement of enzyme mediated peptide self-assembly.

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Tailoring crystalline structure in N-doped TiO2 thin films: application to photocatalytic and biological reactions

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Photocatalytic reactions at the surface of titanium dioxide (TiO2) have been attracting much attention in view of their practical use in environmental, energy and biomedical applications [1]. However, the bandgap of TiO2 corresponds to maximal wavelength absorptions in the UV. Thus, the development of photocatalysts exhibiting high absorption under visible light should allow a more efficient use of the solar spectrum. The objective of this study was to obtain TiO2 doped with nitrogen (N-doped) by plasma sputtering in a helicon reactor. Changing the impinging ion energy resulted in changes in the crystalline nanocrystals from anatase to rutile. Moreover, introduction of nitrogen also resulted in changes in the crystalline structure. Results will be presented on the photon absorbance and interaction with blood simulants.

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A multi-approach of the bacteria non-adhesion phenomenon onto hydrophobic polymeric surfaces

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The concept of synthetic surfaces superhydrophobic appeared only very recently. They are characterized by water contact angles particularly high (greater than 120°) and can reach values up to 160 - 170°, values mostly induced by two factors, chemical nature and roughness. The latter parameter could be ranged from several hundred micrometers to a few nanometers [1-2]. Despite great potential of applications, these original surfaces so called self-cleaning surfaces as non-adherent are still poorly known in the field of bioadhesion.

Biofilm formation depends primarily on the adhesion of microorganisms to surfaces, phenomenon related particularly to the characteristics of solids. Roughness and topography of the support could have a particularly strong influence on proteins and cells adhesion [3-4] and an alteration of hydrophobic / hydrophilic balance of the material could significantly change its ability of bioadhesion and as consequence modifies the biocontamination processes [5].

In order to study the dependence of bioadhesion phenomenon on these material parameters, different polymeric surfaces have been modified until the high hydrophobic character, indeed the superhydrophobicity property was obtaining. For this purpose, polypropylene and polystyrene have been treated by RF or µwaves CF_4 plasma with different volumes, the results were compared according to the density of injected power. The effect of pretreatment such as mechanical abrasion or plasma activation was also studied. The modified surfaces were shown as hydrophobic, or even superhydrophobic. They were characterized by measurement of wettability and roughness at different scales, *ie* macroscopic, mesoscopic and atomic ones. It has been shown that a homogeneous surface at the macroscopic scale could be heterogeneous at lower mesoscopic scale. This was associated with the crystallinity of the material and induces a bioadhesion of certain bacteria on this type of surface materials despite its strong hydrophobic character.

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Superiority of plasma chemistry over surface topography-characteristics of human osteoblast-like cells

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Introduction: Both, surface roughness and chemical modifications can influence the interaction of osteoblasts with titanium (Ti) surfaces [1]. We have observed that osteoblastic responses to smooth titanium can be improved by a positively charged surface [2, 3]. In this report plasmachemical treatments of topographically modified Ti and their effects on cell morphology are introduced.

Materials & Methods: Titanium disks (Ti, cp, grade 2), with different roughness: Polished (Ti-P: Ra=0.045 µm), machined (Ti-M: Ra=0.315 µm), and corundum blasted (Ti-CB: Ra=4.14 µm) were used and subsequently plasmachemically functionalized with a thin film (d≤0.1 µm) of microwave plasma polymerized allylamine (PPAAm). In addition, collagen I (Col) was immobilized on PPAAm via the bifunctional linker polyethyleneglycoldiacid (PEG DA) or glutardialdehyde (GDA). Human osteoblast-like cells MG-63 (ATCC) were plated onto the Ti specimens and cultivated in serum-free DMEM at 37°C and 5% CO_2. Cell shape was analyzed by scanning electron microscopy (SEM). The initial cell adhesion (5 min) was characterized by flow cytometry (FACSCalibur). Actin filament organization was observed microscopically (LSM).

Results & Discussion: Plasma-chemical modification with PPAAm enormously improves cell ingrowth into the structured surfaces. The morphology of osteoblasts demonstrates an extremely flattened phenotype on allylamine-modified surfaces and the cells seem to merge with the topography of the surface. Interestingly, we found that not only the cells but also the actin fibers are aligned along the grooves and ridges except for the PPAAm coated surface. Here, cell growth is not directed due to the dominance of the plasma chemistry, thus the cells and their actin fibers can overcome the grooved structure. Modification of the surfaces with PPAAm considerably improves the initial adhesion of osteoblasts. In addition, higher surface roughness enhances this adhesive effect. Cell adhesion is increased nearly 2-fold on both structured Ti surfaces (Ti-M PPAAm, Ti-CB PPAAm) compared to Ti-P PPAAm. In contrast, collagen I immobilization improves the cell adhesion only slightly in this initial phase. Altogether, it is striking that the positive charges seem to be dominant over the typical extracellular matrix protein collagen concerning initial cell adhesion. The PPAAm-layer is attested to be long term stable and sterilizable via gamma irradiation. We hypothesize that the treatment of implant surfaces with PPAAm is a promising method for improving cell-material-interaction.

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Evolution of thermal properties and secondary structure of collagen with atmospheric plasma jet treatment

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Collagen type I is the most abundant extracellular matrix protein in the animal kingdom and it is widely used as a biomaterial for tissue regeneration and implantation [1]. To improve strength and durability, collagen may be cross-linked by chemical (glutaraldehyde) or physical methods (gamma rays, UV irradiation). Chemical cross-linkers are potentially cytotoxic and lead to calcification of fibers, decreasing the durability of the bioprostheses. UV treatments have been investigated to cross-link collagen although irradiation can cause both stabilization and destabilization of the collagen structure.
In this study, we have investigated the effects of non thermal atmospheric pressure plasmas as they are not yet used for the treatment of collagen fibers, but already successfully used in various other biomedical applications. We used a low temperature plasma jet generated in ambient air [2] and producing various active species (excited species, free radicals, charged particles and photons covering a large spectrum from UV up to visible) that are in contact with type I collagen fibers under both freeze-dried and hydrated states.
To quantify the effect of the plasma treatment on the thermal properties and secondary structure of type I collagen, samples have been characterized by Differential Scanning Calorimetry (DSC) and Fourier Transform Infra-Red (FTIR) Spectroscopy.

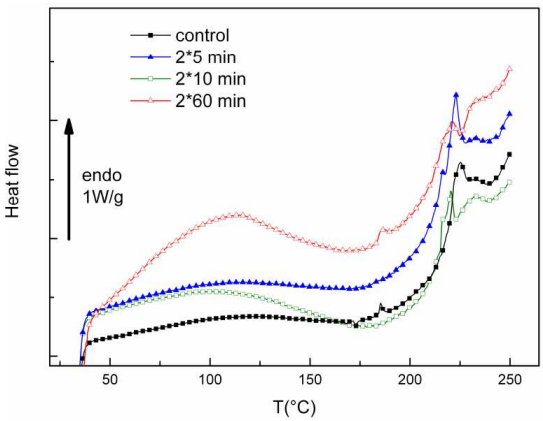

Figure 1 : *DSC first scan (20 °C/min) of freeze-dried collagens*

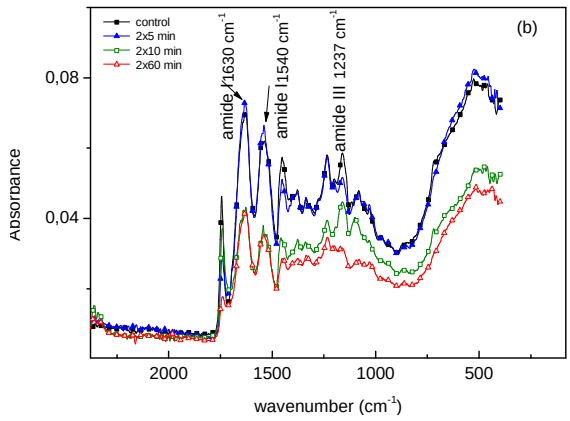

Figure 2 : *FTIR spectra of control and plasma treated collagen.*

Our studies show that the destabilization of the triple helical structure is the main event for the longest exposure times in the freeze-dried state. For hydrated samples, the cross-linking phenomenon becomes predominant for the longest exposure times. It is shown from FTIR analysis, slight modifications in absorption band synonymous of the preservation of the integrity of the triple helical structure of collagen[3].

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3D electrospun PET nano-fibrous mats with plasma-polymer coating for vascular graft applications

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In tissue engineering of vascular grafts, scaffolds that simulate the mechanical and morphological properties of the extracellular matrix beneath endothelial cells and that possess similar 3D nanofibrous structure are required. This inspired the idea to generate 3D nano-fibrous electrospun mats as substrates with similar compliance and morphology [1]. In addition, since the formation of a complete and stable endothelium is required to prevent thrombus formation inside small diameter vascular prostheses, a suitable surface treatment is needed to provide the requisite strong cell-adhesion. Here, functionalization by cold plasma appeared to be ideal [2]. The abovementioned substrates were prepared from poly(ethylene terephthalate) (PET) by electrospinning, then coated with a thin plasma polymer film prepared from mixtures of ethylene (C2H4) and ammonia (NH3). The surface chemistry, morphology, and mechanical properties of optimized mats were characterized using XPS, SEM, Mercury Intrusion Porosimetry, and tensile tests. The functionalized substrates were then seeded with human umbilical cord vascular endothelial cells (HUVEC) to evaluate *in vitro* cell-adhesion. XPS measurements confirm the presence of plasmadeposited nitrogen-(N)-rich coating on the substrates and show that N concentration decreases with increasing depth into the (ca. 90 μm-thick) mat. This reveals that plasma species penetrate deep inside the porous structure. SEM micrographs show a randomly interconnected open structure of fibers with smooth morphology, even after plasma coating. The average nanofiber diameters were ca. 521 nm and 565 nm for untreated and plasma-coated mats, respectively. The overall porosity was 87 %, ideal for the envisaged application, and it did not change significantly after coating. The tensile strain was reduced somewhat after coating, as expected, but the tensile stress was raised and coated mats appeared somewhat stiffer. *In vitro* experiments show that plasma-coated electrospun mats promote HUVEC adhesion. In summary, nanofibrous plasmacoated PET mats can provide scaffolds with suitable morphological, mechanical and biocompatible properties adapted for cell-adhesion and vascular graft applications.

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Silver nanoparticle loaded antibacterial polymer mesh using plasma polymerization process

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Some specialized applications of polymers include biomedical devices and healthcare products made of various forms of polymers. For example, patches in the form of fabric meshes made of synthetic polymeric material are being widely used as support to repair hernias in a surgical procedure. These support polymer patches are sewn over the weakened area in the abdominal wall after the hernia is pushed back into place. The support mesh decreases the tension on the weakened abdominal wall, reducing the risk of hernia recurrence [1]. Prevention of adsorption and growth of micro-organisms on polymer surfaces is prerequisite for the biomaterials to prevent the post-surgical infections. However, synthetic or natural polymers themselves do not have intrinsic antibacterial properties. One way to circumvent this drawback is to coat the polymer with silver nanoparticles which cumulate the well known antibacterial properties of silver and structural properties of nanoparticles, whose large specific surface area as compared to conventional materials allows a small concentration of silver nanoparticles dispersed to the polymeric substrate to exhibit an excellent antimicrobial efficacy [2].

This work reports on the use of plasma processing for incorporation of silver nanoparticle on polyethylene terphtalate (PET) mesh in order to achieve antibacterial property. Polyacrylic acid was polymerized on to polymer substrate by Plasma Enhanced Chemical Vapour Deposition (PECVD) process to introduce carboxylic groups, which act as the anchor for silver nanoparticles synthesized by chemical reduction method using $NaBH_4$. Plasma polymerized acrylic acid (PPAA) chains acts as a capping agent as well as stabilizing agent for the silver nanoparticles. Silver nanoparticles loaded polymer samples were characterized by UV-visible spectroscopy, field emission scanning electron microscopy (FESEM), energy dispersive x-ray (EDX) and XPS techniques, showing the presence of ~1.0 at. % of silver nanoparticles composed of 79% zero-valent (Ag^o) and 21% oxidized nano-Ag (Ag^+). The plasma processed PET meshes samples were tested for antibacterial activity against two bacterial strains, *Staphyloccocus aureus* (Gram positive bacteria) and *Escherichia coli* (Gram negative bacteria). Qualitative and quantitative tests showed that silver containing PPAA-PET meshes exhibit excellent antibacterial property against the tested bacteria with percent reduction of bacterial concentration >99.7%, compared to the untreated PET mesh.

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Late Ar-O_2 afterglow for amino acids treatment

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Ar-O_2 afterglows are very efficient media to inactivate bacteria [1]. Yet, little is known on the way active species of these media interact with basic chemical functional groups that take part in the composition of living materials. In Ar-O_2 afterglows, one finds at relatively high concentration ground state molecular oxygen $O_2(X^3\Sigma_g^-)$, vibrationally excited states of $O_2(X, v \geq 1)$, the singlet state $O_2(a^1\Delta_g)$ which is metastable and oxygen atoms. If the pressure is high enough, ozone O_3 must be included. Other species like $O_2(b^1\Sigma_g^+)$, $O(^1S)$ or $O(^1D)$ can be found but at much lower concentrations. These active species may react or not with given chemical functional groups. If we can determine the way they do, we might expect to predict how the simplest components of life, the amino acids are modified when they are treated by an Ar-O_2 afterglow. Amino acids contain various groups like C-C- and C-H simple bonds, saturated or unsaturated rings, the -COOH acid, -OH alcohol, -SH thiol functions and the -NH_2 amine group. Knowing which active species reacts with which functional group, one could predict the way amino acids would react in an Ar-O_2 afterglow. In recent works [2-4], we used model molecules (hexatriacontane $C_{36}H_{74}$, stearic acid ($C_{18}H_{36}O_2$), and biphenyl $C_{12}H_{10}$). We could draw the following conclusions:

- non linear effects occur: if the initial temperature varies from *e.g.* 333 K to 353 K, chemical modifications undergone by materials can be very different,
- O_2 is not inert and can react with radicals formed by other processes,
- consequently, O is not always responsible for material modifications,
- $O_2(a^1\Delta_g)$ does react with rings,
- chain mobility plays an important role: thick or thin films can behave very differently,
- crystallinity matters: depending on the chains orientation, etching rate may change.

These first studies will be continued with –NH_2 containing molecules before testing given amino acids and comparing predictions from the matrix data thus obtained.

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Plasma Synthesis of Superparamagnetic Iron Oxide Nanoparticles

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There is currently intense interest in the use of superparamagnetic iron oxide nanoparticles for biomedical applications, including as contrast agents for magnetic resonance imaging and as targeted agents that can be heated by applying an alternating magnetic field, killing cancer cells by hyperthermia. Conventionally these nanoparticles are synthesized by wet chemical methods. However plasmas and other gas-phase synthesis methods have a number of potential advantages over wet chemistry, including higher production rates, the avoidance of impurity residues, the avoidance of the need to manage and dispose of hazardous solvents, and the avoidance of the need to remove surfactants before adding layers that impart additional functionality to the nanoparticle.

A number of researchers, dating to the early 1980s, have reported plasma synthesis of iron oxide nanoparticles. However in none of these studies were the measured magnetic properties of the produced powder satisfactory from the viewpoint of biomedical applications. Here we report synthesis of iron oxide nanoparticles using a DC argon-helium thermal plasma with injected ferrocene vapor and oxygen. The powders produced have a mean particle size below 10 nm, and are superparamagnetic, with measured magnetic properties (saturation magnetization ~40 emu/g, coercivity ~25 Oe) that are far superior to results previously reported for any plasma process.

After exiting the plasma reactor the iron oxide nanoparticles are coated with very thin layers of silica, using photoinduced chemical vapor deposition, driven by a xenon excimer lamp that emits at 172 nm. Tetraethylorthosilicate (TEOS) vapor is used as the coating precursor. SiO_2 coatings improve the nanoparticles' stability, suppress agglomeration, and can serve as an excellent substrate for additional surface layers or biofunctionalization. Preliminary results are presented for the effect of operating parameters on coating thickness, surface chemical composition, magnetic properties, and stability of the nanoparticles in aqueous dispersion.

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Cell repulsive/cell adhesive behavior on films deposited by an Atmospheric Pressure DBD fed with TEGDME aerosol

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Atmospheric pressure Dielectric Barrier Discharges (DBD) are new, challenging technology for surface modification, combining the benefits of an atmospheric operation mode with those of cold plasma. The interest in DBDs is growing also for thin films deposition and treatments for biomaterials [1, 2]. Our research is aimed to plasma-deposit PolyEthyleneOxide (PEO)-like coatings with DBD technology, with tunable properties from cell-adhesive (low retention of PEO structure) to non fouling (high retention of the PEO structure). Depending on their chemical composition such coatings can be synthesized with unique resistance to protein adsorption and cell-adhesion in water media, or with swelling properties for drug delivery systems. It is possible to tailor the biological response from cell adhesive to cell repulsive surfaces controlling the percent of ether groups (C-O-C, PEOcharacter) in the films, as already done in low pressure plasmas [3].

An homemade DBD reactor was used [4] fed with TetraEthylGlycolDiMethylEther (TEGDME) aerosol, chosen as suitable organic precursor for the coating deposition. A constant He flow (3.15 slm; aerosol flow) was addressed to generate the aerosol with a constant output atomizer (TSI, 3076); a variable He flow was used as carrier to transport the aerosol into the discharge. The effect of the applied voltage, V_a (6.5-8.5 kV_{pp}) and of the total flow, Φ_{TOT} (8-10 slm), i.e. of the dilution of the aerosol precursor in the plasma, was evaluated on the coating composition with water contact angle, profilometry and X-rays Photoelectron Spectroscopy (XPS).

In our defined conditions, the main parameter to achieve a significant modulation of the C-OC content was the total flow, i.e. the aerosol amount introduced in the discharge area, reached by changing the flow rate of the He carrier (+/- 1 slm) at a constant aerosol flow (3.15 slm). In this case, the chemical composition of the coating was nicely tuned resulting in an excursion of the PEO-character from 50 to 70 % at 10 slm and 8 slm, respectively. Once water stability was assessed on polycarbonate substrate by a proper modulation of the chemical composition in a multi steps approach, PEO-like samples were used for cell adhesion experiments. The cells behaviour on the modified surfaces is related to the percentage of ether groups in the coating: coatings with a ether content of 70 % deposited at 8 slm displayed very good non fouling cell repulsive properties, while cell adhesive film were obtained for an ether content of 50 % at 10 slm.

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Plasma-generated Species and its Effect on Surface Chemistry and Morphology of Polymers exposed to Atmospheric Pressure Plasma - A Prospective Application for Biomedical Purposes

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Nowadays the use of non-thermal physical plasmas in the field of medicine is intensively investigated for sterilization, wound healing, and surface modification of biomedical materials. It is well known that a number of reactive species, including UV photons, positively and negatively charged ions, electrons, and radicals, emitted by plasmas can have very different impacts on the exposed substrate. Therefore, a fundamental understanding of the plasma-based mechanisms in the gas discharge as well as on the substrate surface is required for appropriate applications of plasma sources.

The influence of atmospheric pressure plasma on the chemical und morphological surface properties of bio-relevant polymers has been investigated. Besides changes in the O/C ratio of the plasma-exposed polymer, followed by the creation of oxygen-containing functionalities, significant alteration of the surface topographies, in particular roughening of the surfaces, were observed. Especially, the admixture of oxygen to the argon plasma led to an increased etching of the surface resulting in the formation of etching depths of several micrometers. Furthermore, this study analyses the correlation between the plasma-based modification on polymeric surfaces and the reactive species emitted by the gas discharge. For the plasma diagnostics optical emission spectroscopy (OES) and two-photon absorption laser-induced fluorescence (TALIF) were applied. The obtained results indicate a correlation of the etching depth and the surface roughness with the concentration of oxygen atoms. Moreover, it has been found, that the observed radial etching profile is due to the presence of plasma-generated oxygen species. Deduced from these results multiple applications of the used plasma jet are feasible. Additionally to plasma-based bio-decontamination, a promising application of this plasma device could be the removal or etching of organic substances, e.g. biofilms, from surfaces. Since, biological remnants of biofilms (dead bacteria) after conventional cleaning procedures are capable to entertain inflammatory processes in the adjacent tissues, the complete removal and not only the killing of pathogen is mandatory. Hence, this contribution further shows the efficacy of non-thermal plasma on etching of 7-day old *Candida albicans* biofilms depending on the operating gas and the treatment time.

XPS investigation of adsorption of albumin on polymer surface

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Plasma is often used to improve biocompatibility properties of various polymer implants especially in the case of cell growth. Cells are normally kept in a protein-rich solution. Therefore, interaction of proteins with a polymer surface is an important step in adhesion of cells. The influence of surface hydrophilicity/hydrophobicity on adhesion of protein albumin to polymer polystyrene (PS) was studied. The polymer surface was made hydrophilic or hydrophobic by treatment either in an oxygen plasma or in tetrafluoromethane plasma respectively. The rate of adhesion of albumin was studied by X-ray photoelectron spectroscopy (XPS) after incubation of samples in the albumin solution for different periods ranging from 1 s to 1.000 s. Measurements of the intensity of nitrogen peak that is originating from the adsorbed protein layers for samples after different incubation showed some important conclusions. Namely, the results have shown that the adhesion of protein appears already in the 1 s of incubation. The quantity of adsorbed protein was slightly higher for both plasma treated samples than for untreated one. After 100 s of incubation this difference has disappeared. The results clearly show that proteins are the first macromolecules reaching the polymers surface, because adhesion of cells appears much later. Therefore, this adsorbed protein layer may govern further adhesion of cells.

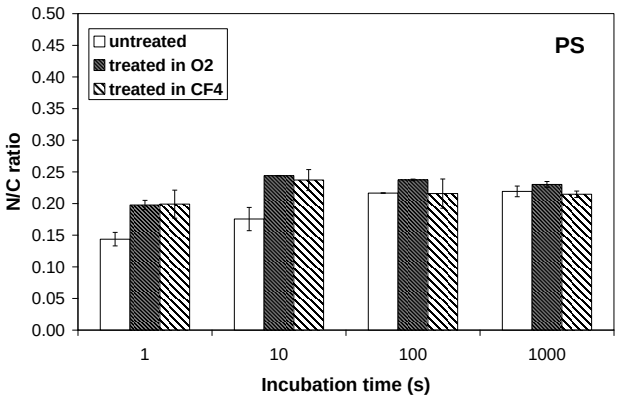

Figure 1: *Comparison of N/C ratio after incubation in albumin solution for untreated polystyrene samples and those treated in O_2 and CF_4 plasma.*

The authors acknowledges the financial support from Slovenian Research Agency through the project L7-4035 (Toward ecologically benign alternative for cleaning of delicate biomedical instruments).

Treatment of *Paulownia tomentosa* seeds in the low pressure CCP reactor

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Previous studies of the effects of non-thermal low pressure plasma on seeds as well as detailed experimental setup can be found elsewhere [1]. In order to learn how plasmas affect human tissues full elucidation of mechanisms of plasma effects on simpler living systems would be helpful. Here we present results of air plasma treatment of *Paulownia tomentosa* seeds in the cylindrical asymmetric CCP reactor at 200 mTorr. Significant improvement of germination is observed and the effect is strongly depending on the duration of plasma exposure. After the treatment XPS and SEM EDXS analysis were performed. From the SEM EDXS images we can see the porous structure of the seeds (see Figure 1).

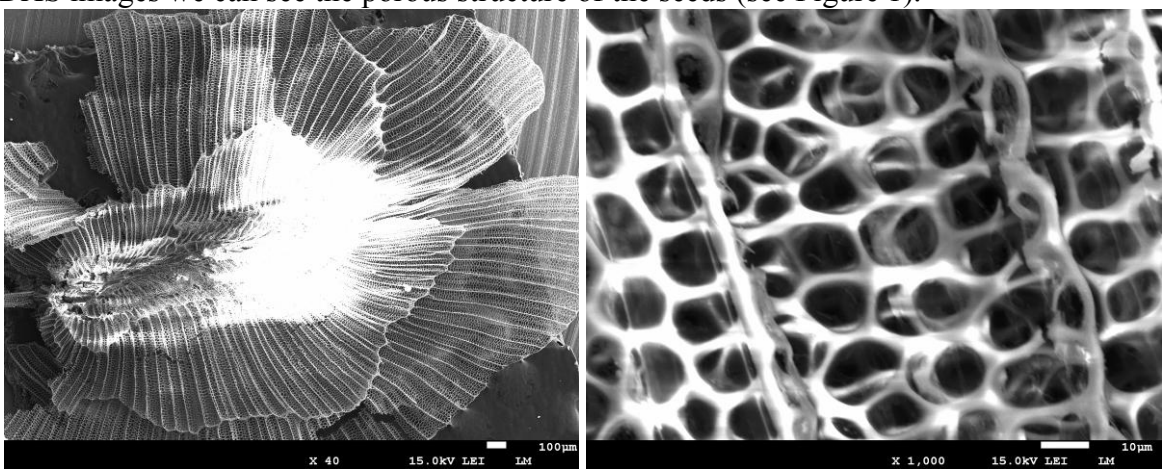

Figure 1: *SEM EDXS on Paulownia tomentosa seeds (*x*40 left,* x*1000 right, 15 kV)*

Based on the XPS results we can see that O/C ratio is increasing with the treatment time which leads to the conclusion that the air plasma is inducing the surface oxidation of the seeds. For shorter treatment times (1, 5 min) N concentration at the surface is increased, as well as, potassium concentration which cannot be observed in control samples. The effect on the germination increase can be explained by increase in N and O concentrations on the seed's surface after plasma treatment. Further optimization of the plasma effects on the seed can be achieved by adjusting the power, pressure, gas composition and the distance of the samples from the powered electrode.

This research has been supported by the MES Serbia, project III41011 and ON171037.

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Bacterial inactivation using corona discharges applied in water

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Cold plasmas, including the corona discharges, are already used to inactivate bacteria on surface or to decontaminate gases [1]. Apply these discharges on water is more difficult because the electric field intensity must be higher and prevent corona to arc transition. Nevertheless, previous preliminary studies showed the efficiency of theses corona discharges on bacterial decontamination [2] [3] [4].
Abou-Ghazala *et al.* [5] have shown that these discharges applied in water caused a greater reduction in *Escherichia coli* than *Bacillus subtilis*. Moreover, they have shown that these discharges are inefficient on spores.
The present work is a study of the effects of corona discharges applied in contaminated water with *Escherichia coli* or *Bacillus subtilis* var niger (under vegetative and spore form).
We used a Marxbank generator delivering pulses of 200 ns with a voltage of 60 to 90 kV.
Contrary to Abou-Ghazala *et al.* [5], our results show a greater reduction in *Bacillus subtilis* var niger under vegetative form than *Escherichia coli*. The mechanisms seem to be different on these two strains: a chemical mechanism in *B. subtilis* and probably a physical mechanism in *E. coli*.
This study reveal that *B. subtilis* var niger under spore form is sensitive to these discharges. Indeed a reduction of 4 $\log_{10}$ is observed after 10000 discharges (80 kV, 4 Hz, 200 ns). This reduction seems to be due, at least in part, to shock waves induced by these corona discharges in water.

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Influence of Ozone on Suspended Microorganisms caused by DBD Treatment: A Comparison with Ozone Gassing

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The treatment of liquid with a surface dielectric barrier discharge (DBD) under atmospheric pressure in air results in pH decrease and generation of nitrate (NO_3^-), nitrite (NO_2^-) and hydrogen peroxide (H_2O_2) in water. As well as, suspended microorganisms were inactivated within a few minutes [1] [2]. Furthermore, ozone (O_3), dinitrogen oxide (N_2O), carbon dioxide (CO_2) and nitric acid (HNO_3)/peroxynitrous acid (ONOOH) were detected by FT-IR in the gas gap between the plasma and the liquid surface [2].
Ozone is known for its antimicrobial effects [3] [4] but other active species from the plasma are candidates for biological decontamination, too. In order to resolve the role of O_3 in the plasma-liquid interaction a comparing study has been performed. Using an ozoniser and a FT-IR spectrometer different ozone concentrations as reached during direct plasma treatment of liquids (145 – 1900 ppm) were generated in a separate discharge chamber (without liquid sample) and the ozonized gas was blown over the liquid surface in a downstream petri dish. The pH and the H_2O_2 concentrations were estimated in 5 ml ozone treated water, as well as, antimicrobial effects on *E. coli* and *S. aureus* (10^7- 10^8 cfu $\cdot$ ml^{-1}) suspended in physiological saline were investigated.
It was found a pH decrease to 4.7, but lower generation of H_2O_2 than in direct DBD treated water. Inactivating effects on the microorganisms are weaker in case of ozone gassing. Consequently, the observed effects of the DBD treatment were not caused by ozone alone.The generation of protons (H^+) and H_2O_2, as well as, the antimicrobial effects may result from reactions of ozone with water. Presumably, $HO^\bullet$ and $HOO^\bullet$ radicals were produced and may cause the inactivating effects of the microorganisms. Also the pH decrease could be explained as given in equation (1). The H_2O_2 generation is a result of radical recombination as given by equation (2) and (3) [5] [6].

$$HOO^\bullet \leftrightarrow H^+ + O_2^{\bullet -} \quad (pKa = 4.8) \qquad (1)$$

$$2\ HO^\bullet \leftrightarrow H_2O_2 \qquad (2)$$

$$2\ HOO^\bullet \leftrightarrow H_2O_2 + O_2 \qquad (3)$$

Additionally, other presumably nitrogen-based reactive species may play a role in the effects of plasma-liquid interaction. Therefore, further investigations have to be done.

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Inactivation of enterohemorrhagic *Escherichia coli* (EHEC) by non-thermal atmospheric pressure plasmas

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Foodborne illnesses occur worldwide and in any environment. They are caused by the consumption of raw and microbial contaminated food, mostly by Gram-negative bacteria like *Escherichia coli* and its pathovar EHEC (enterohemorrhagic *Escherichia coli*) as well as *Listeria monocytogenes* and *Samonella* spp.. The last EHEC epidemic 2011 in Europe, especially in Germany, was caused by EHEC strain O104:H4. To reduce and/or prevent microbial contaminations, cleaning and hygiene of food is indispensible. Due to many disadvantages of the current used methods, the industry is in need of alternatives. One possible alternative could be physical plasma. It is used worldwide in manifold applications and is a flexible and highly adaptable tool, maybe for decontamination of food. Therefore, two very different non-thermal atmospheric pressure plasma techniques, a DBD setup and a microwave driven discharge, were investigated for their antimicrobial capacity against *E. coli* strain K-12, O157:H- as well as O104:H4. The used plasma sources led to the inactivation of the bacteria *E. coli* (K-12), EHEC (O157:H-) and EHEC (O104:H4) for a level of ≥3 to 5 log steps within 9 minutes treatment time (Figure 1). The gained results are very promising, however, further investigations with other foodborne pathogens should follow and the influence to the food quality must be considered. [1]

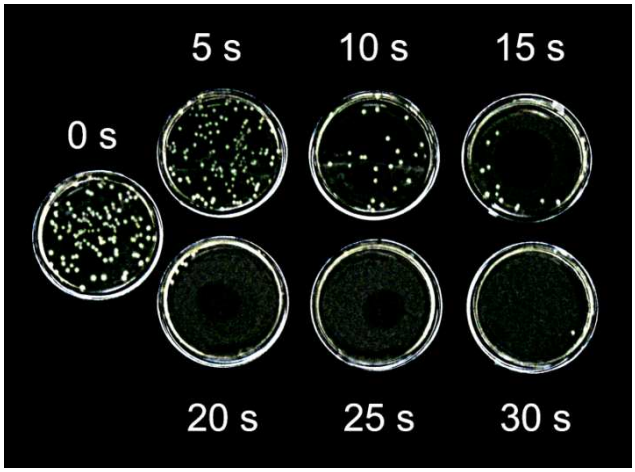

Figure 1: *Inactivation of E. coli K-12 by surface DBD*

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Analysis of the long-time lethal Effects of Plasma treated Sodium Chloride Solutions

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Nowadays used sterilization techniques such as heat and radiation as well as sterilization through chemicals are limited by high temperature, harmful radiation or cytotoxic effects, respectively. Consequently, it is not possible to use heat sterilization for heat-sensible materials and radiation sterilization for living tissues. Also, chemicals such as ethylene dioxide show often toxic effects in human cells. An alternative method for decontamination of heat-sensible drug containing solutions is the sterile filtration but it is also limited by the diameter of the pores. These limiting features and increasingly resistances of bacteria against common used antiseptics or antibiotics lead to the research for alternative methods for decontamination and sterilization. Over the past few years, it has been crystallised that non-thermal atmospheric pressure plasma can be used effectively for sterilization and decontamination processes especially for heat-sensitive materials and substances. [1] Recent investigations have been done to investigate the plasma liquid interaction with a special focus on decontamination processes. These investigations have shown that plasma treated 0.85 % sodium chloride solution has germicidal effects. [2] It is assumed, that the inactivation of bacteria is depending on acidic pH and the formation of low-molecular chemical species such as nitrate, nitrite, and hydrogen peroxide.

The aim of this work was to investigate the long-term antimicrobiological effects of plasma treated physiological sodium chloride solution with respect to the stability of reactive species, which are generated by plasma treatment. Plasma exposure to a 0.85 % sodium chloride solution with a ceramic surface dielectric barrier discharge results in acidification of the liquid as well as in formation of nitrate, nitrite, and hydrogen peroxide. The plasma induced generation of reactive species were determined by wet chemical reactions following by measurements of the absorption to quantify the amount. Germicidal effects have been investigated with a suspension of *Escherichia coli*, exposed to plasma treated sodium chloride solution. To characterize the biological effects as well as the stability of the generated reactive species the application and incubation time was systematically varied. Delay in application time brought a reduction in bactericidal efficacy which could be compensated by longer incubation times. Analytical investigations have shown a decrease in the concentration of hydrogen peroxide as well as nitrite within 24 h; pH value and the concentration of nitrate were stable over 24 h.

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Atmospheric-Pressure Cold Plasma as New Strategy in Disinfection of *Fusarium spp*

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Fusarium is a widespread fungus distributed in soil, plants and many other organics. Recently, infections caused by *Fusarium* species have been increasing in frequency among human, especially invasive fungal infections in immunosuppressed patients. Cutaneous infection will lead to red or gray macules, papules, pustules and subcutaneous nodules, and it is even lethal to some patients with damaged immune function [1]. Most *Fusarium* infections fail to respond to clinical antifungal therapy, and some effective antifungal agents usually result in patient's neutropenia.

In this study, a direct-current, atmospheric-pressure, He/O2 (2%) cold plasma microjet (PMJ) was used to disinfect 10 clinical isolates of *Fusarium spp* (five isolates of the *F. solani* and five non-*F. solani* isolates: three *F. oxysporum* and two *F. proliferatum*), both in air and in distilled water. Effective inactivation was achieved both in air and in water within 6 min of plasma treatment. The inactivation was verified by a XTT test. Three kinds of strong reactive oxygen species, which were believed to be the lethal factors generated in the plasma treated distilled water, were detected by electron spin resonance (ESR) spectroscopy, namely hydroxyl radical (.OH), superoxide anion radical (.O2 -), and singlet oxygen (1O2). **.**O2 – is shown to be the precursor of .OH. The concentrations of 1O2 and .OH are evaluated by comparing the ESR signals from plasma microjet (PMJ) treated samples with that from different concentrations of 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO) in water under identical experimental conditions. This study may provide a novel approach for clinical therapy for *Fusarium* cutaneous infection.

Work supported in part by Bioelectrics Inc. (U.S.A.), the Peking University Biomed-X Foundation and China International Science and Technology Cooperation (2008KR1330 - "Cold Plasma induced biological effect and its clinical application studies")

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Inactivation of Plant Pathogenic Fungi by Non-Thermal Atmospheric-Pressure Plasma Jet

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Non-thermal atmospheric-pressure plasma is very effective and convenient in deactivation of micro-organisms[1][2]. In this work, we suggest a simple non-thermal atmospheric-pressure plasma jet system, and evaluate the effect of the system through in vitro and in vivo test.

The plasma jet system consists of a control box and a micro plasma-jet nozzle. The plasma-jet nozzle has four components; a thin Ni anode, a porous ceramic insulator, a stainless steel cathode, and an acetal case. In order to generate the plasma jet we used nitrogen gas. The gas flow rate and the input voltage were maintained at 5 L/min and 20 kV_{p-p}, respectively.

In order to evaluate the sterilization effect of the system, we treat the Pectobacterium carotovorum and Staphylococcus aureus on the surface of agar and potato with plasma jet for 5 and 10 min. After plasma treatment, the treated surface is analyzed using fluorescence microscope. Both the survival rates of Pectobacterium carotovorum and Staphylococcus aureus are less than a few percents after treatment for 10 min. In our study, proposed system effectively inactivated fungi onto agar and potato. We expect the non-thermal atmospheric-pressure plasma will be practicable treatment method for agricultural industry.

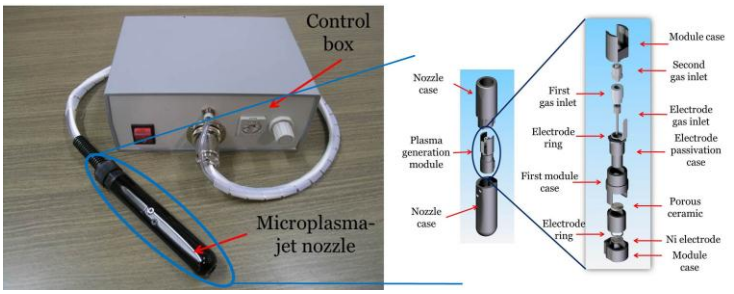

Figure 1: *The image of plasma-jet system. The inset is the schematic image of nozzle.*

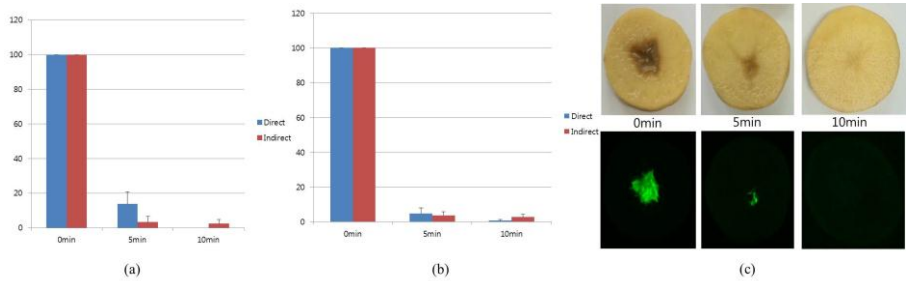

Figure 2: *The survival rates of (a) Pectobacterium and (b)Staphylococcus and (c) the image of potato surface after the plasma treatment.*

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Chemical Modifications in Non-Thermal DBD Plasma Treated Water and Antimicrobial Effects

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Plasma medicine is an emerging area of biological and medical applications, and non-thermal atmospheric plasma is a major focus of it. Most literatures are pertinent to direct plasma treatment. Previously our laboratory has reported that antimicrobial effect of non-thermal dielectric-barrier discharge plasma using floating electrode (FE-DBD) is much more effective than other traditional methods, and able to inactivate bacterial pathogens in both the planktonic and biofilm forms [1]. We also reported that FE-DBD technique of plasma application generates reactive oxygen species (ROS) that are significantly responsible lipid peroxidation, and eventual DNA damage, and that the known antioxidant was able to prevent these changes significantly [2]. The aim of present study is to characterize non-thermal DBD plasma treated water for its chemical properties which are playing major role on antimicrobial effect Among the possibilities are decreased pH, ROS and RNS that are generated in plasma treated water, and comprehend possible mechanisms responsible for making plasma treated water stable for extended period of time. Our findings suggest that non-thermal DBD plasma treatment of water generates ROS, acidified nitrates, and strong antimicrobial effect. Our findings also showed that non-thermal DBD plasma treated water (for 3 minutes), inactivates all the pathogens to 10^8/mL as well as in biofilm forms. The pH was dropped to ~2.00. The plasma treated water could sustain its antimicrobial property for weeks at normal atmospheric conditions. Amount of H_2O_2, HNO_3 and HNO_2 in water that are generated by 3 minutes of non-thermal DBD plasma treatment were determined as 50 mg/L, 3.86 mM, 0.26 mM respectively by using UV-vis spectroscopy, GC-MS and hydrogen peroxide assay kit (National Diagnostics, Atlanta, GA). In conclusion, non-thermal DBD plasma treatment of water generates strong oxidative species, which contribute microbial inactivation. Low pH and generation of nitric acid and nitrous acid might be responsible for chemical stabilization of generated species or their products. For the better understanding of underlying mechanisms of antimicrobial effect, and stabilization of non-thermal DBD plasma treated water, studies are underway.

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Inactivation of various microorganisms with the N_2-O_2 discharge flowing-afterglow

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Sterilization of medical devices (MDs) by gaseous plasmas is an alternative solution to conventional sterilization techniques based on heat, ionizing radiation and chemicals (O_3, ethylene oxide, H_2O_2, etc.). The temperature of our flowing-afterglow system being lower than the glass-transition temperature of most polymers, it is therefore possible to use it to disinfect/sterilize thermo-sensitive MDs. As concerns exposure to ionizing radiation from radioactive sources, it induces changes in the bulk of the materials, this technique further requiring a large and secured dedicated building, resulting in high costs. In the case of chemical treatment, it is generally necessary to ventilate the treated MDs for a few hours because they remained impregnated with toxic residues. In contrast, the plasma disinfection/sterilization systems that are developed in our laboratories do not generate toxic residues, thus needing no venting, which reduces total operation time. It is therefore safe for both the patient and the operator. It should not cost more than standard chemically-driven sterilizers. In this presentation, we describe the operation of the N_2-O_2 flowing afterglow discharge, at reduced pressure (p = 5 Torr), where the plasma is maintained by a surfatron, powered by a microwave generator delivering 120 watts at 2.45 GHz. We shall focus on the study of the optimization of the UV radiation intensity (200-400 nm), which is the main biocidal agents, though the role of particles (radicals, ions…) cannot be excluded [1, 2]. Figure 1 shows, as an example, the survival curves of various bacterial spores subjected to our flowing-afterglow system.

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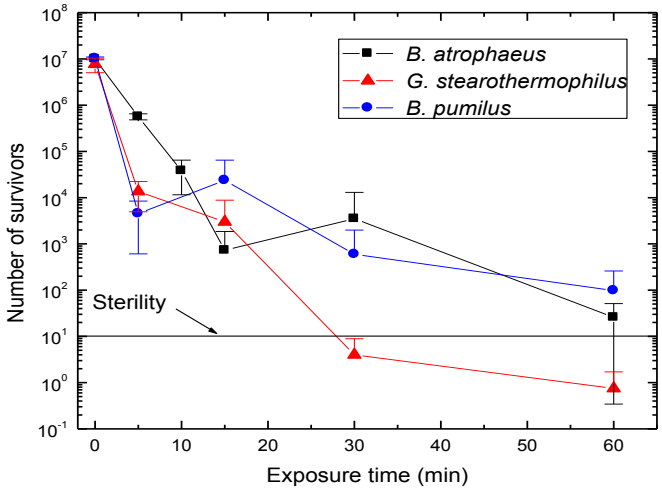

Figure 1: *Survival curves of dried spores (B. atrophaeus, G. stearothermophilus and B. pumilus) deposited on polystyrene Petri dishes and exposed to the N_2–O_2 flowing afterglow.*

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Decontamination of pathogenous prions and pyrogen molecules by the flowing afterglow of a reduced-pressure N_2-O_2 cold-plasma

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The current methods of sterilization of medical devices are not able to inactivate the prion proteins responsible for the mad-cow disease. These methods are also unable to inactivate completely pyrogen molecules such as Lipopolysaccharide (LPS) and Lipoteichoic acid (LTA) respectively from Gram-negative and Gram-positive bacteria. Knowing that our sterilization system, based on a low-pressure N_2-O_2 discharge flowing-afterglow, is efficient for the inactivation of microorganisms such as bacterial spores [1] and bacteria, we wanted to evaluate the decontamination potential of this system for prions and pyrogens.

We demonstrated by *in vitro* (on polystyrene strips) and *in vivo* (on steel inserts in mice) experiments that our sterilization system can decontaminate prion proteins [2]. Furthermore, we present preliminary results showing that it is possible to reduce the pyrogenic activity of LPS and LTA molecules through exposure to the N_2-O_2 discharge flowing-afterglow.

This work was financed by the Ministère du Développement Économique, Innovation et Exportation (MDEIE) of the Gouvernement du Québec and by the Conseil de Recherches en Sciences Naturelles et en Génie (CRSNG) of Canada.

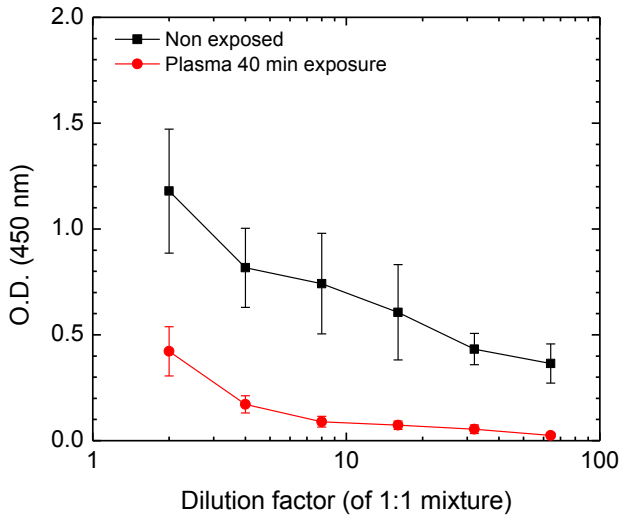

Figure 1: *Immunoreactivity (determined by ELISA) of homogenate mixture made from BSE-positive and BSE-negative bovine brains, coated on polystyrene surface wells at different dilutions, after 40 min exposure to the N_2-O_2 discharge flowing afterglow as compared to non-exposed samples (control).[2]*

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Sterilizing Air Plasma and Aesthetic Microwave Plasma Devices at Atmospheric Pressure

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Low temperature plasma has strong possibilities of application, for instance, sterilization, tooth whitening, skin treatment and others [1, 2]. Thanks for synergetic characteristic of plasma elements like electron, radical, UV light and electric fields, the plasma can do better work compared with conventional sterilization methods [3]. But plasma sterilization methods also have disadvantages. Hydrogen peroxide plasma has long process time and a bulk vacuum system, and atmospheric air plasma can cause damage on sterilization objects by high concentration of ozone. In this study, atmospheric air plasma sterilization with low concentration of ozone has been proposed. Portable plasma source driven by low frequency (~10 kHz) generates atmospheric air plasma inside a chamber. An ozone filter, that strongly grabs ozone molecules, is placed at the front of the plasma source to prevent sample damages by high ozone concentration. To enhance the sterilization efficacy and reduce the ozone production amount, low concentration of hydrogen peroxide is sprayed into the chamber before and during the sterilization process. Its sterilization performance has been verified with biological indicator disk placed at the center of the chamber. Compared with the low frequency atmospheric air plasma that has a good sterilizing ability, microwave (~GHz) plasma has a strong potential to be applied in biomedical applications such as skin treatment and dental applications because it generate lots of reactive species, and can be driven by a low voltage and consequently provides a great safety [4]. Although a lot scholarly progress has been made in the past ten years for this issue, it is still far from the commercialization of a microwave system for biomedical applications due to bulky and expensive equipment. Reducing gas and power consumption is challenging issues of the commercialization of the microwave plasma system. Portable microwave system that has six/twelve plasma jets those can be driven by low microwave power and low Ar gas flow rate has been developed. Plasma generator's structure has been optimized for this requirement.

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Treatment of *Weissella confusa* biofilms with low temperature plasma jet at atmospheric pressure

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Several devices of low temperature plasmas at atmospheric pressure have already shown their antimicrobial activity. However bacteria within a biofilm are more resistant to the plasma treatment than the planktonic or adherent ones [1, 2, 3]. The aim of this study was to evaluate the efficiency of a new plasma jet driven by a DC-corona discharge [4] to inactivate biofilms and adherent cells of Gram-positive bacteria. The plasma is generated directly in ambient air used as carrier gas without any admixture of rare gas flow and produces a large variety of active species at a temperature not exceeding 27°C [5]. *Weissella confusa* was selected as a model. These lactic acid bacteria, isolated from a food matrix, excrete a polysaccharide polymer (dextran) when sucrose is present [6]. Exopolysaccharides (EPS) play a major role in the protection of the microbial cells against environmental stresses. In addition, EPS are important components of the extracellular matrix of biofilms [7].

Biofilms or adherent cells were treated with the plasma jet for different exposure times. The antimicrobial efficiency of the plasma treatment was evaluated against adherent cells and 48 h-old biofilms grown with or without sucrose. Bacterial survival was evaluated using both Colony Forming Unit enumeration and tests of LIVE/DEAD Baclight Bacterial viability. The experiments show the ability of the new plasma jet device to inactivate the bacteria. An increased resistance of biofilm is clearly observed. The resistance is also significantly higher with biofilm in presence of sucrose, which indicate that dextran could play a protective role.

Overall, this work demonstrates the good potential of this new device of low temperature plasma jet to inactivate Gram-positive biofilms.

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Bactericidal effect in different gas compositions using Surface Micro-Discharge (SMD) plasma

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The usage of cold atmospheric plasma for biomedical application is a rapidly growing field of research with a wide application spectrum. Here atmospheric plasmas, as the application of which has been derived and inspired from the semiconductor fabrication research as well as the space research, have been utilized to inactivate microorganisms such as bacteria, spores and viruses. There are still many unclear mechanisms, e.g. how the cold atmospheric plasma interact and inactivate microorganisms. One possible answer is that the reactive species play an important role by making the cell wall of microorganisms permeable and penetrate to the inside.

The bacteria samples, Escherichia coli inoculated on agar, are treated by a Surface Micro-Discharge (SMD) electrode at atmospheric pressure. The electrode consists of a grounded planar metal plate, and a metal mesh with a dielectric in between. The plasma discharge is produced on the mesh electrode side by applying high voltage in the kilovolt range (peak-to-peak) at several kilohertz. The bacteria samples are placed 6mm away from the electrode.

The SMD-electrode is placed into a vacuum sealed chamber. Using this chamber, the gas composition for the plasma environment can be manipulated by leading different gas mixtures into the chamber. At a constant flow rate of 2slm, the chamber is filled with the gas mixture after less than 10 minutes. During this filling process, the exhaust valve of the chamber is open so that the pressure in the chamber remains constant at the ambient pressure.

The plasma is ignited in different gas conditions, by changing the ratio of oxygen and nitrogen. The electrical property of the discharge is then studied for different conditions. Compared to the bacterial experiment, using E.coli as a testing microorganism, the possible major and minor players for bacterial inactivation by the atmospheric plasma is discussed.

Effects of Atmospheric Pressure Plasma on Cellular Components: An Insight into Bacterial Destruction Mechanisms

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Atmospheric pressure non-thermal plasmas have proven to be effective in the eradication of bacteria in both planktonic and biofilm modes of growth [1]. These observations make this a promising approach for surface decontamination of medical devices and even viable tissues. However, the exact mechanisms of bacterial cell destruction mediated by cold plasmas are not fully understood. Cell killing mediated by the plasma is thought to be a complex and heterogeneous process, which involves a sequence of reactions [2]. In the present study, interactions of atmospheric pressure non-thermal plasma with different bacterial cell components are being explored in order to identify cell components most vulnerable to plasma exposure. Coupling this with the knowledge of plasma chemistry will help in identifying the specific reactions leading to cell death.

In this study, the efficacy of a 20 kHz atmospheric pressure dielectric-barrier type plasma jet [3], operating in helium and oxygen, was evaluated against a set of clinically significant bacterial strains in both of their planktonic and biofilm forms. Optical diagnostics for relevant reactive oxygen species have also been applied for direct correlations; absolute densities of metastable singlet delta molecular oxygen and ozone, relevant reactive oxygen species have been measured [4]. All planktonic bacteria were inactivated within four minutes of plasma exposure. Although 48-hour old bacterial biofilms, of the same strains, were more resistant to the plasma treatment, all biofilms were still completely eradicated within ten minutes.

Isolated bacterial plasmid DNA has also been exposed to the plasma and analysed using gel electrophoresis. Changes in plasmid structural conformation were quantitatively assessed and the rates of single and double strand breaks were calculated. Catalytic activity of certain bacterial enzymes was also evaluated after plasma exposure using fluorogenic assay, which allows the determination of the maximum retained enzyme activity after each plasma exposure. Peroxidation of lipid content of bacterial cells was also studied to evaluate the effect of plasma exposure on the phospholipid bilayer in the cell membrane. Furthermore, cell wall integrity and change in membrane permeability were assessed by measuring the leakage of Adenosine-5'-triphosphate (ATP), while electron microscopy studies are underway to examine the morphological changes to the cell surface following plasma exposure.

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The Promise of Plasma Medicine in the Post-antibiotic Era

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For decades we have enjoyed the positive impact of effective, relatively safe antibiotics to help control infections within the hospital and in the community despite increasingly resistant microorganisms. It now appears, however, that there are few drugs within the pharmaceutical development pipeline to address the most resistant current strains. We have a genuine fear of entering the Post-antibiotic Era in regard to these pathogens.[1] The organisms of most concern include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. (dubbed the ESKAPE pathogens [2]), as well as increasingly virulent *Clostridium difficile*.

Efforts to address this challenge include the development of programs to preserve the efficacy of currently available drugs through prudent antibiotic stewardship, to encourage and reward targeted new drug development by the pharmaceutical industry, and to support the timely recognition and appropriate isolation of patients with resistant strains of bacteria to limit their potential spread within medical institutions and the community at large.

Recent and ongoing advances in plasma medicine offer hope for pushing back the advent of this Post-antibiotic Era and – should it eventually arrive – for dealing with infections for which no traditional medical therapy exists and for which surgical intervention alone might not be feasible. Studies have confirmed the ability of atmospheric non-thermal plasmas to kill many of the above organisms which are becoming increasingly resistant to available antibiotics.[3] In addition, the effectiveness of various plasma medicine applications to treat infected wounds without retarding healing is becoming well-established.

Newer areas of investigation into the delivery of non-thermal plasmas for intraluminal and intracavitary applications promise to broaden the range of infections which might potentially benefit from this technology to include empyema, peritonitis, and sinusitis. Studies have explored the role of plasmas in sterilization and environmental decontamination as well.[4]

Experiments into the beneficial role of non-thermal plasmas in the treatment of malignancies may lead to advances in delivery technologies that can be adapted for use by those seeking to eradicate infections. It may well be that our current Antibiotic Era may one day, in retrospect, be viewed as the Pre-Plasma Medicine Era.

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Cellular and molecular responses of a filamentous fungus *Neurospora crassa* to plasma

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Although plasma is an efficient means of microbial sterilization, mechanism of plasma effect on microorganisms still needs to be clarified. In addition, a limited number of studies are available on eukaryotic microorganisms such as yeast and fungi in relation to plasma application [1][2]. Thus, we investigated cellular and molecular aspects of plasma effects on a filamentous fungus, *Neurospora crassa* by making use of argon plasma jet at atmospheric pressure [3]. The viability and cell morphology of *N. crassa* spores exposed to plasma were both significantly reduced depending on the exposure time when treated in water. The intracellular genomic DNA content was dramatically reduced in fungal tissues treated in water with plasma. Dramatic reduction in pH of water after plasma exposure was observed and this might produce detrimental effect on fungal spores. However, direct plasma treatment resulted in more severe effect on fungal spores than plasma treated and acidic water, indicating that factors directly from plasma could affect fungal viability and other responses. Interestingly, we discovered that the transcription factor *tah-3* gene was involved in generating fungal tolerance to a harsh plasma environment.

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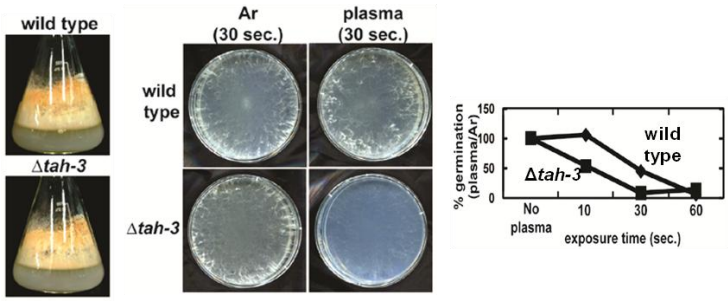

Figure 1: Responses of the *tah-3* deletion mutant to plasma. Growth in normal condition (Left panel), Growth on Vogel's Minimal media (middle panel), and spore germination rate (Right panel)

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Inactivation of *Penicilium degitatum* spores by reactive oxygen radicals employing atmospheric-pressure oxygen radical source

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Reactive oxygen species (ROS) including ground-state oxygen atom, hydroxyl radical, excited-state oxygen molecule, and so on, are effective to inactivate microorganisms. It is important to investigate the roles of ROS based on quantitative analysis of the gas phase. We reported that the spores of *Penicillium digitatum* were rapidly inactivated by high density non-equilibrium atmospheric pressure plasma (NEAPP) [1][2]. In this study, we have investigated the efficiencies of ROS on the inactivation of the spores of *P. digitatum* by using an atmospheric-pressure oxygen radical source. The absolute densities of ground-state oxygen atom and excited-state oxygen molecule were measured by vacuum ultraviolet absorption spectroscopy using a micro-discharge hollow cathode lamp and deuterium lamp.

$O_2/(Ar+O_2)$ flow rate ratio of the oxygen radical source was changed between 0 to 1.2 % in the chamber purged with Ar gas at atmospheric pressure as shown in Fig. 1. The densities of O (^3P) and O_2 (^1D) were from 10^{14} to 10^{16} cm^{-3}. At the $O_2/(Ar+O_2)$ mixture flow rate ratio of 0.6 % , O (^3P) density was the highest, while the D value estimated by the colony counting method was the lowest. On the other hand, O_2 (^1D) density increased monotonically with increasing $O_2/(Ar+O_2)$ flow rate ratio. Since the D value is in inverse relation to the inactivation efficiency, these results indicated that ground-state oxygen atom is the dominant species in the inactivation of *P. digitatum*.

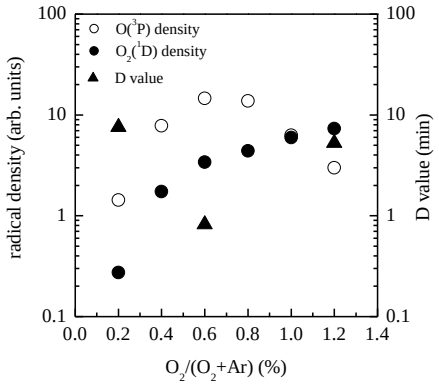

Figure 1: *Densities of $O(^3P)$ and $O_2(^1D)$, and D value as a function of $O_2/(Ar+O_2)$.*

This work was partly supported by the Knowledge Cluster Initiative (Second Stage), Tokai Region Nanotechnology Manufacturing Cluster, and a Grant-in-Aid For Scientific Research on Innovative Areas, “Frontier Science of Interactions between Plasmas and Nanointerfaces” (No. 21110006) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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Removal of dental plaque biofilm on titanium discs using different plasma devices and settings

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Dental biofilms plays a major role in the pathogenesis of peri-implantitis. Biofilm removal is a prerequisite for a successful therapy of peri-implant lesions; In this study we evaluated different plasma sources with different gas mixtures concerning biofilm removal.
We assessed the biofilm removal of kINPen08, (1,8 MHz, 2-6 kVpp, 5-8 slm), with and without an additional metal cap to exclude the influence of charge carriers but to let radiation and reactive species pass and a needle discharge (13 kV with argon or 10 kV with helium, 5 W with both gases) with 5000 sccm argon, 5000 sccm argon+6.5 sccm O_2, 5000 sccm argon+50 sccm O_2, 5000 sccm helium, 5000 sccm helium+6.5 sccm O_2 and 5000 sccm helium+50 sccm O_2 plasma against subgingival multispecies anaerobe biofilm grown on titanium discs *in vitro*. The biofilm was stained with Mira2ton® and treated with plasma for 1, 3 and 5 min respectively. Efficacy of plasma treatment was determined by the microscopically captured, unstained area without biofilm.
The biofilm removal of kINPen08 was very intensive and the treated surface was clean without protein residues. The other devices remove biofilm, too, but in a sparser way. This removal rate was under the detection rate of our measurement system, because here, we also measured stained bacterial protein residues. The biofilm removal is a time-dependent process. The longer the treatment, the greater the ablated surface (Fig. 1). Moreover, the higher the oxygen admixture, the higher the biofilm free surface. This was the case for both gases, helium and argon. Furthermore, the biofilm removal is increased by adding oxygen.

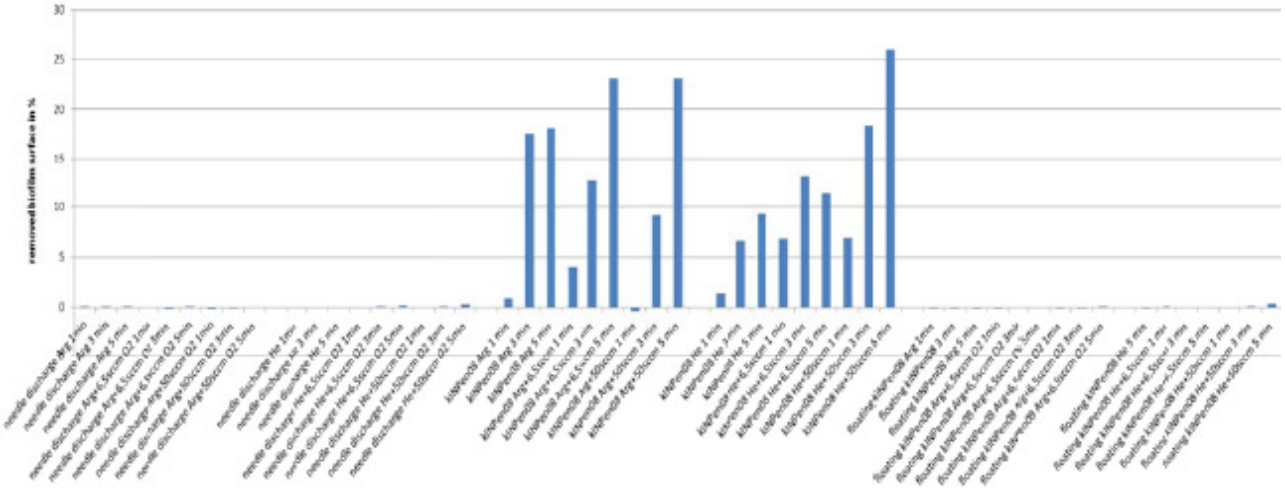

Figure 1: *Removed biofilm on the surface in % of the whole disc surface*

KINPen08 is the most potent plasma source concerning biofilm removal. Because of the high heat generation of this plasma device a new needle discharge plasma device was developed. To ensure patient safety a kINPen08 with a metal cap was used as well. Both safety devices did not remove the biofilm in the same extend like kINPen08. Therefore it is necessary to combine the plasma process with mechanical biofilm removal.

UV-C Emitting Phosphors under Plasma Excitations: A Biocidal Effect?

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This paper presents a preliminary study in order to investigate the biocidal activity of an UV-C phosphors light emission under plasma excitation.

In this purpose, a small Dielectric Barrier Discharge (DBD) lamp prototype has been developed in collaboration with Saint Gobain Society. The design principle of this lamp is presented in figure 1 where two quartz plates are separated by a gas gap (mixture of Ne/Xe 50%). The quartz plates present the dielectric. An internal phosphor coating, calcium pyrophosphate ($Ca_2P_2O_7$) doped with Pr^{3+}, generates UV-C emissions and a conducting grid is deposited on both external sides (parallel and plane electrodes) to apply the voltage and initiate the gas discharge. The power supply can generate square or sinus waveform with frequency up to 100 kHz and a 2 kV maximum voltage.

The choice of the phosphor coating has been justified in our previous work [1] where phosphor efficiencies were compared in a dedicated experiment chamber filled with a Ne/Xe 50% mixture at 250 mbar. In these conditions, the highest temperature process (α-$Ca_2P_2O_7$:Pr 2%Na2%) was identified to be the most efficient.

The first step of this study is a parameters approach based on different excitations (waveform, frequency and power) with spectral investigations on the UV-C emission leading to the choice of a standard configuration. Once the different parameters are fixed, the biocidal effect of the UV-C emission on Escherichia coli is studied for different exposure periods and concentrations.

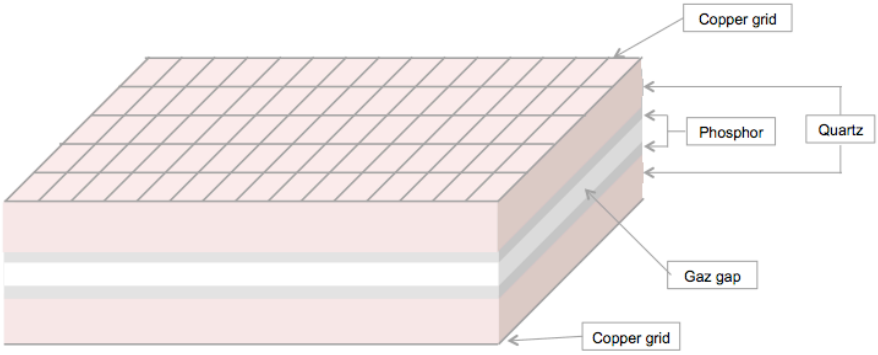

Figure 1: *The DBD lamp prototype design principle (80 mm × 120 mm ×6 mm)*

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Different modes of protein inactivation by atmospheric pressure plasmas

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Atmospheric pressure plasmas are known for their fast inactivation of microorganisms, but the specific mechanisms are not well understood. We investigated different biological macromolecules regarding their susceptibility to different plasma components to deepen our understanding of plasma-induced damage inside a cell. We employed a specific atmospheric pressure plasma jet configuration, called X-Jet [1], to split the effluent into photons and particles. Furthermore, all experiments were performed in a He atmosphere to minimize secondary effects of ambient air as well as allowing VUV radiation emitted by the plasma to reach the surface.

Proteins are key players in life. Information on their composition is stored in genes on the DNA level, these are transcribed into RNA, which in turn serves as translational template for protein synthesis. Nearly all cellular processes are based on protein catalyzed reactions and protein interactions. Reflecting the diversity of their functions, proteins exist in a multitude of forms, as well as featuring diverse levels of sensitivity towards different kinds of damage. Therefore an array of different model proteins should be used to study plasma-induced protein damage.

We present the effects of plasma treatment on three different proteins. The protein GapDH is an essential part in the central energy metabolism. Its active form consists of four subunits linked via thiol groups, which are prone to oxidation. Enzyme activity can be measured spectroscopically *in vitro* via the reduction of its energy donor NAD^+ to NADH. It was shown that plasma emitted (V)UV radiation inactivates GapDH very slowly. Treatment with emitted particles is more effective, whereas treatment with the undivided effluent proved to be most efficient for inactivation. As a second model protein, RNase A was used. RNases in general are among the most stable proteins known. Activity of RNase A was measured by monitoring spectroscopically the degradation of its model substrate cCMP. None of the plasma treatments with effluents (photons, particles or combined) had any significant effect. The same experiments performed with an atmospheric DBD plasma source [2] showed that direct contact with plasma inactivates RNase A samples within 5 minutes. The third employed protein is mCherryRED, which consists of a very stable β-barrel structure sheltering a single chromophore. When excited with light at 562 nm mCherry emits red light with a maximum at 607 nm. Relative fluorescence was measured during a time-course experiment with the untreated control set to 100% fluorescence. Interestingly, all jet configurations were effective in protein decoloration, but the protein regained its fluorescence after 18h of incubation. Only after long treatment times no regeneration was observed.

References

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