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Potential Interactions between Cold Atmospheric Plasma/Plasma-activated Medium and the ROS/RNS-Controlling System of Tumor cells

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Malignant transformation of cells requires NADPH oxidase (NOX)-dependent generation of extracellular superoxide anions. These drive the proliferation, but also cause the elimination of malignant cells through the HOCl and the NO/peroxynitrite signaling pathways. These intercellular signaling pathways induce apoptosis selectively in malignant cells, due to site-specific concerted interaction of defined reactive oxygen and nitrogen species (ROS/RNS). Tumor progression requires the expression of membrane-associated catalase. This enzyme interferes with HOCl signaling through decomposition of H₂O₂, and with NO/peroxynitrite signaling through oxidation of NO and decomposition of peroxynitrite. Membrane-associated catalase has been found on all lines of bona fide tumor cells and represents a promising target for novel antitumor strategies. Inactivation of tumor cell-specific membrane-associated catalase reactivates intercellular ROS/RNS-dependent apoptosis-inducing signaling and leads to autocrine apoptotic selfdestruction of tumor cells.

Model experiments with defined ROS and RNS led to the conclusion that singlet oxygen that was either contained in CAP or derived through the interaction between PAM constituents might lead to site-specific inactivation of catalase, followed by tumor cell-specific generation of secondary singlet oxygen and subsequent inactivation of further catalase molecules. This then allows for subsequent reactivation of intercellular ROS/RNS-dependent apoptosis-inducing signaling [1]. Only on the first sight, this model seemed a) to be in contradiction to the model on the dependence of CAP action from aquaporins [2] and b) to be independent of subsequent immunogenic cell death and activation of a cytotoxic T cell response [3, 4]. The analysis of existing experimental data from several groups and the alignment of site-specific mechanisms, defined by chemical biology and cell biology, allows to establish an updated comprehensive model [5] that includes the concepts from references [1-4] in a rational way. This model includes several biochemical amplification loops related to the generation of secondary singlet oxygen, catalase inactivation and ROS/RNS signaling. In addition, local enhancing effects of aquaporins, a positive feed-back of HOCl signaling on immunogenic modulation, as well as a feedback loop from activated T cells to catalase inactivation are suggested. The understanding of these cooperatively acting mechanisms might be instrumental for the establishment of synergistic effects that should allow tumor treatment even at low doses of CAP or PAM.

References
Plasma generated reactive oxygen and nitrogen species for treating prostate cancer

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Non-equilibrium plasmas, operated at ambient atmospheric pressure and temperature, are very efficient sources for highly reactive neutral particles e.g. reactive oxygen and nitrogen species (RONS [1,2]), including atomic oxygen and nitrogen, hydroxyl radical, superoxide, singlet delta oxygen, nitrogen oxides, charged particles, UV-radiation, and electro-magnetic fields. Individually many of these components have been implicated in therapeutics, including cancer therapy. RONS are known to play a crucial role in biological systems, such as signaling, and generating oxidative damage to a variety of cellular components, which can ultimately lead to cell death [3]. Plasmas have the advantage of delivering these simultaneously providing potentially superior processes.

The generation, control, and transport of the various plasma components to the biological target is complex and important. In the core plasma a large, but defined, number of species can be created. As the plasma interfaces with ambient air, humidity or liquid layers, new reactions and species of varying lifetimes can be created. Energy dissipation at these interfaces is important and to date not fully understood. Measurements and simulations under this atmospheric pressure environment are challenging, primarily due to the multi-phase, strongly non-equilibrium with large gradients (e.g. in electric field), high collisionality thus short-lived species and micron length scales [2]. This requires the application of advanced techniques for both measurements and models. In this presentation quantitative concentrations of various plasma generated reactive species (e.g. O, O$_3$, OH), their transport into liquids [4] and subsequent action on prostate cancer cells will be discussed.

We have plasma treated both purified tumour cells freshly extracted from prostate cancer patients, and matching non-tumour cells from a distant region of the same prostate [3,5]. Freshly isolated primary tumour cells act as a near patient model, which has recently confirmed differences in pharmacological susceptibility as compared with established cell lines. To determine the mechanisms of cytotoxicity, we have also explored the immediate and longer-term effects on gene expression.

This research was funded by UK EPSRC, Leverhulme Trust, York Against Cancer, and a donation from Ron and Beryl Gatenby.

References

Activation control of microorganisms using neutral radical irradiations

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Recently, the applications of non-equilibrium atmospheric-pressure plasmas (NEAPPs) have been intensively studied in agricultural and medical fields.[1] NEAPPs can inactivate microorganisms such as Escherichia coli and spores of fungi. In our previously works, we found that the ground-state atomic oxygen O(3Pj) generated by a commercially available atmospheric-pressure radical source (Fuji Machine MFG. CO., LTD. FPA-10) can be a key species in activation of microorganisms with a Penicillium digitatum spores. Moreover, we found quantitatively that ground-state atomic oxygen O(3Pj) was the dominant factor responsible for inactivating spores of P. digitatum and Aspergillus flavus and niger.[2-4] In addition, we have investigated the effect of atomic oxygen radical treatment on the fungal-spore activation by monitoring amylase production of Aspergillus oryzae (A. oryzae), which is very beneficial microorganism and employed for decomposing starch to glucose in the process to produce Japanese sake. We irradiated the oxygen radicals to A. oryzae spores in the similar manner to the inactivation processes described above. As a result, the germination rate was increased by 13 % and amylase activity secreted from the irradiated A. oryzae spores were increased by ~100 %.

Moreover, we clarified that the change from promotion to repression of the growth of the budding yeast cells can be controlled through the dose of oxygen radicals using atmospheric-pressure oxygen-radical source.[5] Furthermore, we have investigated the effect of nitric oxygen radicals on the proliferation of budding yeast and optimized treatment conditions. NO densities were measured using UV absorption spectroscopy and the proliferation was evaluated with microscope with cell-counting chamber. From these results, we observed around 20 % increase of the number of yeast cells at a NO radical dose of ~ 1× 10¹⁹ cm⁻³. The promotion effects were also observed for the fibroblast cells.

From these results, it was concluded that the activation of microorganisms can be successfully controlled through dose of oxygen or nitrogen oxide radicals with high repeatability. The control method using neutral radical irradiation has a potential to be applied for a proliferation-promotion process of cultured cells as well as microorganisms.

Acknowledgements

This work was partly supported by MEXT-Supported Program for the Strategic Research Foundation at Private Universities (S1511021) and a project for Promoting Research Center in Meijo University.

References

Plasma Medicine Applications – Focus on Skin

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The ability to produce cold plasma at atmospheric pressure conditions was the basis for the rapid growth of plasma related application areas in biomedicine. Plasma comprises a multitude of active components such as charged particles, electric current, UV radiation, and reactive gas species which can act synergistically. Anti-itch, antimicrobial, anti-inflammatory, tissue stimulating, blood flow enhancing as well as proapoptotic effects were demonstrated in in vivo and in vitro experiments and until now no resistance of pathogens against plasma treatment was observed. The combination of the different active agents and their broad range of positive effects on various diseases, especially easily accessible skin diseases, render plasma quite attractive for applications in medicine.

For medical applications two different types of cold plasma appear suitable; indirect (plasma jet, plasma torch) and direct plasma sources (dielectric barrier discharge - DBD). 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 (Cinogy GmbH, Duderstadt, Germany) 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 non-conducting layer avoids the transition of the gas discharge into a hot arc by limiting the current. The biological tissue itself (i.e. the skin) acts as the counter electrode. In contrast, the atmospheric pressure plasma jet (APPJ) kINPen® MED (INP Greifswald / neoplas tools GmbH, Greifswald, Germany) consists of a hand-held unit for plasma generation, a DC power supply (60 V) and a gas supply unit. The APPJ is generated due to a centered electrode which is surrounded by a second round electrode and expands to the surrounding air at the end of the nozzle, driven by an argon gas flow. The argon gas flow was set to 5 standard liters per minutes (slm). The kINPen® MED is applicable primarily for small-point treatments and very useful for the treatment of small gaps. Both devices are CE-certified as a medical product to treat chronic wounds in humans, showed efficacy, and a good tolerability.

Recently, the use of plasma in cancer research and oncology is of particular interest. Plasma has been shown to induce proapoptotic effects more efficiently in tumor cells compared with the benign counterparts, leads to cellular senescence, and – as shown in vivo – reduces skin tumors. To this end, we introduce a first Leibniz professorship for plasmabiotechnology in dermatology world-wide and establish a scientific network for the investigation of the efficacy and safety of cold atmospheric plasma in dermato-oncology. Hence, plasma medicine especially in dermatology holds great promise.
Cell Membrane Transport Activated by Gas-Liquid Interfacial Plasmas for Future-Oriented Gene/Drug Transfer Device

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It is indisputable that non-equilibrium atmospheric-pressure plasmas (APPs) have promising potentials of medical treatments such as wound healing, cancer treatment, blood coagulation, and so on. Among many medical applications, transfer of membrane-impermeable molecules (drugs, proteins, and genes) using APP has attracted attention because the conventional methods have many difficult problems (e.g. cell damage). In this study, gas-liquid interfacial atmospheric-pressure plasmas (GLI-APPs) were used to activate cell membrane transport toward developing future-oriented minimally-invasive and highly-efficient gene/drug transfer devices.

In an attempt to identify dominant factor(s) in APP-activated cell-membrane transport, the concentration and distribution of plasma-produced reactive species in the gas and liquid phase regions were measured. These reactive species are classified in terms of their life-span: long-lived (e.g., $\mathrm{H}_2\mathrm{O}_2$), short-lived (e.g., $\mathrm{O}_2^-$), and extremely-short-lived (e.g., $\mathrm{OH}$). As one of the noteworthy results, the horizontally center-localized distribution of $\mathrm{OH}_{aq}$ corresponded with the distribution of the transferred cells upon short-time APP irradiation. These results suggest that a high dose $\mathrm{OH}_{aq}$ for a short term is likely one of the dominant factors in the plasma-activated cell-membrane transport.

Furthermore, it has been verified that some APP-produced reactive species enhanced the molecule uptake mediated by transient receptor potential (TRP) channel(s). The time-course changes in cytoplasmic calcium ion concentration $[\mathrm{Ca}^{2+}]_{i}$ and drug-simulated fluorescent material (YOYO-1) uptake in cells stimulated with plasma-irradiated solution were observed using live cell imaging systems. While administration of plasma-irradiated solution to cells in culture resulted in gradual and sometimes oscillatory increases in $[\mathrm{Ca}^{2+}]_{i}$ after a relatively short lag period (~70 s), YOYO-1 uptake were enhanced after a relatively long lag period (> 600 s). The maximum values of both $[\mathrm{Ca}^{2+}]_{i}$ and YOYO-1 uptake after the plasma stimulation decreased with increasing aging time and completely blunted in the presence of ruthenium red, which is a non-competitive pan inhibitor of multiple TRP channels. Multiple measurements of components in plasma-irradiated HEPES-buffered saline (HBS) indicated that prolonged releases of $\mathrm{O}_2^-/\mathrm{ONOO}^-$ via the formation of HEPES-derived radicals, which deactivated with the aging time, were realized at a 10,000-fold lower cost compared with conventional chemical methods. Thus, it was likely that the prolonged releases of $\mathrm{O}_2^-/\mathrm{ONOO}^-$ in the plasma-irradiated HBS induced both the calcium response and YOYO-1 uptake mediated by TRP channel(s).

When chemistry meets biology – plasmas in medicine


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Following the positive clinical outcome, especially in the field of wound healing, cold physical plasmas have sparked fundamental research seeking for answers. Recent studies using advanced cell/tissue/animal models led to a deeper insight into the functional consequences of a given plasma treatment and substantiated the awareness that plasmas interact with cellular redox signaling pathways [1, 2]. Due to their complexity and cross activation potential, understanding which plasma-derived component “does the job” is hampered. This situation does not benefit from the growing but yet insufficient knowledge on gas-liquid interfacial reactions and transport mechanisms. Hence, despite much advance, plasma physicists and engineers still await an explicit outcome-driven guidance by the life scientists defining the “ideal species”.

Here, in a series of approaches small molecules have been used to trace the presence of plasma derived reactive species in liquids [3, 4]. Such, the chemical potential of each species is emphasized over their mere presence, naturally biased by the reactivity of the chosen trace molecules. The data obtained allow a clear distinction between plasma sources, working parameters, and liquid composition. Of interest, a profound difference of the chemical impact was found for direct and indirect (separate) treatment modes pointing at the relevance of short-lived species. Beside the use to determine the chemical footprint of a plasma source, keen interest is given to the data’s relevance in vivo.

Cellular redox signaling exploits predominantly the thiol group and its various oxidation states in order to modulate activity, location, and lifetime of proteins. In addition, covalent modifications of tyrosine are used to change enzyme activities. The chemical impact observed on cysteine and phenol in vitro suggests that a plasma treatment in vivo will significantly attack such structures via the short-lived species and such the translation of the chemical signal into a biological one. It remains to be clarified, how the interpretation of this signal is predetermined by the cell or tissue treated and how specificity can be achieved.

This work was supported by German Federal Ministry of Education and Research (BMBF) (Grant No. 03Z22DN11 & 03Z22DN12.

The plasma care© - a cold atmospheric plasma device for the treatment of wounds


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It is well-known that chronic infected wounds are commonly colonized with microorganisms and that the wound healing process is delayed if more than four different bacterial species are found on a wound.

Cold atmospheric plasmas – partly ionized gases, which produce a reactive mix of electrons, ions, excited atoms and molecules, reactive species and UV light – proved their use for the treatment of chronic infected wounds by effectively inactivating bacteria independent of the specie and their resistance level against antibiotics1,2.

The plasma care© is a small, mobile, handheld and accumulator-driven medical device, which will receive CE-certification in October 2018 and which uses cold atmospheric plasma for wound and skin disease treatment.

For the plasma care© a new generation of thin film plasma source – a further development of the Surface Micro-Discharge Technology – was invented, which allows plasma production at low voltages of 3.5 kVpp and low power consumptions of around only 1 Watt.

To ensure that the produced cold plasma is safe for usage on patients with wounds an extensive pre-clinical study was carried out to evaluate the therapeutic window in which bacteria and fungi are efficiently inactivated by the plasma without harming human eukaryotic cells and tissue. This study therefore included

- extensive efficacy tests on bacteria and fungi (planktonic cells and biofilm)
- safety tests on ex vivo human skin
- viability investigations using primary keratinocytes and fibroblasts
- as well as mutagenicrity tests.

The results show, that a safe therapeutic window exists and that the newly developed thin film plasma source fulfils all requirements for the usage on wounds. The plasma care© device will be available on the market in November 2018.

1 Isbary et al. BJD 2010
2 Isbary et al. BJD 2012
Thiol Chemistry as a Molecular Tool in Plasma Medicine

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Cold atmospheric plasmas have arrived in the medical sector. Clinical trials to treat skin diseases and chronic wounds are underway. Different plasma sources are tested, ranging from dielectric barrier discharges (DBD) operated in air to plasma jets with a high gas flux of noble gases with molecular admixtures. All plasma sources have in common that they generate reactive oxygen and nitrogen species (RONS) but the quantities differ from plasma source to plasma source. It is almost impossible to compare different plasma sources per se. Comparison of different sources would allow us to gain a better understanding and to identify general mechanisms of plasmas by pinpointing dominant effects of some species over others. Ultimately, this knowledge would help designing application-specific plasmas.

Thiols are an interesting target for plasma. The sulfur is readily oxidized and structural or activity changes are expected after plasma treatment. Furthermore, thiols are used by the human body’s redox system and are thus also of interest for studying the impact of plasma treatment on the human redox system. First experiments were performed with the amino acid cysteine, treated by a DBD for different discharge parameters and conditions [1]. The established cysteine model was further used to compare two different well-characterized plasma jets, namely the COST jet [2] and the kinpen [3]. A spatially resolved investigation of a plasma-physically uniform surface DBD showed differently pronounced chemical modifications on the model cysteine. In the next step, the model complexity was increased by using the redox pair Glutathione (GSH) and Gluthathione Disulphide (GSSG), the most important redox buffer in organisms responsible for detoxification of intracellular reactive species [4]. The presented results were obtained by FTIR and Raman spectroscopy, mass spectrometry, and supported by molecular dynamics simulations.

References

Use of transporting discharge and plasma gun for surface processing of polymers for biomedical application

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Since a few decades atmospheric pressure plasma jets have been used for surface modification of polymers, deposition of different inorganic coatings as well as organic plasma polymer films [1]. However, for biomedical applications, very often surface modification of complex shapes may be required such as, for example, inner walls of vascular grafts or catheters. In this work, the potential of surface modification of films and tubes inner wall with a helium transported discharge and a plasma gun has been investigated [2-4]. This type of discharges allow both admixture of monomer, upstream and downstream of the reactor. The development of nano thick polyethylene glycol (PEG) coatings by using Diethylene Glycol Methyl Ether (DEGME), via plasma polymerization is presented. UHMWPE films and HDPE tubes were used as the substrate. Different experimental conditions were used to induce modification in the inner surface of the tubes as well as the substrates placed outside the jet. Surface characterization was performed with the aim of understanding the surface modification effects by means of FTIR, AFM, XPS and contact angle measurements. The results have shown that PEG coatings have been successfully deposited inside the tubes. The spectral analysis of PEG coatings deposited at different locations of downstream tubes show substantial changes in the chemical composition of the coatings with an increase of the distance from the powered electrode. Also, cell adhesion or non-adhesion of PEG like coatings with respect to cells were studied. Better cell non adhesive properties with respect to cancer cells were observed for coatings which were deposited near the high voltage electrode. The possibility to deposit such coatings inside tubes is quite an interesting result for inside catheter tubes, used for evacuation of pathogen biological liquids but also for food applications. A transporting discharge and also plasma gun at atmospheric pressure were used for the treatment of UHMWPE films and HDPE tube. The results show that in different configurations, the two competitive processes, i.e., crosslinking and functionalization, can be optimized in order to limit the ageing inside the tube. Our results indicate that the helium transporting plasma has a potential to modify the surface properties the films and inner wall of long tubes especially relevant for biomedical applications.

References

Evaluation of severity of inflammation after hemostasis with non-thermal plasma using an in vivo molecular imaging technique

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In vivo molecular imaging is a technique that facilitates non-invasive visualization of in vivo molecular processes such as the expression, interaction, and degradation of genes and proteins. In molecular imaging research, various signals including fluorescence, bioluminescence, nuclear magnetic resonance, ultrasound waves, radiation, etc., are detected and converted into images. Nuclear medical molecular imaging involves administration of a radioactive imaging probe to a living subject followed by non-invasive detection of the distribution of the radioactive probe; thus, information regarding biological functions related to the mechanism of probe accumulation is acquired in a highly sensitive, quantitative manner. This technique plays a central role in various areas of in vivo molecular imaging research. Nuclear medical molecular imaging is an established clinical diagnostic imaging technique, used for e.g., for diagnosis of cancer, blood flow analysis, etc., and it facilitates transfer of basic research findings to clinical practice.

High-temperature coagulation, a technique commonly used for intra-operative hemostasis currently, has the disadvantage of delayed healing at the hemostasis site due to heat injury. Hemostasis by non-thermal plasma has been reported recently; it has shown a hemostatic effect even in anticoagulant (warfarin)-treated mice whose blood showed resistance to coagulation. Non-thermal plasma hemostasis is expected to exhibit a rate of healing faster than that noted with high-temperature coagulation hemostasis due to its minimal thermal effect. However, the mechanisms and in vivo kinetics of the healing process remain unknown. Herein, we evaluate the inflammatory response after non-thermal plasma hemostasis, and the subsequent healing process by nuclear medical molecular imaging with the radioactive probe, 2-deoxy-2-[¹⁸F]fluoro-D-glucopyranose (¹⁸F-FDG), which is known to accumulate at sites of inflammation. In this talk, we present the results of our study and discuss how nuclear medicine molecular imaging can contribute to innovations in plasma medical science.

This work was supported in part by a Grant-in-Aid for Scientific Research on Innovative Areas (Research in a proposed research area), Plasma Medical Innovation (KAKENHI No. 25108508, 15H00895) from the Japan Society for the Promotion of Science.
PrinciPAL - understanding biological effects of plasma activated liquids

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The exposure of aqueous solutions to non-thermal plasmas results in the generation of reactive chemical species in the liquid phase which are biologically active and have demonstrated anti-microbial and cytotoxic activity. These plasma activated liquids (PALs) are of interest for decontamination of surfaces, wounds or food products as well as applications in cancer treatment while their off-site production, storability and ease of administration in liquid state can be advantageous over a use of direct plasma discharge.

The translation of plasma chemistry to biological response occurs via the liquid interface at the extra- and intracellular level. An identification of the key liquid-based effectors is therefore important for the elucidation of the underlying mechanisms in both direct exposure of cells to plasma discharge and their treatment with plasma activated liquids. The cause-effect relationships remain incompletely understood and display high degrees of freedom due to differences in plasma sources, discharge and treatment parameters, liquid characteristics and the cellular targets. Reactive oxygen and nitrogen species generated in the plasma discharge are involved in numerous cellular processes and are believed to be key mediators of the observed biological effects in liquid.

Investigations of PALs derived from different types of liquids and a range of plasma devices provide insight into the distinct reactive species which can be generated in a plasma activated liquid [1,2]. Such platform of plasma liquid chemistries is employed to investigate the effects of selected reactive species on various pro- and eukaryotic cells as well as their cellular building blocks such as proteins and lipids in isolation [3,4]. The translation of extracellular reactive species to intracellular signal and resulting cellular effect is tracked through a range of biochemical assays and molecular probes, giving insight into the roles of cellular redox status, intracellular pH and activation of cell signalling pathways.

Elucidating the plasma-liquid-cell relationship will allow a more directed approach to the use of plasma in biomedical applications including cancer treatment, disinfection or wound healing and may permit the tailoring of plasma and PAL chemistries to selected targets. A detailed understanding of the principles underlying PAL-mediated biological effects on both prokaryotic and eukaryotic organisms is essential in order to harness this new technology.

This work was supported by Science Foundation Ireland (SFI) under grant number 15/SIRG/3466 and 14/IA/2626.

Effective Delivery of Plasmid DNA to the Skin

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The easy accessibility of skin makes it an excellent target for gene therapy applications. It is easily accessible and effects to the tissue can be easily monitored. We have been exploring various approaches for delivery including gene electrotransfer (GET), plasma as well as the combination of the two. These in vivo methods are simple and direct for delivering genes for therapy and can be accomplished in a minimally invasive way. Potential clinical applications of skin gene therapy include the treatment of cutaneous disease, wound healing, DNA vaccination, as well as the alleviation of some systemic disorders. We previously demonstrated that GET was an effective tool for delivering plasmid DNA to a variety of tissues including skin. Recently, we have begun evaluating the use of cold plasma as a delivery tool for plasmid DNA. Plasma assisted gene transfer is an area that is gaining more attention, since it is a non-invasive method and it minimizes potential discomfort of treated area [1]. The atmospheric pressure plasma discharge is an effective approach for delivering plasmid DNA for both in vitro and in vivo applications [2, 3].

In this current study, we have been exploring the use of cold plasma alone or in combination with GET. We investigated plasma assisted delivery and the combination both in vitro in a full thickness skin construct as well as in vivo in a guinea pig model. We utilized a novel atmospheric pressure plasma jet (APPJ) which is operated in air. Delivery was assessed utilizing two plasmids each encoding a different reporter gene (luciferase or green fluorescent protein). Following injection of the plasmid, the area was exposed to either plasma, GET or the combination. Expression was assessed either using a Caliper IVIS whole body imaging system (luciferase) or histologically (GFP). Sites treated with plasma, GET or the combination, had higher levels of expression than just injecting the plasmid alone. Expression lasted for approximately 2 weeks. Plasma assisted delivery can enhance the transport of plasmid DNA both in vitro and in vivo. The main advantage of this method is that plasmid DNA delivery is performed without contact to skin, thus it eliminates temporary pain in the treated area. Study is continuing to determine if levels could be increased further.

This work was supported by NIH Grant no. R01EB018956 and the Frank Reidy Research Center for Bioelectrics

Atmospheric pressure air discharges generate cold plasmas rich in production of reactive oxygen and nitrogen species (RONS) that play crucial roles in multiple biomedical, food processing and agriculture applications, such as disinfection, induction of cell proliferation, antitumor effects, stimulation of seed germination or plant growth. Plasma activated water (PAW), i.e. water or aqueous solutions treated by such plasmas, also demonstrate antimicrobial or cytotoxic effects. The plasma-generated RONS are transported from the gas phase through to the liquid and induce formation of secondary RONS in water. This is typically accompanied by acidification and antimicrobial effects that can last in the PAW for several hours/days after plasma treatment [1].

We tested various self-pulsing DC-driven air discharges regimes: positive and negative streamer corona and transient spark (TS) in various configurations and gas mixtures. Water was activated either indirectly or by a discharge directly into the water surface. The water electrospray was even more efficient, allowing for very efficient mass transfer of plasma-generated RONS into water [1-2]. The production of RONS in PAW can be controlled by the physical discharge properties and gas mixtures, which determine the PAW chemical and antibacterial properties.

The antibacterial effects were tested on E. coli in water and correlated with the RONS generation and time evolutions. They are stronger with direct than indirect plasma treatment when bacteria are only incubated in PAW. Antibacterial properties of PAW decay within hours but can be prolonged when cooled or frozen. PAW showed a great potential for some medical therapies e.g. of periodontal biofilms, endodontics, urinary tract infections, or open wounds. Direct TS treatment of various biomolecules (e.g. protein or DNA) show efficient denaturation and changes in their secondary structure. Indirect TS treatment of cell culture media decreased viability of cancer cell (HeLa, A375 melanoma) without recovery, while normal cells (Vero, HEK293T) partly recovered after initial inhibition, which opens new potential for cancer therapies [2].

TS air discharge was successfully tested to induce antimicrobial effects in food products, such as fruit juices and significantly extended shelf-life time of the juice without a negative impact on its chemical (pH change, contents of organic acids, polyphenols, sugars) and sensory properties. RONS in the PAW may act like signal molecules inducing the seed germination and also as the essential nutrients for plant growth. We measured the increase of germination rate of wheat seeds and dry weight of seedlings. Enhanced plant growth was tested on several plant species (lettuce, radish, tomato, wheat): the growth parameters, photosynthetic pigments, net photosynthesis rate, and activity of antioxidative enzymes were analyzed. We observed different responses to PAW in dependence on plant species. PAW can be used in certain conditions as an environmentally friendly fertilizer.

This work was supported by VEGA 1/0419/18 and APVV-0134-12 grants.

Air plasma inactivation of clinically relevant single and mixed species biofilms

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Bacterial biofilms are collections of microorganisms embedded in a self-produced matrix of extracellular polymeric substance (EPS) [1]. The EPS provides the biofilm with a multilayered system of defense making them highly tolerant to disinfectants and antimicrobials agents. Biofilms formed by opportunistic pathogenic bacteria (such as Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa) play a pivotal role in the formation of persistent medical device-associated infections and chronic infections [2].

Because conventional antimicrobial agents in many cases cannot efficiently eradicate biofilms, alternative strategies need to be developed. The efficacy of cold atmospheric pressure plasmas (CAP) in controlling clinically relevant biofilms has been widely reported [3]. The main advantage of CAP decontamination is the simultaneous generation of a diverse array of reactive species that aim to multiple targets within the biofilm. The understanding of the fundamental plasma-biofilm interactions which may drive persistence in bacterial populations still have to be fully understood.

The plasma source considered in this study employed a surface barrier discharge (SBD) electrode housed within a sealed chamber. Biofilm samples were placed 3 mm beneath the energized electrode (semi-direct treatment). To isolate the effects of temperature, UV radiation and short-lived reactive species, a mechanical pump was used to draw the effluent from the treatment chamber in to a second enclosure where further biofilm samples were exposed (indirect treatment).

Biofilms grown on polymeric coupons were subjected to both semi-direct and indirect treatments simultaneously and in triplicate. Two plasma powers were considered: a low-power discharge (P = 8.4 W) and a high-power discharge (P = 12.2 W) to provide a variation in the generated gas phase chemistry. Comparison of the microbial reduction between the semi-direct and indirect treatments under the same power conditions revealed considerable differences, with the semi-direct treatment being considerably more effective. To explain these differences a computational air plasma model was used to investigate the gas phase chemistry as a function of spatial position downstream of the discharge electrode.

This work was supported by the UK Engineering and Physical Sciences Research Council [grant EP/N021347/1].

References

In this talk, two main categories of plasma sources - plasma jets [1] and dielectric barrier discharges [2] interacting with dielectric surfaces will be discussed. The use of these sources in biomedicine results from the efficiency of producing reactive species and their ability to treat biological surfaces having convex, concave or sloping topography. We will discuss the plasma ability to propagate along nonplanar and rough surfaces and distribute reactive species along the surface. We will address the basic mechanisms of the production of reactive oxygen and nitrogen species in different discharge configurations. We will also discuss results from a computational investigation of the effect of conductive and dielectric surfaces on the plasma jet (bullet) propagation, plasma bullet reflection and the back-bullet evolution (Fig. 1).

The use of APPs in biomedicine often involves a thin layer of liquid covering the surface (tissue) being treated. We will address a few questions in this regard on how charged species, neutrals and radicals are transferred from plasma to the liquid phase.

This investigation is based on the 2D computational results using the modeling platform nonPDPSIM, which solves transport equations for charged and neutral species, Poisson’s equation for the electric potential, the electron energy conservation equation for the electron temperature and Navier–Stokes equations for the neutral gas flow.

The author is grateful to Prof. M. J. Kushner (Univ. of Michigan) for initiating her interest to this topic. This work is supported by the Russian Foundation for Basic Research under Grant 17-52-53044.

References
Intracellular responses in apoptotic cells to reactive species in plasma treated liquids

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The reactions that occur in liquids irradiated with non-thermal plasmas have been studied. In indirect irradiation, plasma acts as a source of a rich variety of reactive species, such as OH and NO. Light can also play roles in plasma-induced effects. Since the interaction is mostly limited in liquid reactions, long-lived species are regarded as participating in interaction occurring at biological surfaces.

In cancer therapy, for example, modifications of liquids in contact with cells or tissues play important role in the resulting effects. Together with effects on cell membranes resulting from plasma-induced changes in electric fields, responses to the reactive species also occur. We have studied radical kinetics and electrical effects to fully elucidate these phenomena [1-15]. Controlling these reactions is difficult due to the nonequilibrium states that are thermodynamically unfavorable. To overcome this challenge, the dynamic behavior of plasmas must be characterized in situ at all hierarchical levels (i.e., liquid, cell, tissue, organ, body).

The authors would like to thank N. Kurake, R. Furuta, Y. Kurokawa, D. Kanno, Y. Hosoi, S. Maeda, T. R. Brubaker, T. Tonami, S. Yamaoka of Nagoya University for technical assistance; and K. Takeda, T. Ohta, and M. Ito of Meijo University; K. Nakamura, H. Kajiyama, H. Hashizume, T. Tsutsumi, H. Kondo, M. Sekine of Nagoya University for fruitful discussion. This work was supported by JSPS-KAKENHI Grant No. 24108001, 24108002, and 17H02805.

References

Progress and Opportunities in Cancer Immunotherapy

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Immune-based therapy has offered the possibility of safe and effective cancer treatment since William Coley began experimenting with a mixture of heat-killed bacteria to stimulate the body’s “resisting powers” more than 100 years ago. For decades, that potential remained unexploited as our understanding of cellular and molecular immune processes lagged behind our collective desire to manipulate them. In the last five years, we have seen an unparalleled increase in the number of cancer therapy approvals focused on immunological mechanisms of action. These are being applied in a variety of cancers, employing several different approaches, the most notable of which are checkpoint inhibitor blockade and cellular therapy using genetically modified immune cells.

The immune system employs multiple systems of checks and balances to help ensure that immune responses appropriately target pathogenic microorganisms and toxins, without wasting resources on harmless invaders or causing autoimmunity. However, these also limit antitumor immunity and several “checkpoint inhibitor blocking” therapies have now been developed to manipulate these processes and shift the balance towards effective immunity. The first checkpoint inhibitor therapy, ipilimumab was FDA approved in 2011 for melanoma, and now several checkpoint inhibitors have been approved for multiple disease indications.

Adoptive cell therapy using patient immune cells engineered to express cancer-targeted receptors are a much newer therapy, and the first two treatments were FDA approved in 2017 for patients with certain B-cell malignancies. By engineering and expanding patient immune cells to produce enormous quantities of cancer-targeted cells, these treatments have produced remarkable response rates in patients whose disease is progressive and refractory to all other therapies.

While these therapies have made a profound impact for a subset of cancer patients and more experimental treatments are expected in the near future, several challenges remain before we see a meaningful reduction in the ~9 million deaths worldwide each year from cancer.

Innovative solutions that engage a patient's immune system to treat cancer and prevent its recurrence may be needed to fill this unmet medical need. Indeed, chemo- and radio-therapies traditionally viewed as ablative are now known to rely in part on immune-mediated mechanisms to treat patients. Similarly, plasma-based therapies are also showing promise as immune-modulating interventions which may be developed and exploited to improve anticancer immunity and patient outcomes. Combination approaches employing established and novel therapies may finally deliver on cancer immunotherapy's 100-year promise to eliminate cancer.
Experimental studies of nanosecond-pulsed DBD in atmospheric air: fast imaging and spectroscopy

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Dielectric barrier discharges (DBDs) are non-equilibrium low-temperature discharges. Uniform dielectric barrier discharges have many potentially transformative industrial applications, including uniform thin-film deposition, surface modification of polymers, sterilization of biological samples, treatment of living tissues and cells for their advantages of low gas temperature, moderate power density, uniform energy distribution, controllability of chemical composition and so on. Uniform DBDs are traditionally generated at special conditions (e.g., low pressure, rare gases), and in atmospheric air are of filamentary nature. Recent developments in pulsed power generation technology allowed controllable application of fast-rising short (nanosecond) high voltage pulses for generation of pulsed discharges. In our preliminary studies we have been able to perform fast imaging of the discharge development on nanosecond time scales in atmospheric air, and show transition of DBD from filamentary to uniform mode. We show that the discharge uniformity may be achieved in the case of strong overvoltage (provided by fast rise times), when anode-directed streamers are formed. Here we present our results on fast ICCD imaging of DBD in atmospheric air for uniformity analysis, as well as time- and space-resolved temperature and local electric field measurements using OES. We also report on measurements of ROS delivery into liquid.

This work is funded by the NSF/DOE Partnership in Basic Plasma Science and Engineering (DOE grant DE-SC0016492, PI: Dobrynin).
Plasma-Induced Long-Term Immune Effect on Cancer Tumors in Mice

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Plasma treatment on cancer tumors is expected to activate immune response and cause systemic effects [1–3]. Our previous study demonstrated an anti-tumor immune response induced by nanosecond pulsed streamer discharge in vivo [3]. In [3], mouse melanoma tumors (B16-F10) were subcutaneously injected into the right and left hind legs of mice, and only the right-side tumors were treated using the streamer discharge for 10 min per day for 3–7 consecutive days. The plasma treatment delayed the growths of the non-irradiated left-side tumors as well as those of the irradiated right-side tumors from the day next to that of plasma irradiation. The rapid abscopal tumor-suppression effect suggests the activation of innate immune response. In addition, much amount of IFN-γ cytokine was observed in the spleens of plasma-treated mice using ELISA. It suggests the activation of adaptive immune response as well. If the plasma treatment induces tumor-specific adaptive immune response, a long-term systemic effect is also expected. In the present study, the long-term systemic effect was examined using re-challenge experiment, and CD4 and CD8 positive cells in the re-challenged tumors were counted using flow cytometry.

C57BL/6 mice were subcutaneously inoculated with B16-F10-luc cells to their right hind legs. After 4 days, tumors were treated with the nanosecond streamer discharge for 10 min per day for 5 successive days. The tumors were surgically resected after the last plasma treatment. Two weeks after the resection, the mice were subcutaneously re-inoculated with B16-F10-luc cells to their left (not right) hind legs. Two weeks after the re-inoculation, 40% of the mice in the plasma treated group (2/5) had tumors, while 100% of the mice in the control group (3/3) had tumors. This suggests that the plasma treatment induced long-term anti-tumor systemic effects in mice.

The re-inoculated tumors were surgically resected two weeks after the re-inoculation and analyzed using flow cytometry. The results showed that the ratio of CD8 positive cells to the tumor infiltrated lymphocyte was much higher for the plasma treated group than the control group (P = 0.0005). This could be the result of the immune response to the tumor-associated antigen strengthened by the plasma treatment.

This work was supported by JSPS KAKENHI Grant Numbers 24108003, 16H04312, and 16K14208.

References

Plasma jets for cancer treatment and dermatology

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Plasma jets are widely used in the new research field of plasma medicine because of their versatility; their working power can be adjusted over a very large range from fractions of milliwatts to tens of watts; they can be operated in pulsed mode or in AC mode over a very large range of frequencies (few Hz to GHz); The operating plasmagen gas of jets may also be varied in order to tune the nature of reactive species \cite{1}; above all they provide an effective treatment without any mechanical “hard” contact, between the gaseous plasma jet and the treated living tissues \cite{2}. Jets designed for cancer treatment \cite{3} are not necessarily the same as the ones designed for dermatology or cosmetology as the flux of Reactive Oxygen and Nitrogen Species (RONS), of UVs, of heat and the amplitude of the electric field has to be adjusted to the specificity of the target.

In the present work we report the use of 3D prints plasmas jets in two main fields: i) skin grafting in dermatology, ii) tumor reduction in oncology. In these two topics, in-vivo experiments are presented as well as comparisons with in-vitro and ex-vivo results. Chemicals produced by these 3D single-jet and multi-jet are quantified in the case of in-vitro experiment. Depending upon the power of the plasma jet, a special care may be paid to the heat transfer to living tissues using numerical simulation.

In the case of dermatology, a low power single-jet is used to evaluate in-vivo potentialities of accelerating skin healing after skin grafting on mice. This is completed by a in-vitro and ex-vivo study of angiogenesis. It is shown that angiogenesis is strongly enhanced in-vivo leading to functional blood vessels. Plasma treatment is effective though the skin barrier.

In the case of tumor reductions, high power multi-jet and cooled multi-jet have been used on TC1 and CT26 orthotopic and ectopic tumor implanted subcutaneously on mice; a careful monitoring of the skin temperature has been performed during plasma treatment. Tumor reductions were observed when the skin temperature exceeds 45C.

Modeling and numerical simulations play an important role in plasma medicine research, as in many other disciplines of sciences and technologies. In plasma medicine, a typical system involves a plasma device, which produces a plasma, and a biological system that interacts with the plasma directly or material surfaces or media that were treated by the plasma in advance. Modeling and numerical simulation of such plasmas have been widely performed by various authors. On the other hand, modeling and numerical simulation of biological systems are still rare as typical biological systems are too complicated to be modeled mathematically. In this presentation, we shall review three systems for which the authors have developed models and performed numerical simulations. The first system is transport and chemical reactions of chemically reactive species, including ions and electrons, introduced or generated in water exposed to a low-temperature atmospheric-pressure plasma (APP). The governing equations are reaction-diffusion-advection equations coupled with Poisson equation. The rate constants, mobilities, and diffusion coefficients are obtained from the literature. The gaseous species are given as boundary conditions and time evolution of the concentrations of these chemical species in pure water is solved numerically as functions of the depth in one dimension. The second system is deposition of a thin film on or surface modification of a biomaterial by plasma treatment. For this purpose, we use molecular dynamics (MD) simulations to estimate the film growth and the formation of some functional groups such as primary amines. The third system that we shall discuss is a set of metabolic reactions of Escherichia coli (E. coli) exposed to a plasma-induced stress. The computational model is based on the flux balance analysis (FBA), where the fluxes of the metabolites in a biological system are evaluated in steady state, i.e., under the assumption that the fluxes do not change in time. The fluxes are determined with linear programming to maximize the growth rate of the bacteria under the given conditions. Although FBA cannot be directly applied to dynamical responses of metabolic reactions, the simulation still gives insight into the biological reactions to exogenous chemical species generated by an APP.
Dermatology deals with the entire spectrum of disease processes: infectious, neoplastic, degenerative, inflammatory, auto-immune and others. What makes skin diseases special is that they are on the outside and therefore readily accessible. Visual inspection, diagnostic studies, treatments can be performed without physical barriers, thus skin diseases can also serve as a model for disorders of other organs. Plasma treatments are mostly administered directly, making the skin an ideal target, and also allowing the skin to be used to gain information about the effects of plasma on various pathologies in general. The specifics and options of treating skin diseases using plasma will be highlighted through the example of our experiences developing effective treatments for neoplastic and other skin conditions. Current results, future possibilities will be discussed.
Resistance of cancer cells to targeted therapies is often associated with altered cell metabolism. This provides a rationale to target cancer metabolism for therapeutic intervention. Current small molecule inhibitors of cancer metabolism are effective against one or two specific metabolic enzymes, and their efficacy may be compromised by metabolic plasticity with which cancer cells evade inhibition at a single metabolic point. Restriction of metabolic plasticity is therefore central to the effectiveness of metabolism-targeting strategies. While this may be addressed by using several different inhibitors simultaneously, such combinatorial strategies carry the risk of unknown toxicity. It is unknown whether simultaneous inhibition of multiple metabolic regulators may be possible with a single drug.

We hypothesize that simultaneous inhibition of multiple metabolic enzymes is feasible using diverse exogenous reactive oxygen and nitrogen species produced with cold atmospheric plasmas (CAP) and that this can be achieved preferentially against cancer cells. In this paper, we show selective lethality of CAP against drug-resistant cell lines of melanoma, breast cancers and leukemia. Using chronic myeloid leukemia (CML) as an example, we show that a 60 s exposure to CAP induced simultaneous inhibition of multiple glycolytic enzymes (GLUT1, HK1, HK2, PDK1, PDK3, PKM2 and LDHA) and AKT/mTOR/HIF-1α signaling in BCR-ABL1-expressing cells that are resistant to tyrosine-kinase inhibitors. This broad-spectrum targeting was effective in inducing significant apoptosis in mouse BCR-ABL1-expressing cells and primary CD34+ cells from CML patients with limited toxicity to their corresponding normal cells.
Helium plasma as a tool for interacting with cells and pathogens
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This contribution reviews recent activity of the Padova group on the use of helium plasmas in plasma medicine. Following the initial emphasis on disinfection of the cornea [1], the research activity has developed along several research lines, which cover the topics of wound healing, cancer treatment and non-thermal coagulation.

Plasma source characterization from the physical and chemical point of view has been performed, comparing two different sources: a RF source for indirect plasma treatment [2] and a Dielectric Barrier Discharge jet for direct treatment, specifically designed for non-thermal blood coagulation applications. The comparison has included an assessment of disinfection properties. The specificity of helium as working gas has been emphasized by mass spectrometry measurements, which hint to the importance of metastable excited states.

The wound healing activity has seen a set of in vitro tests, which have shown the ability of a RF indirect treatment to stimulate cell proliferation and migration, processes which are related to an increase of intracellular ROS level [3]. Subsequently, an in vivo study on large animals (sheep) has been performed, showing the ability of the plasma treatment to significantly reduce bacterial charge on the wound, to reduce inflammation, to promote the regeneration of cutaneous annexes, such as hair follicles and glands, and to lead to an anticipated induction of blood vessel formation.

The work on cancer treatment has been carried out in vitro, using primary cells cultivated from tissue samples of patients affected by laryngeal and lung cancer. The plasma treatment has been shown to lead to an increased RONS level in cells, with a stronger effect observed in cancer cells than in healthy ones. As a consequence, apoptosis is induced in a remarkable fraction of cancer cells, with a preferential effect with respect to healthy ones. This result could be enhanced by combining the plasma treatment with incubation with a molecule known to increase the ROS level in cells.

Finally, the first results of a project on non-thermal blood coagulation induced by the direct interaction with a helium plasma jet will be reported. In vitro studies have shown that the applications of the plasma indeed accelerates coagulation. The result has been confirmed by in-vivo tests on animal models.

References
Immune-Recognition in Plasma Oncotherapy

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Cancers are the second leading cause of death in developed societies.¹ It is well established that aberrant tumor growth goes along with evasion of cancer cells from immune control.² Specifically, innate and adaptive immunity eradicating on-setting malignancies in some cases selects for cancer cells of low immunogenic profiles. This fuels tumor cell evolution towards variants that subvert immune recognition in order to proliferate and metastasize. A second mechanism of cancer cells is their re-programming or selective attraction of immune cells towards tumor growth-supporting phenotypes including myeloid-derived suppressor cells, M2 macrophages, and regulatory T cells. This concludes three lessons: i) tumor cells can be specifically recognized by immune cells, ii) tumor growth can be limited by appropriate immunity, and iii) this system can be interfered with, making it addressable with tailored therapeutic approaches.

Next to surgery, there are three main approaches in oncology. The first focuses on drugging or selectively killing all cancer cells over non-cancer cells. The second aims at limiting immunosuppression in the tumor microenvironment. The third goals either new or more powerful adaptive immune responses, which can be facilitated by adoptive transfer of immune cells or eliciting immunogenic cancer cell death (ICD). The concept of ICD proposes that cancer cells can die in an inflammatory fashion to sufficiently activate antigen-presenting cells. This allows adaptive immunity to be established against neoantigens derived from mutated tumor proteins in tumor cells.³ Strikingly, all three process have been linked to redox control.

Cold physical plasma not only generates a variety of reactive species that contribute to redox signaling pathways. It has been also suggested to an antitumor effector in each of the three approaches in oncology mentioned above. The concept of immune-recognition in plasma oncotherapy and experimental data in support of this hypothesis will be shown and discussed.

This work was supported by the German Federal Ministry of Education and Research (BMBF) (Grant No. 03Z22DN11 & 03Z22DN12).

References

**Effects of plasma on microbial differentiation and secretion**

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We have examined the potentiality of plasma technology in activating cellular processes and functions in beneficial microorganisms. As an example, a plant growth-promoting bacteria (PGPB) [1] treated with micro DBD plasma (~1.5 W power) showed the enhanced multiplication and motility. Plasma treated bacteria promoted the germination rate of rice seeds, plant growth and rice yield more greatly than non-treated bacteria. We also examined the effect of micro DBD plasma on cellular differentiation and protein secretion in *Aspergillus oryzae* (a filamentous fungus fermenting soybeans and rice) [2]. Our preliminary data show that spore germination percentage slightly increases, and production and secretion of alpha-amylase and protease are enhanced after nitrogen plasma treatment.

This work was supported by the National Research Foundation of Korea (No. 2016K1A4A3914113, 2016R1D1A1B03934922) and the National Fusion Research Institute.

**References**

Delivery and Stabilization of Plasma-Generated Species for Disinfection of Produce

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The growing concern for food safety is the cross contamination of fresh produce that leads to the outbreak of foodborne illnesses [1]. The microbiological load of these pathogenic organisms, such as Escherichia coli and Salmonella, is considered significant requiring disinfectants to reduce microbial growth without affecting the quality of the produce [2]. This problem becomes more difficult when processing cut produce due to the reduced protection from its outer protective layer, the cuticle. Current sterilization strategies involve the use of both chemical and physical methods, however have limited efficacy due to the adverse effects on produce quality [1]. Non-thermal plasma has shown antimicrobial activity against pathogenic organisms on produce with minimal degradation of produce quality [3-5]. The challenges for applying non-thermal plasma are the high buffering capacity of bulk liquids and the presence of organic compounds that reduce treatment efficacy. Presented are two methods to overcome these limitations. First is the treatment of micron-sized water droplets for the disinfection of microorganisms on produce stored in relatively large volumes. Second is the formation of nitrosothiols, allowing for the stabilization and transport of plasma-generated nitric oxide through organic load solutions (OLS) to inhibit microbial growth, where plasma treatment alone cannot as shown in figure 1[6].

![Figure 1 Plasmatron Treated NAC Solutions Inactivate Escherichia Coli Suspended in OLS.](image)

This research is supported by the USDA-NIFA Program on Enhancing Food Safety through Improved Processing Technologies (A4131) grant number 2015-68003-23411.

References

Overview of Plasma Farming in Korea

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Plasma technology has a great potential of an innovative technology to meet the requirements of future agriculture and food because it does not only inactivation of harmful microorganisms but also activation of seedling growth, functional metabolites, and useful microorganisms. Since the early 2000’s the studies on plasma application for agriculture and food have been investigated by various research groups in Korea including universities, national institutes and companies. The project of ‘Plasma Farming’ through the National Fusion Research Institute (NFRI) has been running since 2014 and given opportunities to many of plasma experts to involve the plasma farming and to collaborate with other experts in agriculture and food. ‘Plasma Farming’ is the comprehensive plasma application to the entire agricultural phases from farm to table, which includes whole animal processes for livestock and fishes as well as whole plant processes for crops, fruits and vegetables.

Here we would like to introduce a brief overview of plasma farming program and our R&D consortium in Korea. I will also summarize the interesting studies in the fields of cultivation, postharvest, and food safety and finally discuss future plans.

This work was supported by R&D program of “Plasma Advanced Technology for Agriculture and Food (Plasma Farming)” through the National Fusion Research Institute of Korea (NFRI) funded by the government funds
Transport of reactive oxygen and nitrogen species across native and oxidized phospholipid membrane

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Reactive oxygen and nitrogen species (ROS and RNS, i.e., RONS) play an imperative role in plasma medicine [1], although the underlying mechanisms are still unknown. Until now, most computational studies have focused on the effects of ROS [2,3]. The role of RNS is also important in order to gain insights into the processes of plasma treatment. Indeed, plasma-generated RNS (in combination with ROS) can interact with the cell membrane, and possibly enter into the cell interior, eventually causing there oxidative damage.

In this study we perform umbrella sampling (US) molecular dynamics (MD) simulations [4] to calculate the free energy profiles (FEPs) of RONS across native and oxidized phospholipid bilayers (PLBs), i.e., model systems for the cell membrane. Specifically, we compare the FEPs of ROS (i.e., OH, HO₂, H₂O₂ and O₃) with those of RNS (i.e., NO, NO₂, N₂O₄) penetrating through native and 50 % oxidized PLBs.

Our simulation results suggest that the ROS (except O₃) experience high free energy barriers to reach the center of the bilayer (i.e., hydrophobic lipid tail region) in both native and oxidized cases, whereas these barriers are significantly lower for RNS and O₃, showing higher penetrating capability of these species across the membrane.

Fig. 1. FEPs of RONS across the native PLB.

References

Various solutions of plasma jet (or needles) devices have been carried out for disinfection application. Many difficulties such as high disinfection level, minimal duration of treatment, shape ratio or complex geometry of the tools must be overcome [1]. Their common characteristic is the requirement of a high voltage source as input. Obviously, it is justified by the necessity to reach the plasma ignition voltage value of several kVolt. In some applications, this high voltage source can represent a safety problem, an unsuitable electromagnetic interference source and also a prohibitive solution for compactness specifications.

The piezoelectric property is the capability of some materials to develop an electric potential when mechanical stress is applied on it. This particular property is used in numerous applications such as actuators, sensors, or electrical transformers for several decades [2]. This latter configuration is able to offer huge voltage gain leading to a high voltage on the piezoelectric material surface, high enough to ignite and maintain a cold plasma discharge in neutral gas flow [3-4]. The very high material relative permittivity over 1000, the non-uniform electric potential distribution, the local step-up voltage stage near the plasma location, are the most significant particularities of such plasma generator.

The proposed Piezoelectric Plasma Jet (PPJ) working with argon was developed relying on this principle. The preliminary evaluation of its bactericidal performances was carried out with two different bacteria strain: P. aeruginosa and S. aureus using an adapted EN 13697 protocol. This standard protocol implies to operate at ambient temperature (~20°C) and atmospheric pressure.

The preliminary results have shown the capability of significant bactericidal effect crossing the detection limit value over 6.7 log reduction. These promising results have been obtained with 2.5W input power, on both bacteria strains in 10 minutes exposure on the inoculum with 100ul a droplet water deposit.

*This work was supported by the French National Research Agency ANR-15-CE19-0025.*

**References**

Cold atmospheric plasmas (CAPs) have demonstrated to be quite useful tools in biomedical applications and their relevance in medicine is getting more and more recognized. Drug delivery in cancer cells combining low cytotoxicity and high efficiency remains to be optimized with physical techniques. Several studies have shown that plasma could be used to deliver chemotherapy [1] or nucleic acids [2] via enhancement of cell permeabilization. We investigated the parameters needed for helium plasma to induce cell membrane permeabilization on cancer cells for drug delivery purposes. 10,000 plasma pulses were used at a frequency of 100 Hz for 100 s with an applied voltage of 14 kV. Both plasma electric field and endocytosis involvement in molecular uptake were also studied [3]. Kinetics of plasma-induced permeabilization were studied. Highest permeabilization efficiency was observed when propidium iodide was added after treatment (up to 40% of propidium iodide positive cells in both HeLa and 4T1). No significant toxicity was observed in these conditions as the viability in HeLa cells was measured to be of 80%. Doxorubicin was used as an anticancer drug. A treatment combining doxorubicin and plasma decreased the concentration of doxorubicin needed to achieve 50% of cytotoxicity by enhancing doxorubicin uptake in cancer cells.

References

On the development of a portable, easy scalable and flexible large area treatment device based on textile for medical purpose

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At the moment there is a variety of cold atmospheric plasma (CAP) sources used for research and only some of them are approved as medical products according to Directive 93/42/EEC for plasma medical application. One of these sources is the atmospheric pressure plasma jet the kINPen® Med or the dielectric barrier discharge (DBD) the PlasmaDerm®. For both types the basic biological effects were investigated and the inactivation of a microorganism was shown [1,2]. A main field of application for CAP is the chronic wound care. Nowadays CAP is used only in some clinical centers. The common way to treat wounds at the moment is to bandage them with dressings for example composed of gauze, special films and foams [3].

To enhance this therapy, we present a DBD surface discharge plasma device based on a flexible textile mesh and wire electrodes without any floating electrodes. In this work, we describe aspects of development on the way to a medical plasma source with aspects like high voltage connection and power supply. Furthermore we present an overview of the plasma components investigated in reference to DIN SPEC 91315 [4] during the development and an outlook for a CE conform system (e.g. cytotoxicity) with a portable power supply. With this CAP we can treat big chronic wounds with an area of 100 cm² in seconds instead of minutes and are able to achieve 3 log reduction of bacteria (e.g. E.Coli). With this cost effective single use CAP we expect to take the next step of spreading plasma devices as ordinary tools for chronical wound care.

Fig 1. l: one of the first laboratory prototypes, c: prototype with spacer and HV-connection, r: plasma ignited on textile surface

Calcium and Reactive Species are Required for Plasma Effectiveness in Enhancing Limb Development

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We previously reported that cold atmospheric plasma (CAP) generated reactive oxygen species (ROS) enhanced survival, growth and elongation of limb buds in organ culture after a single 10s treatment. The observed effects were attributed to an increase in intracellular ROS and associated signaling pathways, including the Wnt signaling pathway. The possibility that CAP is enhancing limb bud survival, growth and elongation in a calcium dependent manner is both likely and intriguing, as the roles of intracellular ROS and calcium are intricately associated in activating signaling pathways and effecting cellular responses. In this study, we investigated how the CAP treatment-associated enhancement of limb bud development are controlled by intracellular and extracellular calcium. To investigate the role of calcium in driving the observed CAP effects on limb development, intra- and extracellular calcium was manipulated by the use of chelation and calcium free media, inhibition and activation of calcium related channels and release of calcium stores in the endoplasmic reticulum. Flow cytometry showed that CAP enhanced both intracellular calcium and ROS. Western analysis was used to determine the signaling pathways involved. We show calcium is required for the CAP / ROS stimulated enhancement of limb development (Fig 1.) through the p38 signaling pathway.

![Figure 1](image_url)

**Figure 1** Consecutive images of digit development in an E12.5 embryonic mouse limb. CAP-treated autopods in DMEM with calcium, DMEM with EDTA and BGJb (calcium-free) media at 0, 24 and 96 hours. The limbs 96h limbs were stained with Alcian blue to highlight the cartilaginous elements. Results show that CAP promoted limb elongation only in DMEM media with calcium.

**Acknowledgements**

This work was supported by NIH R01 EB 013011 (Freeman)
Comparison between dielectric and metallic setups for atmospheric pressure plasma multijets

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Atmospheric pressure plasma jets have been extensively studied in the last decade. Thanks to the production of high transient electric field and reactive nitrogen and oxygen species, many applications have been found from material processing to biomedical field. One of the remaining challenge is the size of the jet. At atmospheric pressure, the plasma plume diameter on a target is of the order of a millimeter leading to difficulties where larger areas must be treated. In order to treat extended wounds, to sterilize wider surfaces or to address large-scale processing applications, one needs to develop plasma jet arrays.

The reactor used in this work is a Plasma Gun made of a high voltage electrode inside a glass capillary, surrounded by a grounded electrode. A power supply feeds it with 14kV microsecond pulses from single shot to 4 kHz. The pulsed atmospheric pressure plasma stream (PAPS) [1], composed of an ionization wave front followed by a plasma column, originating from this dielectric barrier discharge, propagates in helium inside the capillary. A linear multijets assembly is added at the end of the capillary. Plasma multijets production have been studied varying following parameters: material of the assembly, size of holes, their distance, wall thickness...

This work focuses on the differences between a dielectric and a metallic assembly. ICCD camera recordings show how the ionization wave propagates inside a transparent dielectric assembly, igniting the jets one after another.[2] However, as the PAPS reaches a metallic one, it transfers its electric potential to the assembly. Then, no plasma propagates into due to the absence of electric field but new plasma ionization waves are generated at each hole at the same time. These two different ways of ignition trigger differences in intensity and homogeneity between the jets. The presence of species was also investigated via EOS or optical filters. A Schlieren setup has been also used to visualize gas flows with and without plasma with different gas flow rates, sizes and numbers of holes. With large enough holes the drop of pressure in the different holes becomes visible as jets are shorter and shorter. Additionally to the ionic wind in each jet, as already studied in [3], interactions between them add a new feature. Repulsive forces bend the jets, more strongly for the first and last ones and in negative polarity. Electric fields measurements run with an electro-optic probe, made by Kapteos and based on Pockels effects, with both assemblies will be compared.

X.D. acknowledges his grant funding Thermofisher Scientific INEL/Région Centre Val de Loire.

References

Nanosecond-Pulsed Dielectric Barrier Discharge Plasma Accelerates Astrocyte Regrowth and Neurite Regeneration Following Physical Trauma In-Vitro

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The central nervous system (CNS), consisting of the brain and spinal cord, has a limited capacity to regenerate following injury or disease [1]. This lack of regenerative capability represents a huge burden, both financially and on the quality of life for patients and caregivers. Following injury or neurodegenerative disease, successful regeneration often requires reformation of long-distance communication fibers, called axons. Long-distance axonal regeneration does not occur in the mature brain, and in particular, following severe injuries, the formation of a glial scar inhibits axon regeneration and cell migration [2]. Strategies to augment axonal regeneration and modulate the post-injury microenvironment are actively being investigated to promote healing and facilitate functional regeneration. Non-thermal plasma treatment is being explored as a tool to promote tissue regeneration by activating endogenous biological processes to promote cell regrowth, differentiation, and/or proliferation [3, 4]. In the current study, we found that when neurons and astrocytes were treated with moderate levels of dielectric barrier discharge plasma, neural regeneration in-vitro was increased due to direct neuron stimulation, as well as indirect stimulation of astrocytes in non-contact co-culture with the neurons.

Financial support was provided by the National Institutes of Health [U01-NS094340 (Cullen) & F31-NS090746 (Katiyar)] and the Department of Veterans Affairs [RR&D Merit Review I01-RX001097 (Cullen) & BLR&D Merit Review I01-BX003748 (Cullen)].

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Applications of dielectric barrier discharge (DBD) based atmospheric pressure plasma jets are often limited by the relatively small area of treatment due to their 1D configuration. Here we present the first results demonstrating generation of 2D plasma “bullets” and 2D plasma jets permitting fast treatment of large targets. Imaging of pulsed DBD in He and ionization wave propagation along the dielectric surface show that DBD evolution starts with formation of transient anode glow, and only then continues with development of cathode-directed streamers. The anode glow can propagate as an ionization wave along the dielectric surface outside of the discharge gap. We show that plasma “bullets” propagation is not limited to 1D geometry (tubes), and can be organized in a form of a planar plasma jet, or other 2D (or even 3D) shapes.

This work was partially supported by the NSF/DOE Partnership in Basic Plasma Science and Engineering grant (DOE grant DE-SC0016492, PI: D. Dobrynin).
Combined Effects of Cold Plasma Components in Chronic Wound Treatment

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Introduction: Cold plasmas are complex mixtures of free electrons and ions, UV radiation, visible light, heat, and many excited species. In particular, the excited oxygen and nitrogen species together with UV radiation and electric fields are responsible for the biological effectiveness of the plasmas. These cold plasmas affect the cellular redox balance and, depending on the composition and duration of treatment, can be adjusted to either stimulate or kill cells. Therefore, well-adjusted cold plasmas are suitable for killing bacteria, while still stimulating wound healing processes. Multi-resistant germs show the same reduction rates as non-resistant strains.

Methods: Recently, we performed the transfer of the basic results into the clinical practice of wound treatment. Therefore, we analyzed wound fluids of more than 30 type II diabetic patients and examined for their cellular and soluble components. Besides the reduction of wound size, we analyzed the bacterial load of those exudates and in parallel the content of cytokines, growth hormones as well as repair enzymes, too. In addition to the analysis of molecular markers, we also applied hyperspectral measurements of plasma treated wounds, in order to analyze hemoglobin, tissue oxygenation as well as near infrared perfusion, to evaluate the efficacy of cold plasma treatment.

Results: Cold plasma treatment could stimulate wound healing, accompanied by a reduction of wound contamination, a significant diminished inflammation as well as a positive influence of on the level of Matrix-Metallo-Proteinases (MMP). Wound size was reduced in more than 80 percent of all patients. While the total load of microorganisms could be lowered in all wounds, there was no significant difference in the composition of the bacterial load at beginning of plasma therapy compared to the end of plasma application. However, cytokines such as IL-6 and IL-8 were significant reduced – indicating a reduced inflammation. Furthermore, cold plasma treatment led to a significant reduction of MMPs – known to have a negative effect on chronic wounds at elevated levels. The application of hyperspectral technology as a diagnostic tool for the evaluation of plasma treated wounds revealed a significant improvement of microcirculation, which was accompanied by elevated levels of tissue oxygenation, further enhancing wound healing processes.

Summary: Cold atmospheric pressure plasmas are useful tools for the treatment of chronic wounds. Besides anti-microbial effects plasma can stimulate wound healing by accelerating cell proliferation via modulation of signaling molecules. Furthermore, cold plasma reduced the amount of MMPs further promoting wound healing. Finally, plasma treatment leads to an improvement of microcirculation and therefore to an elevation of oxygen and nutrition within the tissue. Therefore, all factors: reactive species in combination with UV-light and electrical fields promote healing of chronic wounds and are unmatched by any other treatment option.
Role of scavengers to influence the antibacterial effectiveness of plasma-treated physiological saline

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From basic research using mammalian cells it is well known that reactive oxygen and nitrogen species (RONS) play a key role for biological plasma effects [1]. Among other methods, this was tested by addition of scavengers, i.e. chemical substances that are able to react with and inactivate RONS in a more or less specific manner [2]. Moreover, it is well known, too, that plasma treatment of aqueous liquid is useful to generate an antibacterial effective solution [3]. The aim of this study was to investigate whether in general and on which RONS the antibacterial activity of plasma-treated liquid is based. For plasma treatment of 5 ml physiological saline solution (0.85\% NaCl), a surface dielectric barrier discharge (surface-DBD) arrangement in the upper shell of a 60 mm Petri dish was used [3]. After plasma treatment over different times, various scavengers were added and, after 2 min shaking, test microorganism \textit{Escherichia coli} K-12 (DSM 11250) was added. After 5 min exposure time, the total viable cell count was determined. As scavengers were used: the enzymatic scavenger catalase as well the non-enzymatic scavengers dimethyl sulfoxide, ascorbic acid, hypotaurine, ebselen, and sodium thiosulfate. Without scavenger addition, a complete \textit{E. coli} inactivation (initial concentration \(\sim 10^6\) cfu/ml) was realized using 2-3 min plasma-treated liquid. Using ascorbic acid, hypotaurin, ebselen and sodium thiosulfate, a complete loss of antibacterial effectiveness of plasma-treated saline was found. Addition of dimethyl sulfoxide resulted in a partial reduction of antibacterial effectiveness. Thus, no selective scavenger specific inactivation was found. Several cross reactivity of the scavengers cause this. Consequently, using this experimental setup, any identification of RONS being specifically responsible for the antibacterial activity of plasma-treated physiological saline was not possible. However, surprisingly the lowest inhibiting activity was found using catalase, the only enzymatic scavenger with the most selective scavenging activity for hydrogen peroxide (H\(_2\)O\(_2\)). Therefore, the most important finding of this study is that H\(_2\)O\(_2\) seems to be not the main component responsible for antibacterial activity of plasma-treated liquid.

References

Impact of Cold Atmospheric Pressure Plasma on Microbial Burden in Chronic Wounds of Diabetic Patients

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Recent results show that the use of cold atmospheric plasma (CAP) is promising for the treatment of chronic wounds [1]. However, there is still uncertainty about how such treatment affects the microbiota of those wounds. Therefore, the aim of the present study was to find out if the CAP influences the quantity of microbial populations and the presence of different microbes in chronic wounds of diabetic patients.

Methods: Since the beginning of 2016, in “Competence Center Diabetes” in Karlsburg (Germany), foot ulcers of diabetic patients are being routinely treated with plasma using kINPen MED® (neoplas tools GmbH). The study was performed from June to September 2017. Seven ambulant patients who were in different treatment stages were used. The patients were treated usually once in every two weeks. Before and after plasma treatment, swab samples (N=23 each) were collected and transported on ice to the laboratory. The fresh samples were serially diluted and plated using spiral plate method on six types agar: Columbia agar (CBA with 5% sheep blood, SB) for total aerobic bacteria, Schaedler agar (SBA with 5% SB) for total anaerobic bacteria, Columbia CNA agar (CNA with 5% SB) for gram-positive bacteria, MacConkey agar (MCA) for gram-negative bacteria, Candida chromogenic agar (CCA) with chloramphenicol and Sabouraud glucose agar (SGA) with penicillin and streptomycin for fungi. Apart from SGA (aerob at 30°C), CBA, CNA, MCA, CCA (aerob) and CBA (anaerob) were cultured at 37°C. After 1-2d in case of bacteria and 7d fungi incubation, colony-forming units (CFU) per ml of medium were quantified automatically using a colony counter. After quantification from each agar different colonies were isolated, purified and then cryo-conserved. Obtained cultures were identified via MALDI-TOF mass spectrometry, and 5 strains of each 10 most commonly isolated bacteria were selected for antimicrobial susceptibility testing.

Results: No fungi could be detected on both agar types after macroscopic observation. Apart from gram-positive bacteria, the counts of gram-negative, total aerobic and anaerobic bacteria significantly decreased after plasma treatment. From 535 isolated cultures, 31 bacterial species belonging to 19 different genera were identified and 3 cultures could be identified to only genus level. Ten most often isolated species (ordered by frequency) were Proteus mirabilis (PM), Staphylococcus aureus (StaA), Pseudomonas aeruginosa (PA), Escherichia coli (EC), Klebsiella oxytoca (KO), Streptococcus agalactiae (StrA), Enterococcus faecalis (EF), Serratia marcescens (SM), Enterobacter cloacae (EntC), and Citrobacter koseri (CK). The strains of 6 species (EntC, StrA, SM, CK, EF and PA) were resistant to 3 to 9 antibiotics, of 3 species (PM, KO, and StaA) to only 1 or 2 antibiotics, and apart from one EC strain which was resistant to 7 antibiotics no resistance was detected in 4 EC strains.

Conclusion: The used CAP treatment did not affect the diversity of bacteria, but it could significantly reduce the amount of most bacterial groups. However, in the period between treatments, it seems that bacteria can recover and reach to about the same original amount. Nevertheless, the reduction in wound size and improvement in the overall well-being of the patients could be observed.

Bactericidal property of peroxynitric acid (HOONO$_2$) in cryo-preserved plasma-treated water with the reduced-pH method for effective and safety disinfection

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In this paper, the active component of plasma-treated water (PTW) is discussed. For the plasma disinfection of human body, we have developed the reduced-pH method with direct plasma exposure to bacteria suspension [1], which brings stronger bactericidal activity under the acidic condition (pH < 4.8). This is thought to be brought by hydroperoxy radical (HOO•) generated from superoxide anion radical (O$_2$•) by acid dissociation equilibrium (pKa 4.8). We found that PTW also has strong bactericidal activity under acidic condition and the dependency on pH was almost same. From physicochemical study on chemical kinetics, it was confirmed that lower temperature drastically brings longer half-life period, and the bactericidal activity is kept by cryo-preservation [2]. High performance PTW, corresponding to the disinfection activity of 22 log reduction (against Bacillus subtilis spore), can be obtained by special plasma system equipped with cooling device. This is equivalent to 65% H$_2$O$_2$, which is harmful for human. But, the bactericidal activity is deactivated soon at higher temperature (4 sec. at body temperature), and toxicity to human body seems low. PTW is a sort of ideal disinfectant for human body.

Although PTW has many chemical components, respective components in PTW were analyzed by ion chromatography (IC) [3]. In addition to peaks of H$_2$O$_2$, NO$_2^-$ and NO$_3^-$, a specific peak was detected and only this fraction had bactericidal activity (Fig. 1). This means that active ingredient was successfully purified. Considering the activation energy for deactivation of PTW and other experimental results, we assumed that HOONO$_2$ (PNA: peroxynitric acid) is active ingredient. From IC analysis of chemically synthesized PNA, the same specific peak was seen. From quantum chemical calculation on PNA decomposition, PNA can releases HOO• and the activation energy is almost same. So we conclude that PNA is a key chemical species of cryo-preserved PTW with the reduced-pH method. Although the existence of PNA has been known since a century ago [4], the usage of PNA to disinfection is the first in the world. Based on chemical kinetics, bactericidal properties of this innovative disinfectant were investigated. It is thought that PNA is generated only on the thinner surface of the plasma irradiated solution, where pH is extremely low, and PNA is stored in PTW if the temperature is enough low. Peroxynitric acid chemistry would contribute to plasma medicine.

Fig. 1 Ion chromatograph analysis of PTW

Gastrointestinal hemostasis for anticoagulant dosed model by 3D printed low temperature plasma jet

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In step with the aging of the population, arteriosclerotic diseases such as ischemic heart disease, arrhythmia, cerebral infarction are increasing, and patients which takes antithrombotic drugs tend to increase. And, the necessity of endoscopic therapy such as endoscopic submucosal dissection has increased by advances in endoscopic technology. Although continuation of antithrombotic drugs increases the frequency of bleeding complications after endoscopic biopsy or treatment, withdrawal of antithrombotic drugs increases the risk of complications such as infarction and thrombosis. Therefore, a minimally invasive hemostasis device which can perform endoscopic treatment to patient receiving antithrombotic drug is necessary.

In recent years, heat sensitive material such as living bodies can be irradiated with plasma, since atmospheric low temperature plasma (LTP) can be generated at from room temperature to around 100ºC using argon and helium gases. Using this plasma, the effective blood coagulation were investigated. However, conventional plasma sources are manufactured by machining, there was limitation for miniaturization.

To make new mini-size plasma source, which can be inserted into instrument port of endoscope, we used 3D printer. The designing of metal 3D printing by CAD is flexible, and high accuracy object can be made. Using 3D printing, we confirmed that 3D printed plasma jet can be inserted in instrument channel of endoscope (I.D. 3.2 mm). In addition, the plasma jet can generate stable plasma with various gas such as argon, helium nitrogen and carbon dioxide. And the plasma is touchable and the plasma gas temperature was below 40 ºC. In this study, the hemostasis effect on anticoagulant dosed model was investigated using the plasma jet.

The hemostasis effect on porcine gastric mucosa, which antithrombotic drug (warfarin) is dosed, was investigated. In this setup, carbon dioxide (1 L/min) was selected as plasma gas species, and the PT-INR of pig was 2.1. As a result, the hemostasis effect was confirmed by 90 s plasma treatment as shown in Fig. 1. In this presentation, the details of the experiments and medical application will be presented.

Fig. 1. Hemostatic treatment by low temperature plasma under endoscope
The consequences of well plate geometry and gas flow on plasma jet interactions with liquid media

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Research on plasma activated media (PAM) has demonstrated selectivity in treatment of cancer and normal cells.[1] In these experiments, the PAM is usually a liquid medium in a single or multi-well plate container that is exposed to a plasma jet. Parameters such as the distance between the liquid and the plasma nozzle of the jet or the geometry of the liquid container can affect the chemistry of the PAM, and so the biological outcomes. For example, the liquid containers in experiments have different size and shapes; from a large surface to volume ratios as in Petri dishes to smaller ratios like in centrifuge tubes or 96-well plates. Geometrical parameters of a liquid container such as width and depth of a plate can alter the gas flow, effluent of plasma, or chemical composition of the gas layer above the liquid. For instance, water vapor density can be higher in a deep plate compared to above a Petri dish. Such geometrical parameters can affect the transport of plasma produced species to the surface of the liquid and eventually, the chemistry of the PAM.

To investigate these possible geometrical dependencies on PAM, we modeled an atmospheric pressure helium plasma jet with two ring electrodes embedded in a dielectric material operating in ambient air – a configuration often called the plasma pencil.[1] The top electrode is powered by approximately 100 ns pulses and the bottom electrode is grounded. In these calculations, the plasma jet is in direct contact with water in a cell culture plate. The modeling platform is nonPDPSIM, a 2 dimensional plasma hydrodynamics model which solves Navier-Stokes equations for neutral gas flow, Poisson’s equation for electric potential, transport equations for neutral, charged species, and electron energy, and radiation transport for photoionization.[2] The reaction mechanism includes 78 gas and liquid phase species and more than 1000 reactions. We will discuss the consequences of the geometry of the plate (e.g., depth, diameter, jet-to-well distance) on the gas flow field, propagation of the plasma ionization wave and activation of the liquid in the well-plate. (See Fig. 1.) Assessments will include production of of long-lived reactive species such as H$_2$O$_2$, O$_3$, NO$_3^-$, and NO$_2^-$ in gas and liquid phase and radicals and short-lived species such as OH$^*$, O$_2^*$, NO$^*$, and ONOOH.

References

Figure 1. The effluent and gas flow of a He plasma jet interacts with water layer in a plate with a radius of 8 mm.
Reactive species in water exposed to the COST plasma jet: Experiments & modelling

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The vast biomedical potential of cold atmospheric pressure plasmas (CAPs) is governed by the formation of reactive species, formed upon interaction of CAPs with the surroundings [1]. In biological milieu, water plays an essential role. The development of biomedical CAPs thus requires understanding of the sources of the reactive species in aqueous media exposed to the plasma [2]. This is especially important in case of the COST RF plasma jet, which was developed as a reference microplasma system, and used in biomedical plasma research [3]. We investigated the formation of the reactive species in aqueous solutions exposed to the COST plasma jet by combining experimental and modelling approaches.

The concentrations of the reactive species in the gas phase plasma were obtained using a 0D chemical kinetics computational model. A 3D fluid dynamics model was developed to provide information on the induced humidity in the plasma effluent. The liquid phase species were analysed using UV-Vis spectroscopy and spin trapping with hydrogen isotopes and electron paramagnetic resonance spectroscopy. The comparison of the experimental trends for the formation of the species as a function of the feed gas and effluent humidity with the modelling results suggests that the reactive species detected in our system, i.e., H₂O₂, OH and H, are mostly formed in the gas phase plasma directly inside the COST jet (Fig. 1). Additionally, using ¹H-NMR spectroscopy we show that the efficacy of the delivery of the species from the plasma jet into liquid samples was independent of the plasma ignition. The results clearly demonstrate the fundamental difference between cross field plasma jets (such as the COST jet) and parallel field plasma jets [4].

This work was supported by the European Marie Sklodowska-Curie Individual Fellowship ‘LTPAM’ within Horizon2020 (grant no. 657304).

References

Anti-cancer capacity of plasma-treated PBS: effect of chemical composition on cancer cell cytotoxicity

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The use of plasma-treated liquids (PTL), simple buffered solutions, like phosphate buffered saline (PBS) in particular, for cancer treatment is gaining increasing interest [1]. In this study, we evaluated the anti-cancer capacity of plasma-treated PBS (pPBS), by measuring the concentrations of NO$^2$⁻ and H$_2$O$_2$ in pPBS, for different values of gas flow rate, gap and treatment time, as well as the effect of pPBS on cancer cell cytotoxicity for three different glioblastoma cell lines [2]. Our experiments reveal that pPBS is cytotoxic for all conditions investigated. For different conditions, the relative concentrations of H$_2$O$_2$ and NO$^2$⁻ are dissimilar. Moreover, a small variation in gap between plasma jet and liquid surface (10 mm vs 15 mm) significantly affects the chemical composition. When the effects of gas flow rate, gap and treatment time are studied, it is found that H$_2$O$_2$ may be a more important species for the anti-cancer activity of pPBS than NO$^2$⁻. Additionally, we found that pPBS might be more suitable for practical applications in a clinical setting than (commonly used) plasma-treated cell media, because of its higher stability. Finally, we also used a 0D model, developed for plasma-liquid interactions, to elucidate the most important mechanisms for the generation of H$_2$O$_2$ and NO$^2$⁻.

Fig. 1. Effect of pPBS on cancer cell cytotoxicity (left y-axis), and comparison with the concentrations of NO$^2$⁻ and H$_2$O$_2$ in pPBS (right y-axis), for 11 different plasma operating conditions.

This work was supported by the Fund for Scientific Research (FWO) Flanders (Grant No. 11U5416N) and the European Marie Sklodowska-Curie Individual Fellowship “LTPAM” within Horizon2020 (Grant No. 743151).

References

Influence of the composition of plasma-activated medium on osteosarcoma

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Over the last few years, significant attention has been paid to biomedical applications of Atmospheric Pressure Plasmas (APP). Plasma chemistry leads to the generation of an abundance of reactive species which are suspected to play a key role in selective cancer cell death \cite{1} without damaging surrounding healthy tissues \cite{2}. The anti-cancer properties of the APP have been described in many cancer cell lines, such as breast, skin, lung, pancreas, cervix and brain cancers and only more recently in bone cancer cells \cite{3-4}. Although the cell death mechanisms are not yet precisely known, this selectivity towards cancer cells is associated in literature to the reactive oxygen and nitrogen species (RONS) generated by the plasma treatment, among other potential actors.

In this work, we aim at comparing the effects of different plasma jets on a variety of cell culture media, and discussing how the differences in liquid media composition and plasma conditions affect bone cancer cell viability and proliferation. To that aim, different osteosarcoma cell lines have been studied (ie. SaOS-2, MG63 \& MC3T3), and compared to different healthy cells involved in the bone regenerative process (ie. pluripotent or from either bone or from connective tissue). This allowed evidencing the selectivity of the medium. Moreover, the modifications in medium composition allowed identifying some key actors involved in the bone cancer cell death mediated by plasma-activated media (PAM). The generation of intracellular RONS triggered by PAM, and in general the biological effects observed are discussed with regard to the different reactive species generated in the PAM (ie. $\text{[H}_2\text{O}_2\text{]}$, $\text{[NO}_2\text{]}$, short-lived RONS).

Fig.1: Effect of different PAM & different compositions on bone osteosarcoma cell viability.

Authors acknowledge the financial support of MAT2015-65601-R project (MINECO/FEDER, EU) and from the ERC under the EU's Horizon 2020 research and innovation programme (grant agreement No 714793).

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Mechanisms of plasma jets impinging upon liquids

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Plasma–liquid interaction is an increasingly important topic in plasma medicine and plasma materials synthesis. A significant focus has been placed on mass transport at the plasma–liquid interface to explain the observed diffusion of reactive oxygen nitrogen species\textsuperscript{1,2}, yet linking liquid flow behavior with plasma jet parameters remains a challenge. In this work, particle image velocimetry measurements in conjunction with infrared and pH imaging were used to identify significant physical, chemical, and electrostatic mechanisms in plasma jet–liquid systems.

A helium, atmospheric-pressure plasma jet was used to treat water-based solutions held in a rectangular quartz cell. The propagation of surface phenomena, notably acidification and evaporative cooling, into the bulk liquid were strongly correlated spatially and temporally via jet-induced advection. Gas flow across the liquid surface accounted for most mass loss in the system. Mass loss was increased during plasma treatment, potentially due to an increased gas temperature at the interface and increased liquid convection resulting in a higher vapor pressure gradient. Recirculation flows in plasma-treated liquids were larger than in helium treatments. We investigate the plasma effects on jet turbulence at the interface and possible liquid-phase mechanisms that attribute to increased vortex size.

Electrostatics played a significant role in interface turbulence in cases where the plasma establishes filamentary discharges to the liquid surface. Electric fields within the liquid were visualized using the propagation of MnO\textsubscript{4}\textsuperscript{-} ions and compared to observed flow patterns.

This work was supported by JSPS-KAKENHI Grant No. 24108002.

References

Physical plasma-treated saline promotes an immunogenic phenotype in CT26 colon cancer cells

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Metastatic colorectal cancer is the fourth most common cause of cancer death. Some current options in palliation such as hyperthermic intraperitoneal chemotherapy (HIPAC) present severe side effects (1). Recent research efforts suggested an adjuvant therapeutic role of liquid enriched with oxidants via exposure to cold physical plasma (2). In view of generating a clinically accepted treatment regimen, we used saline solution for CT26 murine colon cancer cell treatment in vitro. Cells were readily oxidized and showed reduced metabolic activity, cell growth with subsequent apoptosis, cell cycle arrest and an upregulation of marker for immunogenic cell death (ICD). Such can act as danger associated molecular patterns (DAMPs) and can generate an anti-tumor immune response. Such an immunogenic relevant therapy may be associated with prognostic benefit in tumor patients (3). This finding was accompanied with morphological changes such as nuclear swelling, decreased cell roundness, and formation of cell extensions. In real-time deformability cytometry, cells treated with plasma- but not H2O2 saline showed an increased biomechanical stiffness. This was linked to re-arrangement of actin fibers and reduction of motility in treated but viable cells. It is well known that hydrogen peroxide is an important component in this liquid. Peroxide treatment corroborated most but not all findings with plasma-treated PBS but was inferior in inducing toxicity in 3D tumor spheroids. Our results suggest oxidant saline solution to inactivate metastatic colon cancer cells in disadvantage to their metastatic spread. Animal experiments will show the in vivo effect of such a therapy to evaluate the prognostic benefit and whether this should be considered as adjuvant to chemotherapy.

This work was supported by grants funded by the German Federal Ministry of Education and Research (BMBF), grant numbers 03Z22DN11 (SB) and 03Z22CN11 (BF, OO)

References

Direct cold atmospheric plasma (diCAP) enhances microcirculation of the human skin independent of pressure

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Pressure influences the microcirculation of the human skin [1]. Application of direct cold atmospheric plasma (diCAP) with single use electrodes inevitably leads to mechanical pressure on the treated tissue during application. The aim of this study was to discriminate pressure-induced and plasma-induced effects on the microcirculation of healthy human skin.

Microcirculation parameters such as tissue oxygen saturation, blood flow, blood flow velocity and local relative hemoglobin were recorded for up to one hour before and after diCAP-application with optical techniques such as laser Doppler velocimetry and white light spectrometry [2]. Plasma was applied to the dorsal forearm of 10 healthy volunteers for up to 270 s. The experimental setup and protocol was designed to differentiate between pressure- and plasma-induced effects. In addition, physical skin parameters such as pH value, temperature and moisture were recorded.

The study revealed that the pressure stimulus alone did not significantly change microcirculation, while there was a significant positive effect of diCAP treatment. Tissue oxygen saturation, blood flow and local relative hemoglobin were significantly elevated for up to one hour after 270 s of diCAP treatment. Furthermore, skin pH value was decreased after the diCAP treatment. In summary, plasma-induced effects on microcirculation are dominant over pressure-induced effects [3].

This work was carried out within the research project “Plasma for Life” with financial support from the German Federal Ministry of Education and Research (BMBF), National Grant/Award no. 13FH6I04IA. Furthermore, the technical and practical support by CINOgy GmbH and LEA Medizintechnik GmbH is gratefully acknowledged.

References
Effect of cold plasma treatment on the proteome of *Pseudomonas aeruginosa* biofilms

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**Background:**

Cold atmospheric-pressure plasma (CAP) consists of a mix of reactive species and UV that have antibacterial activity. CAP can be used as an antibacterial agent in biomedical and industrial applications. Many contaminations and infections are caused by biofilm-forming bacteria, where bacteria live attached to a surface encapsulated in a protective matrix. *P. aeruginosa* is an important pathogen that forms biofilms and has a high rate of antibiotic resistance. Previous work by our research group showed that CAP treatment can eliminate *P. aeruginosa* biofilms but low doses may lead to the surviving bacteria being more resistant to subsequent plasma exposure by causing DNA mutations in genes important for oxidative stress regulation [1]. We now investigate the effect of CAP treatment on the whole proteome of *P. aeruginosa* biofilms.

**Methods:**

Biofilms were grown on stainless steel coupons in a flow through bioreactor and treated with CAP using the kINPen med (neoplas tools GmbH, Greifswald, Germany) [2]. The kINPen handheld nozzle was connected to a base unit with argon fed at 4.2 slm and plasma pulses were generated at a frequency of 1.82 MHz. LC MS (liquid chromatography mass spectroscopy) was then used to identify differently regulated proteins of whole cell *P. aeruginosa* extracts.

**Results and Conclusion:**

A total of 16 proteins were identified to be affected by plasma treatment compared to the control. Ten proteins belong to ribosomal activity, suggesting that CAP affects translation. Interestingly, CAP also affected bacterioferritin, isocitrate dehydrogenase, trigger factor and a chemotaxis protein. We confirmed that bacterioferritin B plays a role in the bacterial response to CAP by showing that ΔbfrB mutants of two *P. aeruginosa* species (PAO1 and PA14) are more susceptible to plasma-induced cell-death. To our knowledge, this is the first study showing the effect of plasma on the whole proteome of a pathogenic microorganism. Increasing our understanding of the mode of action of CAP-mediated bacterial inactivation will assist the possible use of plasma to complement or replace traditional antibiotics practices, which may slow the spread of antimicrobial resistance.

**References**

Cold Atmospheric pressure Plasma (CAP) treatment to assist the restoration of apical region of root canal in endodontic procedures

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The restoration of the apical region of root canal aims at avoiding a new bacterial colonization in the tooth apex, that may result in a hazardous abscess. Filling materials, such as gutta-percha (GUT), are generally used to completely seal the root apex with the aim of entombing the residual bacteria remaining on the root canal walls or within dentinal tubules (dental substrate) and preventing any contacts with the peri-apical tissues and nerves [1,2]. In the conventional procedures, to achieve a well-adherent apical monoblock, endodontic cements known as sealers (SEA) are applied before GUT filling, despite their cytotoxicity [3]. CAP treatment can be considered as an attractive solution to improve the performances of conventional procedures involved in apical restoration thanks to CAP ability to modify the surface chemistry of dentine [4]. In order to evaluate the enhancement of bonding strength between sealing material and CAP-treated dentine, forty extracted teeth with standardized shape of the only root-canal were used for the experiments. 180s CAP treatment was performed with a DBD He plasma jet, designed for endodontic procedures. The restorative procedure was performed either with direct filling of GUT or with preliminary application of a sealer, comparing the results of untreated or CAP-treated dentine. Indeed, the investigated cases are: G1 (GUT), G2 (CAP + GUT), G3 (SEA + GUT), G4 (CAP + SEA + GUT). The adhesive performances of restorative procedure were evaluated through push-out tests. Moreover, a confocal (CLSM) analysis was carried out to examine the depth of penetration of GUT and sealer in the dentinal substrate. Push-out results show how the procedure G2 leads to adhesive performances comparable with the G3’s ones supporting the idea of a new SEA-free procedure for the apical restoration. Furthermore, the bonding strength for the conventional restoration of the tooth apex is enhanced of +50% by the CAP activation of dentine (G4). These results are also supported by CLSM images, highlighting a higher penetration of filling materials into dentinal tubules in the case of CAP treatment of dentine (G2, G4). The achieved experimental results, combined with ones obtained in the field of root canal disinfection [5], support the exploitability of the DBD He plasma jet in real-life endodontic procedures.

References

Microbial Disinfection of Tubular Medical Devices by Plasma Activated Solutions: Efficacy and Material Safety

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Synthetic polymeric materials such as polytetrafluoroethylene, polyurethane, silicone and polyethylene are widely used in tubular medical devices such as endoscopes and catheters. Polymers are thermolabile and as such they cannot be autoclaved. For disinfecting such medical devices, a number of chemical agents are used as disinfectants, for example alcohol, hydrogen peroxide (H₂O₂) and hypochlorite at high concentrations (few ppm) [1]. However, they are often too corrosive. For example, the combination of H₂O₂ and peracetic acid have been used for disinfection of polymeric lumens but cause functional damage to the polymeric material [2].

This paper reports an investigation of PAS disinfection of microbially contaminated polymeric tubes and the impact of repeated PAS treatment on mechanical properties of the test tubes. Using an atmospheric air plasma sustained on a surface electrode [3], PAS was prepared and its effective dose to eradicate a range of bacterial biofilms was identified. PAS at its effective dose was used to incubate fresh and uncontaminated test tubes for 14 days at 37°C and then the treated tubes were tested for changes in surface morphology, mechanical stress and strains, force at break, and tube elongation at break. In addition, biofilm formation of P. aeruginosa (PA01) on the PAS-treated tubes was studied with a crystal violet assay and compared with untreated polymeric tubes. Our data suggest that prolonged PAS treatment did not impair the mechanical properties of polymeric material of the test tubes and nor enhance the biofilm, adhesion in any significant way. With the same dose, PAS was able to eradicate and remove P. aeruginosa biofilms formed inside test tubes that had been treated with PAS for 14 days.

References
Plasma-polymerized PEG in microfluidics: a real case scenario

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We have recently perfected plasma-polymerized (PP) poly(ethylene glycol), PEG, coatings that resist protein adsorption from buffer and cellular adhesion.[1] Our method, based on electrical measurements in atmospheric pressure Ar dielectric barrier discharges, DBD, enables precise measurements of $E_m$, the energy absorbed per precursor molecule. We have demonstrated the importance of $E_m$ in preparing anti-fouling PP-PEG from diglyme as the precursor (PP-2G).

PP-PEG coatings are known to inhibit possible inflammatory reactions or rejection of an implant following its insertion into living tissue. They can also be used in assay applications, for example for cell capture, where selectivity and purity are key requirements. In this work, PP-2G coatings were applied on micro-fabricated polymer filters with pores (diameter 8 or 15 μm) that permit size-based capture of blood-circulating tumor cells (CTCs) to enhance their selectivity.[2]

Non-specific adsorption of blood components on PP-2G-coated filters was compared to that on pristine- and BSA-passivated ones using (1) BSA-FITC solutions; (2) human blood from healthy donors; and (3) blood from a clinical study involving epithelial ovarian cancer (EOC) patients. As shown in Fig. 1, filters coated with PP-2G displayed greatly reduced non-specific adsorption, mainly of white blood cells (WBCs). Advantages of PP-2G over commonly-used BSA passivation include improved purity, reproducibility, and long-term stability, as we shall demonstrate.

References
Cold atmospheric plasma assisted deposition of nanostructured coatings to reduce biofilm adhesion and proliferation

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In recent years, innovative solutions able to prevent bacterial adhesion to blood-contacting biomaterials have raised significant interest. Beside the reduction of biofilm proliferation, these novel approaches have to preserve the bio- and hemo-compatibility, avoiding blood clots formation on the surface of the biomaterials. Among the innovative strategies developed to reduce bacterial proliferation and biofilm adhesion on biomaterials, the deposition of nanostructured coatings by means of cold atmospheric plasma (CAP) has been demonstrated to be a promising technology [1]. In this work, a CAP assisted process to produce nanostructured coatings composed by silver nanoparticles (AgNPs) embedded in a plasma polymerized HMDSO (ppHMDSO) matrix with antibiofilm and anti-clot properties is presented. Nanostructured coatings were deposited by means of a CAP jet operated in argon and driven by a high voltage generator working at 10 kV and 14 kHz. The produced coatings were composed of three distinct layers: a first layer of ppHMDSO, working as buffer layer; a second layer composed of AgNPs, directly synthetized in the plasma region through the reduction of AgNO₃ aerosol droplets; a third thin protective layer of ppHMDSO, to prevent the dispersion of the nanoparticles and to increase the coating’s biocompatibility. The chemo-morphological analysis of the coatings was carried out by means of Fourier Transform Infrared (FTIR) spectroscopy and Scanning Electron Microscopy-Energy-Dispersive X-Ray (SEM-EDX) analysis. In order to evaluate the biocompatibility of the deposited coatings, hemocompatibility tests were performed by dynamic blood contact and blood cell lysis was evaluated by hemoglobin free assay according to ISO10993-4. After blood contact the biomaterials were stained by hematoxylin/eosin to detect clots. The antibiofilm performance of the coating was evaluated with a contact-test of a 4 strains bacterial culture, and crystal violet staining was used to evaluate the presence of biofilm on the coated surface [2]. FTIR and SEM-EDX analysis confirmed the presence of organo-silicon coatings and of AgNPs incorporated between the buffer and protective ppHMDSO layers. The biological assays outlined that deposited coatings were able to reduce bacterial adhesion and biofilm formation, preserving the hemocompatibility and avoiding clots formation compared to pristine substrates.

References

Immobilization of Quaternary Ammonium Based Antibacterial Monomer onto Dentin Substrate by Non-thermal Atmospheric Plasma

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The antibacterial effects of quaternary ammonium methacrylates (QAMs) included in adhesives are not sustainable due to their no/little interactions with dentin substrate. In this first of its kind study, the use of non-thermal atmospheric plasma (NTAP) brush on immobilization of dimethylaminohexadecyl methacrylate (DMAHDM), a typical QAM, onto dentin bonding substrate, and resulting antibacterial activity against Streptococcus mutans were investigated. A bonding substrate with several-micron-decalcified layer was created from human dentin. DMAHDM was applied onto the demineralized layer with or without plasma exposure. SEM and FTIR spectroscopy were employed to verify immobilization/grafting of DMAHDM onto the substrate. Antibacterial activity of the resulting substrate was assessed by using colony-forming unit (CFU) and confocal scanning laser microscopy. Effects of saliva pellicle treatment and aging process on the DMAHDM immobilized substrate were also evaluated.

The SEM and FITR results demonstrated that plasma-treatment could induce DMAHDM immobilization onto dentin substrate, which was further verified via quantitative IR spectral analysis (i.e. 2925 cm⁻¹/1635 cm⁻¹, 1455 cm⁻¹/1635 cm⁻¹, DMAHDM/collagen ratios). Comparing with non-plasma-treated, the plasma-treated dentin bonding substrate, with CFU 4 log lower, exhibited much stronger inhibitory effects, which were minimally affected by saliva or aging. The DMAHDM-immobilized dentin substrate showed effective and sustained antibacterial characteristics.

In this proof-of-concept study, it was found that NTAP effectively induced immobilization of a quaternary ammonium methacrylate onto dentin bonding substrate within a clinically acceptable treatment time of 30s, generating an antibacterial surface with remarkable and long-lasting inhibitory function. Further investigations should be performed with respect to the NTAP/DMAHDM’s overall effect when incorporated into actual bonding procedures [1-2]. For example, by combining DMAHDM with a dental primer or adhesive, more systematic NTAP studies on antibacterial effects of dental restoration under clinically relevant settings are needed. It is expected that highly reactive particles from NTAP should also induce DMAHDM immobilization in presence of other monomers [1].

This work was supported by Research Grant R01-DE021431 from the National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892, USA

References

Our recent research progress on treatment of medical wastewater by non-thermal cold plasma

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Non-thermal plasma can be applied to deal with medical wastewater containing hazardous or toxic substances including persistent organic pollutants (POPs), antibiotic medicines and bacteria. Recently, we have employed non-thermal plasma technique as an effective advanced oxidation process (AOP) to treat medical wastewater and made a series of progress in the research. In this presentation, we will mainly introduce our recent new research progress on the degradation of norfloxacin based on the method of dielectric barrier discharge (DBD). Using different gases we studied the efficiency of norfloxacin degradation, and found that the plasma treatment efficiency changed in the sequence as helium > oxygen > air > argon > nitrogen. Furthermore, we confirmed that both reactive oxygen species (ROS) and reactive nitrogen species (RNS) made contributions to the degradation of norfloxacin, while hydroxyl radicals played a primary role in the degradation pathway. The effects of other plasma factors/by-products such as UV, hydrogen peroxide and ozone on the degradation efficiency were also investigated. Except for this part of research, in addition, we will also introduce our research progress on the non-thermal plasma treatment for bacterial inactivation and removal of harmful chemicals such as chlorinated organic compounds (e.g. dichlorophenol) and endocrine disrupting chemicals (EDCs) (e.g. bisphenol A) existing in contaminated medical wastewater, illustrating how the plasma treatment results depend on various environmental factors and discussing the involved mechanisms and pathways in the treatment as well.
On the deposition of atmospheric plasma-sprayed bioactive and antibacterial coatings on PEEK substrates

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Atmospheric plasma spray (APS) is a widely known thermal spray technique for coating implants with bioactive materials, with the objective of improving bone in-growth and promote osseointegration [1]. In addition, new strategies have been recently developed to enhance implant functionality, to reduce costs –for instance by using polymer implants [2]– or to improve the patient’s recovery by preventing post-surgical complications related to bacterial infections [3]. However, the utilization of thermal spraying requires different considerations with respect to the differences between the melting point and nature of the substrate and the coating materials. In particular, due to the high temperatures of the plasma and the sprayed powder, thermal load of the polymeric substrate (T_m = 343 °C) have to be prevented whereas in turn, high temperatures are needed to properly melt ceramic, oxide or metallic particles without degrading them or obtaining undesired phases.

In this contribution we present results on the deposition of multilayer coatings comprising bioactive titanium dioxide (TiO_2) and hydroxyapatite (HAp) with admixtures of antibacterial agents (e.g. copper) on poly(etheretherketone) (PEEK) substrates by means of APS. The obtained coatings were characterized in terms of microstructure, chemical composition, and coating adhesion strength by using scanning electron microscopy, X-ray photoelectron spectroscopy, X-ray diffraction, and tensile adhesive strength tests, respectively. Furthermore, cell biological and antibacterial assays were conducted to reveal the capability of the applied methodology to generate bioactive and antibacterial plasma sprayed films while maintaining the integrity of heat sensitive substrates.

Fig. 1. Photograph of a PEEK substrate successfully coated with TiO_2 and Cu-containing HAp.

This work was supported by INP Greifswald e.V. and by Vicerrectoría de Investigación y Extensión of Instituto Tecnológico de Costa Rica, under grant No. CF 1341011.

References

How to increase plasma tolerance in bacteria
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Plasmas for clinical applications are of growing interest. They are used solely e.g. for disinfection in dermatology or in combination with conventional therapies, for instance in treatment of cancer. Nevertheless, the long-time applicability is still not well-studied. One until now underestimated threat is the development of plasma-resistant bacteria. Those strains would substantially limit the application of plasma for treatment of samples, where human cells and bacteria are exposed to plasma simultaneously like in wounds. As plasmas exhibit mutagenic properties, bacterial evolution towards a plasma resistance is facilitated [1].

In a genome-wide screening using a single-gene knockout library (the KEIO collection [2]), 85 genes were identified, which were crucial for survival of low doses of plasma. In a second subsequent screening, these mutants were exposed to stressors mimicking plasma components like hydrogen peroxide, superoxide, or peroxynitrite to assign for plasma factors to which the genes mediate tolerance. Hydrogen peroxide and superoxide were found to be the most potent stressors in this screening indicating these reactive oxygen species to play a major role in the bacterial inactivation by plasma. Additionally, in a rational approach the stress-activated protein Hsp33Ec (heat shock protein 33, [3]) was investigated in depth. It was found to prevent plasma-based protein aggregation in vitro. Further, Hsp33Ec was not inactivated by plasma as most proteins analyzed so far, but activated. In a third line of experiments using the model organism Escherichia coli, artificial and directed evolution was performed to increase the bacterial plasma tolerance by introduction of random mutations. Already after few rounds of plasma exposure, a doubling in tolerance was achieved.

The identification of genes mediating plasma tolerance, the discovery of Hsp33Ec preventing plasma-induced protein aggregation, and the successful increase in tolerance by directed evolution necessitate to rethink long-term applications of plasmas in clinical settings. Unregulated use might result in bacterial strains with a plasma tolerance comparable to eukaryotic cells limiting future applications.

This work was supported by German Research Foundation.

References
In-situ Generation of Reactive Species in Hydrogel Dressing and their Delivery for Wound Biofilm Control

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Wound infection is poly-microbial and there is evidence of bacteria developing into biofilm in infected wounds. As bacteria in biofilm avoid the immune clearance and therapeutic drugs, conventional antimicrobial therapies become less effective when treating biofilm related infections [1]. With a global rise of multidrug resistant bacteria, the new treatment therapies which combat such infections have become urgent in a clinical setting. Recently cold atmospheric plasma (CAP) has been established into many areas of cross-disciplinary research and one of them is wound/ulcer care [2]. Among various products generated by CAP, reactive oxide and nitride species (RONS) are reported for their ability to promote wound healing and importantly to eliminate targeted bacteria via induced oxidative stress and lipid peroxidation [3]. This opens up a new research area for future CAP-assisted wound care.

The primary challenge when applying CAP in wound treatment is how to effectively generate, maintain and deliver reactive species at the dressing-wound interface. Many have reported CAP-assisted wound decontamination using animal models but the potential risks of plasma irradiation on intact skin was not discussed [4]. Recent studies claimed such a direct plasma treatment induced a short term radiation damage to skin and a potential long term cytotoxicity to keratinocytes and fibroblasts [5]. There needs to be a low risk, effective way of plasma treatment on skin.

Here we report a scheme for indirect plasma treatment in which a Helium plasma jet is briefly but regularly used to generate reactive species in a hydrogel dressing. Plasma Activated Hydrogel (PAH) stores long-life reactive species, such as H$_2$O$_2$ and steadily delivers them to the site of infection using mixed-species in vitro wound biofilm model of two clinically important wound pathogens: Staphylococcus aureus and Pseudomonas aeruginosa. Key reactive species generated in PAH are identified and their delivery and effectiveness in eradication of model biofilm bacteria is examined. We show that a regular replenishment, storage and gradual delivery of optimal reactive species in PAH could be the way to keep wound pathogens under control, allowing the local immune system to resume and the wound to heal naturally.

This work was supported by EPSRC funded project no. EP/P003939/1 (Smartwound-plasma) and Medical Research Council funded project no. MR/N006496/1(Development of an infection detecting wound dressing).

References
The Impact of an Air Plasma Jet on Isolates from Human Habitat Objects

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The main field of plasma medicine is the direct application of cold atmospheric plasma on/or in the human body for therapeutic purposes. [1] In this report, we present the experimental results for inactivation of planktonic microorganisms by air plasma jet. Generation of reactive nitrogen species in DC air plasma jet used in the experiment is dominating [2]. Thus, at DC current of 40 mA and air flow of 5 l/min, the concentration of bioactive molecules are NO = 180 ppm, NO₂ = 140 ppm, HNO₂ = 25 ppm. Ozone is minority component in a jet with concentration less than a few of order of magnitude in comparison to concentration of nitrogen species.

The efficiency of plasma exposure is estimated on the percentage of the survived cells in strains of the planktonic microorganisms (gram-positive (Staphylococcus aureus, Bacillus subtilis), gram-negative (Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia, Salmonella enterica), spore (Candida albicans)). Isolates from human habitat objects were used. In each experiment, the total initial concentrations of microorganisms in Petri dishes are 10⁶ CFU/ml. The temperature in Petri dish is controlled and does not exceed 30 °C. The effect of the plasma jet was evaluated by inhibition zones on Petri dishes and in the concentrations of surviving microorganisms determined by colony counting method. The characteristic D-times of inactivation for all cultures were different. Monocultures of S. aureus, P. aeruginosa, B. subtilis, Kl. pneumonia had approximately equal inhibition zones (after 10 minutes of exposure this zone is about 50% of the total area of the Petri dish) with characteristic D-times of about 1.5 minutes. At the same time, D-times for C. albicans, Pr. Mirabilis and E. coli, were from 2.5 min with inhibition zones of 15-25%. The Salmonella’s strain exhibited the greatest resistance with D-time more than 5 minutes with an inhibition zone of about 5%.

The first results of the action of an air plasma jet on the DNA structure are under investigation as well. Assessment of genotoxic properties of air plasma jet is performed in vitro in the comet test on DNA phage λ. It is shown that the action of a plasma jet on DNA for 20 minutes does not lead to its destruction. Results of solely plasma effects and in complex in antibiotics at treatment of wounds are discussed.

This work is partially supported by BRFFR under the grant F17MS-030.

References


Effective Surface Modification of Bone Sialoprotein Mimetic Peptide on Align Nanofibers with Cold Atmospheric Plasma

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An ideal biomaterial should have suitable mechanical properties, biodegradability and biocompatibility, as well as proper surface properties to enhance cell growth. Since designing biomaterials with combined properties is difficult, the surface of biomaterials is usually modified after production. Improved hydrophilicity along with the presence of some functional groups on the surface of scaffolds plays an important role in cell adhesion, proliferation and migration. Various techniques have been used to modify the surface of polymers for both improving hydrophilicity and introducing some functional groups that might serve as biological cues. Recently, some efforts have been undertaken to utilize cold atmospheric plasma (CAP) for surface treatment as an alternative to low pressure plasma. CAP can react with polymers and produce oxygen functional groups on the polymer surface. Modification of polymer nanofiber (NF) surface with bone sialoprotein mimetic peptide, which plays major role on osteogenic differentiation and mineralization as an extracellular matrix protein, may accelerate osteogenic differentiation [1]. In this regard, the aim of this study is to conjugate bone sialoprotein mimetic peptide on PLGA NF, effectively.

NFs produced by electrospinning technique were treated by CAP. Optimum parameters were determined by SEM Imaging. CAP treated, and non-treated NFs were conjugated with glutamic acid (GLU) sequences by using EDC/NHS chemistry. Contact Angle, AFM Imaging, FITC labelling, and cell proliferation assay were evaluated to compare enhancement of conjugation. After incubation of all groups with Simulated Body Fluid (SBF), calcium assay and XRD measurement were assessed to compare mineralization. XPS and FTIR analyses were evaluated to confirm chemical reactions after conjugation. We observed that CAP treatment with optimal parameters increases functional peptide conjugation by maximizing functional carboxylic groups on NF surface. When peptide conjugated nanofibers are compared with non-conjugated nanofibers, it was observed that contact angle was decreased due to hydrophilic characteristic of GLU peptide. Furthermore, 45-second plasma treatment before conjugation decreased contact angle by providing efficient peptide conjugation. Calcium assay and XRD results indicated that NF+Plasma+Peptide group consists the highest Ca amount. Additionally, results of MTT assay suggested that plasma treatment prior to peptide conjugation, improves cellular adhesion and proliferation. XRD and FTIR results confirmed that CAP treatment leads effective peptide conjugation by activating -OH groups on NF surface. These preliminary results will be used in our future works including osteogenic differentiation. FTIR and XPS results are underway.

In conclusion, CAP treatment would be a novel alternative method for surface modification of synthetic NF for conjugating functional peptides.

References

Mitochondrial Complex IV Inhibition and Exogenous Oxidants Potently Synergize in Melanoma Cell Death Induction

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Due to its functional duality in toxicity as well as signaling, research on therapeutic manipulation of reactive oxygen species (ROS) in cancer has been futile. Targeting melanoma cell death with excess amounts ROS, however, is difficult as these cells are highly resistant towards oxidative stress, creating a need for alternative and combinational approaches. Tumor metabolism mainly relies on anaerobic glycolysis. We investigated a potential synergism of mitochondrial complex IV inhibition and exogenous-derived oxidants with both single treatment modalities being essentially non-toxic in melanoma cells.

In this study, we investigated murine (metastatic B16F10 and non-metastatic B16F0) as well as human (SK-Mel 28) melanoma cells, and compared them with murine (primary fibroblasts) and human (HaCaT keratinocytes) non-cancer cells. Mitochondrial complex IV inhibition was realized using sodium azide (NaN3) and potassium cyanide (KCN) and pharmacological inhibitor ADDA5. Exogenous oxidants were generated using cell culture medium that has been treated with the cold physical plasma effluent of an atmospheric pressure argon plasma jet (kINPen). This plasma-treated medium was subsequently applied to different cells. Metabolic activity was assessed by fluorescence quantification of resazurin-to-resorufin transformation. ATP levels were monitored by a luminescence assay. Total cell counts, intracellular ROS levels (DCF), mitochondria membrane potential (TMRE), cell membrane integrity (sytox green) and cellular morphology were quantitatively assessed using live cell and high throughput confocal imaging (Operetta; Perkin Elmer). For tumor cells, also 3D spheroid models were investigated for viability by sytox green staining.

ADDA5, NaN3 and KCN treatment alone failed to induce a significant alteration in metabolic activity in cancer cells. We then exposed the cells pretreated with NaN3 (100-500 µM) or KCN (250-500 µM) to plasma-treated medium. There was a significant inhibition of metabolic activity and rapid induction of cell death 6 h following treatment in melanoma cells compared to cell receiving plasma-treated medium alone. Increase in cellular superoxide levels and decrease in mitochondrial membrane potential and ATP levels was observed preceding cell death. However, no caspase 3 cleavage was observed upon immunoblotting. siRNA mediated knockdown of COX4a led to increased sensitivity of tumor cells towards plasma-treated medium. Furthermore, we validated our findings using a pharmacological inhibitor of complex IV ADDA5 in a tumor spheroid model and demonstrated that complex IV inhibition supplemented with plasma-treated medium, triggered significant cell death via caspase independent mechanism in melanoma cells.

This work was supported by German Federal Ministry for Education and Research.
Cold plasma reactive oxygen species generate oxidative stress but do not trigger apoptosis in yeast

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The mechanisms involved in cell killing by cold plasmas are to date not well understood and dissection of cellular pathways or structures affected by plasma using simple eukaryotic models is needed. We investigate the effects of interaction of cold air plasma with eukaryotic cells of yeasts by comparing several mutants and corresponding wild type strains of Saccharomyces cerevisiae in deionized water subjected to direct plasma and indirect plasma activated water (PAW) treatment by the transient spark discharge with water electrospray.

The cell survival measured by agar plate counts and propidium iodide (PI) staining showed that the cells subjected to direct plasma or to PAW lose their viability; increasing with incubation time. Direct plasma effect was stronger than indirect (PAW). Mutant strains (Δsod1, Δsod2) defective in superoxide dismutase (SOD) were more vulnerable, which indicates that O₂⁻ plays an important role in the cell inactivation (Fig. 1). On the contrary, catalase deficient mutants (Δcct1, Δcta1) did not show viability losses, indicating that low concentrations of H₂O₂ (~0.5 mM) in PAW [1] are not a key inactivation agent. Several in vitro and in vivo studies demonstrated plasma induced apoptosis in cells [2]. The survival of mutants defective in enzymes employed in yeast apoptosis (Δybh3, Δaif1, Δyca1, Δnuc1) did not significantly changes compared to the wild types. The apoptotic sequences induced by plasma in yeast cells were thus not confirmed.

![Graph showing survival of yeast strains](image)

Fig. 1. Survival of yeast strains defective in superoxide dismutases, catalases or programmed cell death pathways in direct plasma treatment. Plotted values represent the survival of mutant strains relative to the survival of wild type (BY4741, BY4742 or CML282) 0 and 60 min post plasma treatment. Supported by Slovak Research and Development Agency APVV-0134-12 and VEGA 1/0419/18.

Diagnostics of Hypochlorite Formation in Plasma Treated Saline Solutions

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The interaction of non-equilibrium plasmas with a liquid state is of great interest in many applications ranging from environmental remediation to material science and health care. Various short and long-lived chemical species are produced by plasma in the liquid either directly, or transferred from the gas phase discharge plasma being in contact with the liquid. These species can react at or penetrate through the plasma-gas/liquid interface and dissolve into the bulk liquid, and can also initiate secondary chemical processes in the liquid. The type and quantity of the reactive species formed by discharge plasma in the liquid depend on the nature and the composition of the working gas and also on the properties of the liquid in the case of contact with plasma. It has been shown that several transient reactive oxygen and nitrogen species such as OH•, O2•-, NO• and NO2• radicals, peroxynitrite may be produced in plasma-treated liquids through post-discharge processes [1]. These species have highly cytotoxic properties and cause prolonged biochemical and antibacterial activity of plasma-treated solutions. However, they are difficult to measure due to their short lifetimes and fast disproportionation in the plasma/liquid systems. It is clear that such short-lived species require in situ and fast measurements and achieving this presents challenges. Up to now only few post-discharge processes were experimentally identified in plasma-treated liquids. Significant attention received peroxynitrite chemistry [2].

In this work we have focused on Cl-related chemistry initiated by plasma in saline solutions. In this case, hypochlorite is assumed to play important role which is a known disinfectant and a part of the family of so-called “free chlorine” compounds. The possibility of OCl⁻ generation in the reaction of O atoms from plasma effluent entering the liquid phase with Cl⁻ ions was suggested [3], however, a direct observation of HOCl/OCl⁻ in the plasma-treated salt solution was not reported. This fact is related to the difficulty directly detect OCl⁻ in the liquid, and also to the possible side reactions of HOCl/OCl⁻ with H₂O₂ and/or NO₂⁻, which are commonly produced in liquids treated by plasma at ambient air. Here we have developed chemical diagnostics for analysis of hypochlorite and other free chlorine compounds in plasma treated saline solutions. We used micro-atmospheric pressure plasma jet (μ-APPJ) operating at the He/O₂ gas flow to promote formation of hypochlorite in the saline by direct reaction between oxygen atom and chloride anion [4]. Kinetics of hypochlorite formation and subsequent chemical processes derived from plasmachemically produced OCl⁻ in plasma-treated saline solutions will be discussed.

References

Optimization of Helium Cold Plasma Jets for New Anti-Tumor Treatments

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Cancer is among the major causes of death worldwide and its rate increases with age, exposure to carcinogens and the current population lifestyle. About 2/3 of patients will be cured due to the combined action of surgery, radiation, and chemotherapy [1]. In addition to these conventional therapies, novel ones have emerged such as the anti-angiogenic therapy and the immunotherapy [1]. Unfortunately, resistance has been observed to these treatments. Sequencing of primary tumors revealed that therapy-resistant clones already existed within the tumor before the treatment. This indicates that the heterogeneity in primary tumors confers a resistance mechanism. Therefore, there is still need to develop new cancer therapies to overcome this problem.

Numerous publications, mainly conducted in vitro on cells in culture, have shown the effectiveness of cold plasma jets (CPJs) to induce the death of tumor cells [2]. CPJs contain reactive ions, electrons, free radicals, UV photons, electric fields, and excited neutral atoms/molecules. Up to date, all CPJs that were developed for antitumor strategies have a common point, i.e., the reactive species are formed when the partially ionized gas interacts with the ambient air and with the biological sample, which is usually a solution or a wet surface. Consequently, the plasma action may be applied through the transfer of reactive oxygen and nitrogen species (RONS) from the gas to the liquid phases, but also via the formation of short- (O, OH, O2*- , 1O2, NO*, NO2*) and long-lived (H2O2, NO2-, NO3-, O3) reactive species directly in the liquid. However, several groups have shown that it is mainly the long-lived species, and in particular H2O2, which gives to the plasma its anticancer properties. The vast majority of studies concerning anticancer properties of CPJs was carried out using monolayer cell cultures (2D culture) and treating the cells either in the culture medium or in a buffered solution. This study is devoted to the interaction between a helium CPJ and a commonly used buffered solution (PBS). The experiments are focused particularly on the impact of the operating conditions (wet or dry shielding environment) and the nature of the working gas (He or He/O2, dry or wet) on the formation of RONS (H2O2, NO2-, NO3-) especially in the liquid phase for the optimization of the present CPJ device for new anti-tumor therapies.

This work was partially supported by the LabEx LaSIPS through the project PHeCell3D.

References

Cold physical plasmas (CPPs) are investigated in various fields of industry and medicine. One of the most promising applications of CPPs in medicine is the treatment of wounds to improve wound healing. While some studies already present promising results from clinical trials, the underlying molecular mechanisms are only understood in part. Wounds are a highly dynamic environment with several processes happening at the same time in a tightly regulated manner. A better understanding of the mechanisms of action of CPPs would allow for a better tuning of plasma conditions to affect desired processes in the wound.

The modulation of several important aspects of the wound healing process by plasma already been demonstrated, both in cell culture as well as in animal experiments. For example, bacterial inactivation, cell proliferation, and cell migration, are triggered by CPP treatment [1]. Based on these observations, the logical next step is the investigation of patient material. Here, clinical studies are already underway. Schmidt et al. tested the effect of CPP treatment on defined wounds [2] and demonstrated a faster wound closure. Furthermore, studies on patients with well described chronic wounds show promising results [3] and are currently extended further.

One major issue is the diversity of patients and the high complexity of a wound regarding its biochemical composition. Proteomics offer a way to observe changes induced by CPP treatment in a more complete and unbiased way than many other laboratory methods. Wound exudates were collected and after kINPen med treatment using swabs. Proteins were purified and digested before being measured on an HPLC/MS system. Collected data were analyzed using bioinformatics to identify proteins and if present post-translational chemical modifications. The latter were of special interest, as modifications introduced by CCP treatment are also known to play a major role during wound healing processes. Proteomic profiling indicated several protein groups correlated to wound healing, such as oxidoreductases, heat shock proteins, and neutrophil- and leukocyte-associated factors. In addition, several interesting modification candidates were found, e.g. methionine sulfoxide, which is described to influence immune cell recruitment [4]. These results allow for a more in-depth understanding how plasma affects wound healing on a molecular level and might help to improve wound treatment protocols and source design.

This work was supported by German Federal Ministry of Education and Research (BMBF), Grant No. 03Z22DN11 & 03Z22DN12.

References
Diffuse coplanar surface barrier discharge and its application on bacteria inactivation

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Abstract

Diffuse coplanar surface barrier discharge was developed to generate a thin layer of plasma in air. In the developed system, parallel strip-like Ag electrodes were covered with a layer of tantalum oxide deposited by reactive sputtering technique. The generated plasma was characterized using OES and ozone analyzer, as a function of electrode gap. Finally, the bactericidal effects of these planar plasmas were tested against E.coli. The results showed that the planar plasma can be effectively applied in bacteria inactivation. The efficiency of the plasma depends on electrode gap.

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Targeting cancer cells with cold atmospheric plasma (CAP) emerged as a promising application in plasma medicine. To understand its mechanisms, we applied plasma treated medium (PTM) and plasma treated deionized water (PTW) on two different pancreatic ductal adenocarcinomas (MiaPaca-2, BxPc3) and pancreatic stellate cells (PSCs) (hPSC128-SV). PSCs closely interact with cancer cells to create a tumor-promoting environment that stimulates local tumor progression and metastasis. We subsequently applied this PTM/PTW at various ratios to both the pancreatic cancer and PSC cell lines. To explain the observed cell death and increased intracellular ROS concentrations after treatment, we also studied the expression of mRNA of several genes, i.e., AKT serine/threonine kinase 1 (AKT1), nuclear factor erythroid 2–related factor 2 (Nrf2), Heme oxygenase-1 (HO-1), mitogen-activated protein kinase 7 (MAPK7), B-cell lymphoma 2 (BCL2), Checkpoint kinase 1 (CHEK1) and DNA damage-inducible transcript 3, also known as C/EBP homologous protein (CHOP), associated with apoptosis, as well as DNA repair, and antioxidant defense. Furthermore, to understand the cellular redox-regulative machinery in cancer cell death, we analyzed the kinase activity profile using PamStation-12 before and after PTM exposed BxPc3 pancreatic cancer cells. These results show a higher expression of redox-regulative associated upstream kinase activity. This study helps to obtain better insight in the oxidative stress factors involved in cancer therapy.

Acknowledgement: This work was financially supported by the Research Foundation Flanders. (FWO; Grant #12J5617N)

References

Modification of hydrogel-based biomaterials by atmospheric pressure plasma to enhance tissue regeneration

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Hydrogels are highly hydrated natural, synthetic or semi-synthetic networks of cross-linked polymer chains whose features such as biocompatibility and in vivo biodegradability, in many cases, make them great candidates for the design of advanced biomaterials. Hydrogel-based biomaterials, have already been implemented in different clinical areas and in wound dressings [1]. Cold atmospheric pressure plasma (APP), including plasma jets or needles, can be used as an effective tool to promote tissue regeneration in wound healing. APP formed in air generates reactive oxygen and nitrogen species (RONS) whose effects on organism stimulate antibacterial behavior and tissue regeneration as a function of the generated concentration. Many wound healing medical devices such as plasters, patches, etc. include hydrogels as components.

APP treatment of biomedical hydrogels could confer them specific features promoting tissue regeneration and therapeutic process. The aim and novelty of this work rests upon the adaptation and the optimization of the methods to quantify RONS usually employed in plasma-treated liquids to alginate hydrogels. Herein, by using different types of APP, we demonstrate that the generation of various species of RONS in hydrogels, such as nitrates and hydrogen peroxides, and their concentration can be tuned by the modulation of the working conditions of plasma treatment. This will be a crucial feature to be able to control the dose of RONS in the hydrogel-based biomaterials and thus its therapeutic effects.

Fig. 1. Influence of gas flow and electrode distance on the [NO\textsubscript{2}–] generated in alginate hydrogels by APP.

Acknowledgement. This project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (grant agreement No714793) and the financial supports of MAT2015-65601-R project (MINECO/FEDER, EU).

References

Effect of cold plasma on different skin cells: comparison of direct and indirect treatment

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The health care expenditure associated with skin injuries ranging from diabetic ulcers, surgical incisions and scald burns through to those that result from fire continue to escalate. Previous studies have demonstrated that treatment with plasma leads to skin regeneration and inactivation of bacteria\textsuperscript{[1]}. The mechanisms are not fully elucidated but the reactive species produced by plasma, like nitric oxide or hydrogen peroxyde are known to play a role\textsuperscript{[2]}.

We studied the effect of our plasma, an AC 50Hz helium jet, on four different skin cell types: keratinocytes, fibroblasts, endothelial cells and macrophages. The only variable parameter is treatment time (1 to 3 minutes or 5 to 15 joules). We compared direct treatment, indirect treatment (with plasma-activated media, PAM) and treatment with long life species at the concentration produced by plasma in order to elucidate their role. \textit{In-vitro} and \textit{ex-vivo} measurement of cellular viability, migration and proliferation enabled to quantify the effects of plasma on a range of treated cells, skin and mouse aorta. The effect of PAM has also been tested in an \textit{in-vivo} model of excisional mouse wound.

In vitro results show that short duration treatment with cold plasma has no impact on cellular viability, whereas longer duration of direct plasma increases cellular mortality. Direct plasma treatment has a positive effect on all cellular migration and proliferation for 1 and 2 min treatment for the three tested cell lines. However, 3 min of direct treatment, indirect treatment and chemical treatment have no impact. Hydrogen peroxide has a small noxious effect on cells but it may be minimized by the consumption of H\textsubscript{2}O\textsubscript{2} by the medium used: indeed H\textsubscript{2}O\textsubscript{2} produced disappeared quickly in DMEM (kinetic of order 2). Ex-vivo experiments confirm \textit{in-vitro} results: direct plasma treatment enhances angiogenesis from the extracted mouse aorta and fibroblasts growth from mice skin. However, PAM has no effect on acute wound healing kinetic \textit{in-vitro} and \textit{in-vivo}.

Our results show that direct cold plasma treatment and not PAM enhances skin cell proliferation, migration and angiogenesis under specific conditions suggesting its potentially beneficial role in wound therapy.

\textit{This work was supported by DGA and Ecole Polytechnique.}

References

Nanosecond pulse plasma generation in complex biological liquids: Effects on bacterial species and blood components

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The field of plasma medicine is rapidly developing with proposed applications in cancer treatment [1], surface decontamination of microbes [2], and many others. Much research is conducted on the applications of atmospheric pressure plasma jets (APPJs) to biomedical applications, often utilizing noble carrier gases and sinusoidal applied voltages in the kilovolt range. Other non-equilibrium plasma generation techniques include the application of higher voltage (10’s of kV) pulses with durations on the order of 10’s of nanoseconds and rise times on the order of nanoseconds. These pulses present unique possibilities including the generation of small-scale plasma discharges directly in liquids. In particular, the application of decontaminating water of microbes using reactive oxygen species (ROS) produced by the plasma shows promise [3].

The Low-Temperature Plasma Science and Engineering Research Group at The Pennsylvania State University is presently pursuing research that marries facets of these concepts: producing small-scale plasma discharges with nanosecond-scale voltage pulses in complex biological fluids including human whole blood in-vitro. This research is the first of its kind with a goal of understanding the interaction and impact of the plasma generation process directly in liquids and related plasma chemistry on the structure and function of blood constituents. This National Institutes of Health funded project also investigates the effect of the plasma chemistry, particularly ROS, on vegetative and biofilm-forming bacteria in blood for a potential future intervention of endocarditis in the cardiovascular (CV) system. Endocarditis is a bacterial infection on native tissue or prosthetic implants in the CV system that has a rate mortality and does not respond well to antibiotics, typically requiring surgical intervention [4].

First experimental results of the project will be discussed including optical emission spectroscopy, ROS assays, early results on bacterial effects, and hematological assays on human whole blood constituent structure and function.

This work was supported by the National Institute of Biomedical Imaging and Bioengineering of the National Institutes of Health under Award Number R21EB024693. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

Endocytosis Dependency of Gene Transfer in Plasma Method and Electroporation

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We have established a gene transfer method using micro-plasma [1], then have been trying to elucidate the transfer mechanism. It has been clarified that 80% of gene transfer is caused by endocytosis [2]. Electroporation and lipofection are widely used for physical and chemical gene transfer method respectively. It is known that the electroporation transfer genes by making pores on cell membranes by electric current, but in recent years it is reported that also in electroporation endocytosis occurs and transfers gene into cell [3]. In this study we clarify the difference of gene transfer mechanism between plasma gene transfer method and electroporation in order to prove the plasma method is highly efficient and low/non-invasive. The target cell was mouse fibroblast L-929. The system for plasma method was developed by ourselves [1], and the commercial apparatus (NEPA21, NEPAGENE) was used for electroporation. As first step we found optimal conditions for each method with these devices. Under the optimum condition, the plasma method achieved the cell viability of 89%, whereas the electroporation achieved only 67%, and the transfer efficiency was 26% for the plasma method and 30% for electroporation (Table-1). In terms of transfer efficiency, electroporation is comparable to the plasma method. As for the survival rate, it became clear that the plasma method is significantly superior to electroporation. These results suggest that the transfer mechanism will be different between them.

Next, it was confirmed how the gene transfer efficiency is reduced by endocytosis inhibitor MβCD under the optimal transfer condition. As shown in Table 1, gene transfer depends on the MβCD concentration in the plasma method, but a significant inhibitory effect of MβCD was not observed in electroporation. In the plasma gene transfection method, it was reconfirmed that endocytosis is caused by electrical factors and chemical factors derived from plasma and that leads to gene introduction. On the other hand, gene transfer by endocytosis was not observed in electroporation. Although this result does not confirm that endocytosis does not occur in electroporation, at least it was suggested that under the optimal introduction condition the transfer mechanism clearly differs between the plasma method and electroporation.

References


Table-1 Effect of MβCD on Gene transfer efficiency of Electroporation and Plasma method

<table>
<thead>
<tr>
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<th>Electroporation (N=4)</th>
<th>Plasma (N=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non MβCD</td>
<td>10 mM MβCD</td>
</tr>
<tr>
<td>Transfer efficiency / %</td>
<td>30.13±3.67</td>
<td>33.90±1.73</td>
</tr>
</tbody>
</table>
Filamentary stochastic non-thermal plasmas dominate the field of plasma medicine. In recent years, standardization of plasma sources and treatment procedures enabled strong progress in the study of plasma induced biological effects. Especially promising results from plasma cancer treatment [1] make evident that new therapies call for a deeper understanding of the underlying principles. The properties of plasmas used in medicine prohibit a simple and comprehensive diagnostic of the discharge kinetics and reactivity.

The requirements for diagnostic techniques are demanding: Time resolution of sub-nanosecond, sensitivity allowing single shot diagnostics, spatial resolution of sub-µm for a large multitude of reactive components including highly reactive atoms and molecules with typically low concentration profiles, fast and high, spatially confined electric fields, and reactive turbulent flow situations in a multiphase environment.

Optical diagnostics known from combustion research and aerospace applications have been developed to meet many of the requirements for a study of filamentary stochastic non-thermal plasmas. In this work, sensitive and non- or minimally invasive diagnostic techniques for electric field measurements and flow field measurements using femtosecond lasers are introduced and demonstrated on a plasma-liquid environment.

For electric field measurements, a femtosecond laser allows to measure electric fields down to 100 V/cm in any gas or gas mixture with a spatial resolution in the µm range and time resolution given by the laser pulse duration [2].

Flow field is measured by femtosecond laser electronic excitation tagging (FLEET) of nitrogen and argon. The laser excitation leads to dissociation of molecular nitrogen. Recombination into electronically excited states of nitrogen provides a source for photon emission long enough to be used as flow marker without the need for particle seeding or other invasive probes [3, 4].

Studies of flow and electric field provide substantial information for control and in-depth understanding of non-thermal plasmas used in medicine.

This work is supported by the Alexander von Humboldt Foundation and Princeton University

References


Non-thermal Plasma Treatment of Pancreatic Cancer- Clinical Considerations
Wilbur Bowne, Marian Khalili, Vandana Miller,

Pancreatic ductal adenocarcinoma (PDAC) is projected to be the second most prevalent cause of cancer death in the United States by 2030 and typically presents with locally advanced, unresectable disease, with few existing treatment options to prolong survival. While immunotherapy has been successful in specific malignancies (melanoma, lymphoma, renal cell carcinoma), there are few immunotherapeutic protocols for advanced PDAC. Non-thermal plasma has the potential to treat unresectable tumors and metastatic PDAC, improving patient outcomes. Previous applications of plasma in oncology have focused on ablative approaches, ignoring its impact on host immunity. In our pilot proof-of-concept studies, we have found that non-ablative plasma in the form of nspDBP produces an immunogenic cascade in PDAC, initiating therapeutic immune responses. If successful, nspDBD can provide a safer alternative to radiation, chemotherapy, and photodynamic therapy for ICD mediated immunotherapy for PDAC and many other cancers.
Plasma oxidation of human epidermal growth factor: Combined computational and experimental study

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Cold atmospheric plasma (CAP) is able to modify cell components (proteins, cell membrane, etc.) through e.g., oxidation [1-5], which leads to a change of their physical and chemical properties.

In this study we show the effect of CAP on the structural and conformational changes in signaling protein human epidermal growth factor (hEGF), by means of computer simulations and experiments. Specifically, we apply different degrees of oxidation to hEGF, determined from combining the simulation results with Fourier transform infrared results. These oxidation degrees correspond to short or longer CAP treatment times. We also perform circular dichroism spectroscopy to verify the conformational changes obtained by the simulations.

In general, our simulation results are in good qualitative agreement with experiments. The obtained results indicate that the oxidized structures become more flexible, due to their conformational changes and breakage of the disulfide bonds, particularly at higher oxidation degrees. We also find that a low oxidation degree has insignificant effect on the binding affinity of hEGF with its receptor, whereas highly oxidized hEGF exhibits less interaction. These results can be linked to the use of CAP for wound healing at short treatment times (i.e., low oxidation degree) vs cancer treatment at longer treatment times (i.e., higher oxidation degree).

Acknowledgement: This work was financially supported by the Research Foundation – Flanders (FWO), grant number 1200216N. The computational work was carried out using the Turing HPC infrastructure at the CalcUA core facility of the Universiteit Antwerpen (UA), a division of the Flemish Supercomputer Center VSC, funded by the Hercules Foundation, the Flemish Government (department EWI) and the UA.

References

Molecular and cellular effects of cold plasma in an acute rodent wound model

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Wound healing is strongly associated with the presence of a balanced content of reactive species in which oxygen-dependent redox-sensitive signaling pathways represent an essential step of the healing cascade. Numerous studies have provided evidence that cold physical plasma supports the wound healing due to the ability to deliver a beneficial mixture of reactive species directly to the cells (1). In this study, a rodent full-thickness dermal ear wound model was employed to investigate relevant signaling pathways triggered by plasma exposure (2-3).

Cold plasma showed a strong wound healing promoting activity during 15 days dependent on plasma treatment time as well as on gender. Plasma exerted significant effects on wound healing including the promotion of re-epithelialization as a consequence of migration of skin cells and inflammatory reactions with the induction of interleukin 6 expression as well as macrophage and neutrophil recruitment at early stages. Wound closure was further linked to an early increase of proliferation as detected by Ki67 expression and an attenuation of apoptosis assessed by DNA nick end-labelling. The ‘pharmacological’ activation of the nuclear E2-related factor (Nrf2) pathway and a strong overexpression of down-stream targets is a promising strategy for the clinical use of cold plasma in wound healing. Through the modulation of anti-oxidative genes and proteins, and the activation of p53 signaling (by increasing of apoptotic, DNA repair or cell cycle arrest gene and protein expression) cells were defended from damages and the cellular redox homeostasis was maintained. Although normal, acute wound healing is non-problematic, the pathways highlighted by our results illustrate that cold plasma is a beneficial tool to promote healing of chronic wounds.


The effect of liquid composition on species produced in plasma-treated liquids - Revealed by means of 2D fluid dynamics modeling

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Plasma-treated liquids (PTL) and plasma-liquid interactions in general are gaining interest in the field of plasma medicine, including in sterilization and cancer treatment [1]. PTL are often more applicable than direct plasma treatment in terms of delivery routes and storability [2,3]. The ultimate goal would be to exactly control the PTL activity, depending on the desired outcome. Controlling PTL activity is related to the amounts of reactive species that are formed in these PTL, as the cocktail of species present in PTL determines its effect [4]. Thus, more fundamental insight is needed in the mechanisms of reactive species generation in PTL.

Recently, we have developed a 2D fluid dynamics model that can be used to study the transport and accumulation of plasma-generated species in aqueous solution during plasma jet treatment [5]. Figure 1 illustrates the concentration of H₂O₂ in gas and liquid phase after 1 minute of plasma treatment. In this contribution, we will present calculation results about the effect of the liquid composition on the generation of species in the liquid during and after plasma treatment. More specifically, we investigated the effect of the initial pH of the liquid and the chlorine concentration on the transport and accumulation of the most important (biologically active) reactive species. Moreover, we studied the stability of the species after plasma treatment in the liquid, which is important in terms of stability and storability of PTL. The results will be compared with measured concentrations in the liquid, to validate the model.

Fig. 1. 2D plot of the number densities of H₂O₂ in the gas and liquid phase, after 1 min of plasma treatment, calculated with the 2D fluid dynamics model using Comsol Multiphysics® [5].

References

Historical Applications of Plasma Medicine

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Although it is not well known, plasma medicine began in the late 19th century with the use of air plasma devices operated with high frequency, Tesla coil-like circuits. [1, 2] In one popular design, the plasma was ignited in the interior of a reduced pressure, glass enclosure: this was referred to as a 'vacuum electrode.' In other manifestations, metallic 'brush-like' electrodes were used to create a filamentary, dispersed air plasma for contact with tissue. Remarkably, medical practitioners in the early 20th century were aware that high frequency currents and associated plasma discharges could enhance local blood flow and blood $O_2$ content, with local germicidal actions from $O_3$ and $NO_x$, often with pronounced analgesic effects. The French gynecologist Paul Oudin speculated in 1910 that his high frequency current, vacuum electrode device stimulated phagocytosis when treating infected gynecological lesions. [3] Thus some of the most recently (re-)discovered plasma medicine mechanisms appear, in fact, to be about 100 years old. Furthermore, there are multiple medical applications reported in this historical literature that appear not to have been considered by the modern plasma medicine community. It is possible that a careful reading of this heretofore mostly unknown literature will lead to new and useful applications of modern plasma medical devices. In this talk, I summarize some of the historical evolution of plasma medicine and offer perspectives on current and future applications in the light of this recently re-discovered history.

References
Multi-pulse Atmospheric Pressure Plasma Jet onto a Reactive Liquid Layer

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The use of non-thermal atmospheric pressure plasma jets (APPJs) for surface-treatment in medical applications is often associated with production of reactive oxygen and nitrogen species (RONS) in a liquid environment surrounding tissue. Activated species in the liquid are both produced directly in the liquid-phase and through solvation from the gas-phase. With a helium and oxygen-admixture jet, production of reactive nitrogen species (RNS) requires entrainment of and dissociation of the surrounding air environment. Fluences of activated species to the tissue underlying the liquid and RONS concentrations within the liquid are therefore functions of operating parameters that enable this mixing of reactive species. These parameters include pulse repetition frequency (PRF), entrainment capabilities of the jet (flow rate and proximity of the jet nozzle to the liquid surface) and proximity of the ionization wave to the liquid surface.

A computational investigation was conducted of a He APPJ with an admixture of oxygen above a reactive liquid layer to characterize RONS production and transport through the liquid while varying system operating parameters. High PRF results in plasma production in previously generated activated gas plumes, in turn resulting in increased densities of aqueous NO₃⁻ₐq coming from a growth of NO and O₃ densities in the gas-phase. A low PRF minimizes the interaction between pulses resulting in larger relative fluences of some reactive oxygen species (ROS) to the underlying tissue layer. The degree of entrainment of surrounding air directly relates to the concentrations RNS. The direct interaction of the plasma plume with the liquid surface relates to the concentrations of ROS.

This work was supported by the National Science Foundation and the Department of Energy Office of Fusion Energy Science.

Figure 1 – From left to right, electron, O, OH and NₓOᵧ radical densities after a 47 ns pulse at -15 kV of a He/O₂ (99.8/0.2) jet into air above liquid surface.
Characterization of Atmospheric Pressure Multijet Plasma Source Effects on Mouse Skin

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Over the past decade, there has been a growing interest in the development of cold atmospheric pressure plasmas for new biomedical applications. This so-called Plasma Medicine concerns various clinical indications, including infectious diseases and recently, cancer treatment [1]. Besides encouraging demonstrations, it has been shown that tolerance and safety issues should be considered with great care for any therapeutic application [2]. A new generation of applicators, able to generate multiple jets from a single primary plasma jet, has been developed and qualified through in vitro experiments. Moreover, they have shown multiple interests (cf. the presentation of Th. Maho and colleagues in this conference).

Using one of these multijet plasma sources, we have proceeded to in vivo treatments on healthy mice skin. Various assays have been carried, on nude mice (hairless mice) and CBl57/6 mice (hairy mice), which skins have different properties. The distance between the nozzle and the skin surface of the treated mouse can be adjusted by applying a spacer (in order to guarantee a constant gap during treatment - spacer not shown). The presence or absence of skin damages caused by the plasma was assessed as a function of time, distance and delivered power.

![Fig. 1: Assays of multijet plasma source from single Plasma Gun on Nude mouse (left) and CBl57/6 mouse (right)](image)

Between the applicator and the target, plasma jets could appear either clearly separated from each other or as a more diffuse discharge. Depending on the distance set between the nozzle and the tissue, different thermal effects and skin damages have been observed. The experimental results obtained from the characterization of these variables and their effects on the healthy mouse skin surface will be presented. These promising results will be used as guidance for the application of multijet plasma on decontamination procedures (cf. the presentation of Th. Maho et al.).

Acknowledgements

This work was supported by the CNRS PEPS project ACUMULTIPLAS and the ITMO Cancer in the frame of the Plan Cancer, project N°17CP086-00.

References

Determination of charge density in an atmospheric pressure plasma jet via electric field measurements and simulations

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Atmospheric pressure plasma jets have been extensively studied for several years as they showed very promising results in numerous fields such as material processing or plasma medicine. What makes cold plasma interesting is the presence of electrons, ions, reactive oxygen and nitrogen species, visible and UV light and high transient electric fields (EF). Even if the latter play a key role, from the production and propagation of the plasma to the efficiency of applications, they are far from being fully understood. This work focuses on the comparison of measurements of EF produced by a Plasma Gun (PG) discharge with an electro-optic probe based on Pockels effect [1] with 2D simulations of atmospheric pressure discharges propagating in a geometry close to the Plasma Gun’s one. Plasma Gun consists in a vertically downward oriented capillary with an inner high-voltage electrode and an outer grounded one. Plasma is powered with µs-duration voltage pulses from single shot to 4kHz.

In [2] the electric field is evaluated by the simulations inside the capillary, in the glass and outside. The aim of this work is to compare the radial profile of the electric field outside the capillary between experiments and simulations to get information on the plasma inside it. The electro-optic probe was placed at different levels alongside the capillary and was getting far from it. Measurements were executed every 5mm over 5cm. At the middle of the capillary a decrease inversely proportional to the distance has been found. It reminds the shape of an EF produced by an infinite uniformly charged cylinder. 2D simulations were in a good agreement finding also the same slope for EF. But simulations permit also to get information on the distribution of charge density and especially to know if the main contribution is from volume or surface charge density. Results show that the radial profile of EF outside the tube is mostly determined by the volume charge density in positive polarity while in negative polarity the surface charge density is dominant. Simulations have provided values of average volume charge density in the order of 7.5 nC/cm³ in positive polarity and of average surface charge density around 1nC/cm² in negative polarity. Near the electrodes, a decrease inversely proportional to the square of the distance has been found, showing that there is an axial position dependence. A time dependence investigation will also be presented.

X.D. acknowledges his grant funding Thermofisher Scientific INEL/Région Centre Val de Loire.

References
Non-thermal plasma as an innovative anticancer strategy on leukemia models

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Antitumor chemotherapy is often hampered by the low therapeutic index of most anticancer drugs and the development of chemoresistance. Furthermore, in leukemia as compared to other kind of tumors, incidence and death rates in patients are really close to each other, pointing out that efficacy of anticancer therapy is suboptimal. Thus, there is a continuous need for new intervention strategies, endowed with a better pharmaco-toxicological profile. Cold atmospheric plasma (CAP) has gained interest as a promising anticancer strategy and earlier studies demonstrated the “non-aggressive” nature of CAP [1]. Several lines of evidence showed that the anticancer activity of CAP mainly depends on the increase in oxidative and nitrosative stress that leads to tumor cell death [2]. However, mechanisms of CAP-cell interaction are not yet completely understood.

In this context, the aim of this contribution is to unravel CAP anticancer effects on in vitro and ex vivo leukemia models achieved within the Italian national project Scientific Independence for young Researchers (SIR), a project that brings together a multidisciplinary team of researchers in the area of Physics/Engeneering, Pharmacology and Oncohematology. The cytotoxic impact of two different plasma sources, a nanosecond pulsed dielectric barrier discharge (DBD) [3,4] and a microsecond pulsed DBD jet, on T-lymphoblastic cell lines was investigated. In particular, we analyzed apoptotic and/or necrotic events, cell-cycle progression, levels of proteins involved in the regulation of apoptosis correlated to CAP characteristic and composition. To explore its selectivity for tumor cells, CAP was tested on normal lymphocytes, as non-transformed counterpart of leukemic cells. Due to the key role of reactive oxygen and/or nitrogen species in the biological effects of CAP, its genotoxic potential was assessed. Furthermore, some preliminary results indicate that CAP was able to induce cytotoxic effects also on leukemia cells cultivated in hypoxia, which plays a critical role in promoting chemoresistance. Taken together, these results contribute to understand the pharmaco-toxicological potential of CAP, thus making the basis to further investigate its anticancer properties.

This work was supported by National SIR Grant RBSI14DBMB (MIUR).

References

Plasma medicine is an interdisciplinary research spreading over physics, chemistry, biology and medical sciences. The review [1] focuses on mechanisms coupling the physics and chemistry of low temperature plasmas to medically relevant biochemistry and biology. Instead of the great progress in this fields, many aspects still remain to be explored. From the viewpoint of modelling, computational biology, i.e. theoretical or mathematical modelling and computational techniques to the study of biological systems [2], should be a powerful tool for the plasma medicine. The computational biology includes the simulations of inner-cellular systems. For example, mitochondrial function in cellular energetic metabolism is one of the most important inner-cellular systems. The essential parts of this system are the tricarboxylic acid cycle (TCA cycle), the respiratory chain (electron transport chain) and the adenosine triphosphate (ATP) synthesis machinery. Figure 1 shows the simplified schematic diagram of the above described mechanisms. The aim of this modelling work is to understand low temperature plasma-induced multiphase reactions and biological processes systematically by quantifying reactive species behaviour and correlating with the plasma induced biological mechanism. In particular, the key issues on the modelling to link the plasma-physics and -chemistry with biological systems will be presented and discussed in the talk.

This work was partially supported by JSPS KAKENHI Grant Number 16K04998.
Similarities and Differences in Gene Transcription in Plasma-activated Medium Treated and Plasma-activated Ringer’s Lactate Solution Treated Glioblastomas

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Plasma medicine is a novel interdisciplinary field that combines studies on plasma science and medical science [1]. Non-thermal atmospheric pressure plasma has been used for medical treatments, such as for cancer, blood coagulation, and wound healing. Direct and indirect treatment of cells with plasma has broadened the applications of non-thermal atmospheric pressure plasma in medicine.

We have previously developed plasma-activated medium (PAM) [2] and plasma-activated Ringer’s lactate solution (PAL) [3] for cancer treatments. We have found some similarities and differences PAM-treated and PAL-treated glioblastomas. We showed that both PAM and PAL exhibited anti-tumor effects on glioblastomas through in vitro and in vivo experiments. We detected little intracellular reactive oxygen species (ROS) in PAL-treated glioblastoma cells, while PAM induced more ROS than did PAL.

To elucidate the mechanisms that PAM induced more ROS than PAL, we investigated early gene expression in PAM-treated and PAL-treated glioblastomas using real-time PCR. Relative expression levels of antioxidant genes, transcription factors, and stress inducible genes were evaluated in PAM-treated and PAL-treated glioblastomas. Interestingly, various differences as well as similarities in gene expression between PAM-treated and PAL-treated glioblastomas have been discovered. We will discuss how PAM and PAL influence on gene regulatory networks in glioblastoma cells and potential therapeutic applications of PAM and PAL.

This work was partly supported by a Grant-in-Aid for Scientific Research on Innovative Areas “Plasma Medical Innovation” Grant No. 24108002 and 24108008, a Grant-in-Aid for Young Scientists (A) Grant No. 15H05430, and a Grant-in-Aid for Challenging Exploratory Research Grant No. 15K13390 from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

Modeling the reaction-diffusion transport of plasma-induced ROS through thin liquid layers

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In many applications of plasma for medical purposes, plasma is applied on the top a thin liquid layer, while the “target cells” are located under the liquid. Transport of various ROS molecules may play the role of signal transduction mechanism. There are two major possible pathways of the signal transduction: through the cells in the liquid layer and around those cells. Understanding the dominant pathway will shed the light on the most efficient way of delivering the plasma impact. In our presentation we discuss numerical simulations of transport of ROS across the liquid layer around the cells. By computing the total flux of the ROS into the target, we show how fast the actuation can be transferred in the presence of multiple chemical reactions. We use a reaction-diffusion model to simulate the transport of ROS molecules. We show the presence of what species may significantly slow down the between-the-cells pathway of transduction. Future comparison of our results with experiments will determine the relative role of the between-the-cells pathway.
Plasma Assisted Delivery of Antibiotics
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Plasma coating of medical devices has been shown to deliver highly biocompatible surfaces based on glycols, amines and other simple functionalities and the technology is well established. Recent research has shown that the combination of a non-thermal, inert gas plasma and a liquid delivery system can produce coatings with more complex biological functionality based on direct plasma deposition of proteins, polysaccharides and mixtures. [1]

Recent research in our labs have shown that it is also possible to directly deposit small molecule pharmaceutical drugs on to a range of surfaces without loss of functionality. To demonstrate this capability, a range of antibiotics have been deposited onto stainless steel substrates and their efficacy tested against both E. coli and P. aeruginosa bacterial strains. The data has clearly demonstrated that the antibiotics remain effective and that the bacterial kill rates are proportional to the minimum inhibitory concentration of each antibiotic. Elution rates were determined in buffered solutions and showed that the bulk of the drugs were eluted over a 7 – 10 day period.

Further work is now underway to determine if plasma deposited antibiotics can inhibit bacterial growth on medical devices in both in vitro and in vivo settings. This could open a path to deliver localized pharmaceutical agents directly to the site of an injury without the use of synthetic linkers, binders or polymers. This would allow for a higher surface concentration of the active agent and reduced complications due to excipient ingredients.

Plot of P. aeruginosa growth over time for control sample and sample exposed to a gentamicin antibiotic coating (ACP-G)

This work was supported by Enterprise Ireland grant number IP/2016/0516/Y.

References
Comparison of a µs-pulsed and a ns-pulsed dielectric barrier discharge for skin permeabilization

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Dermal drug delivery can have several important advantages over the more widely-used strategies of subcutaneous injection and parenteral administration: As a noninvasive, easy-to-use and often pain free method it promises improved patient compliance and avoids drug inactivation due to the first-pass-effect. To improve drug permeation through the stratum corneum, which constitutes the main barrier of mammalian skin, cold atmospheric plasma (CAP) is considered a prospective tool for permeabilization.

This study aimed to elucidate the mechanism of plasma-permeabilization through a comparison of two dielectric barrier discharges (DBDs) with differing pulse characteristics. By means of Franz-cell permeation experiments in combination with transepithelial electrical resistance (TEER) measurements, we studied the overall effect of DBD-treatment on human skin barrier properties. Additionally, we employed electrochemical and redox reactions to directly visualize patterns of permeabilized regions in treated stratum corneum and evaluate the production and permeation of oxidizing species formed in the plasma.

Our results suggested a larger permeabilizing effect of the filamentous µs-pulsed discharge as compared to the more homogeneous ns-pulsed DBD. The density of pores, the permeation of fluorescent model drugs and the decrease in electrical resistance (TEER) were significantly higher for isolated stratum corneum and full-thickness skin samples treated with the µs-pulsed DBD than for samples treated with the ns-pulsed DBD at comparable power densities. This has important implications for the proposed mechanism of skin permeabilization by CAP-treatment.

Plasma-permeabilization is a promising method for future application in dermatology and pharmacology. It potentially holds several advantages not only over established techniques of drug delivery, but also over other innovative methods to enhance dermal drug delivery (electroporation, sonoporation and many more), since recent studies have shown therapeutically beneficial effects [1–3], which may be used synergistically in future clinical applications.

This work was supported by a Georg-Christoph-Lichtenberg grant to M. Gelker, funded by Lower Saxony’s Ministry of Science and Culture (MWK). Experimental equipment was funded by the Federal Ministry of Education and Research (FKZ: 03FH015IX5).

References

Plasma Medicine in Comparison with Nivolumab and other Cytostatic Drugs in Locally Advanced Head and Neck Cancer: Survival Benefit for Patients?

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Patients with infected ulcerations of locally advanced oral cavity carcinoma that not even respond to radio- and cisplatinum-therapy, have poor prognosis and only a few treatment options. One is nivolumab, a recently introduced and in particular promising novel checkpoint inhibitor drug significantly improving survival of this patient population compared to standard single agent therapy with cetuximab, methotrexate or docetaxel [1]. Another treatment option for ulcerated cancer is cold physical plasma, at present mainly as part of a palliative cancer treatment program for the relief of these patients [2]. To receive an initial appraisal whether there is any positive influence of plasma medicine on the survival of the patients as well, we have contrasted in a comparative analysis the first clinical results of a plasma therapy pilot study [3] with the results of the randomized phase 3 chemotherapy trial nivolumab (CheckMate 141) [1]. Figure 1 displays the Kaplan-Meier survival curves of 240 patients treated with nivolumab versus 121 patients under standard chemotherapy by investigator’s choice [4]; and superimposed on this background is the survival curve of 6 patients treated with a jet plasma source (kINPen MED, neoplas tools GmbH, Greifswald, Germany). Eligible patients in all the three groups were of the same kind.

The curves show the percentage of patients still alive under specific treatment in the course of months. With the view to survival benefits, the results call for further clinical plasma trials with a statistically relevant number of patients and two hypotheses to be tested: plasma treatment is in terms of overall survival (i) not harmful to life and (ii) comparable to standard and novel chemotherapy while at the same time advantaged with only very few and no known serious side effects [2].

Figure 1: Survival Curves of patients treated by nivolumab, investigator’s choice or plasma

References

Plasma Processing of Biologically Active Membranes at Atmospheric and Low Pressure for Drug Delivery Systems

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Drug delivery systems (DDS) are intensively studied and developed for their application in the medical treatment of various diseases. By controlling the drug release around a treatment area over a prolonged period of time it is possible to precisely maintain locally the drug concentration within a therapeutic window and avoid overdoses as well as a sub-therapeutic concentration of the drug. Present research work is devoted to developing plasma methods to deposit functional coatings on collagen membranes by plasma processing to fabricate multi-layered DDS. Biocompatible collagen membranes were used as substrates. ε-caprolactone and diethylene glycol dimethyl ether were used as precursors to achieve amphiphilic PCL:PEG films \cite{1}. To fabricate DDS, the first layer was a dense barrier layer (200 nm) deposited in a low pressure capacitively coupled plasma reactor (CCP, 13.56 MHz, 25 W, 0.5 mbar). The second layer was a carboplatin drug, dried from an aqueous solution on the surface of the barrier layer with a drug load of 200 μg/cm\textsuperscript{2}. The third layer was a dense barrier layer deposited at the same conditions as the first layer, to form “sandwich” like structure of DDS. The last top layer was deposited in soft plasmas at atmospheric pressure plasma (18 kHz, 2W, in order to preserve the desired chemical moieties of the precursor). In this paper it will be shown that the compatibility of the mechanical properties of the biocompatible collagen substrate and that of the first dense barrier layer deposited on top of it plays an important role in the drug delivery kinetics of the drug determined by ICPMS. Furthermore the use of low pressure and atmospheric pressure plasmas for the fabrication of DDS will be discussed. Can dense and crosslinked barrier films be fabricated entirely by DBD discharges for DDS? To respond to this question the refractive index of the deposited films in optimized conditions of energy deposit in both CCP and DBD were compared by ellipsometry. Human ovarian carcinoma cell line (NIH:OVCAR-3) was used for \textit{in vitro} measurements of cell interactions with the surface of fabricated DDS. Proposed model of DDS on collagen films prevents migration, adhesion and growth of cancer cells on its surface, and by tuning the thickness of the dense barrier films it is possible to control drug release kinetics and improve the therapeutic effect. \textit{In vivo} experiments were carried out where mice lymph nodes were injected with OVCAR3 cells and after development of a tumour DDS membranes were implanted to evaluate the feasibility of the proposed model.

\textbf{Keywords:} pulsed plasma enhanced CVD, PCL-PEG copolymerization, drug delivery system, DBD

\cite{1} S. Bhatt, F. Valamanesh, J. Pulpytel, R. Lo Dico, A. Baitukha, I. Al-Dybiat, M. Pocard, F. Arefi-Khonsari, M. Mirshahi, Oncotarget, 7, 36, 2016.
Applying cold atmospheric plasma (CAP) for medical applications has revealed enormous potential in view of therapeutic efficacy as shown in various controlled clinical studies [1,2]. However, there are still many open issues with regard to the fundamental mechanisms by which CAP interacts with microorganisms, viruses, mammalian cells and tissues. It is not fully understood how CAP reacts with proteins which play a major role among the components of human and animal tissues [3,4]. Our study highlights CAP-induced effects on the surfaces of the building blocks of proteins, amino acids. Up to this time, only a few studies are dealing with the treatment of amino acids in aqueous solution with CAP, however there are no studies on amino acids in the solid state [5-8]. In this study we investigate two of the most abundant amino acids in the collagen of the human skin, L-Proline (Pro) and trans-4-Hydroxy-L-Proline (Hyp) in the crystalline form.

Pro and Hyp pellets were exposed to CAP generated by dielectric barrier discharge (DBD) operated in ambient air at dry conditions. Surface analysis by XPS revealed a dose-dependent oxygenation of each amino acid. In Pro, the ring structure is cleaved, decarboxylation and deprotonation or deamination occur, also NO$_3^-$ or NO$_2$ groups are added to the surface. In Hyp, NO$_3^-$ or NO$_2$ groups were detected, however there seems to be no evidence for ring cleavage. After 16 days, the analysis of ageing effects of treated Pro and Hyp indicates that plasma induced modifications are to some degree reversible, exhibiting a lower amount of oxygen and the absence of the new nitrogen containing groups. For both Pro and Hyp, ATR-FTIR revealed an attachment of OH and C=O groups in an acid COOH and a ketone group. The aged samples of Pro have still detectable OH and C=O bands. In Hyp, only the C=O band is present.

This work was supported by National Grant no. 13GW0041D.

References

Plasma chemistries in aqueous solutions for biomedicine

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Abstract: This work demonstrated the decomposition products of several amino acids, sugars, and lipids when exposed to nonthermal atmospheric pressure plasma. 20 mM solutions of chemicals were treated with a floating electrode dielectric barrier discharge (FE-DBD) plasma for varying treatment times to investigate possible mechanisms in cell wall degradation and treatment of tissue. The treated solutions were analyzed primarily using GC-MS and NMR. The primary targets of the plasma treatment were found to be the oxygen or nitrogen species. Small molecules, such as acetic acid and lactic acid were observed throughout all solutions as oxidative products from the plasma treatment.

This work was supported by Keck Foundation.
Plasma induced immune-modulations and its application in cancer treatment

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The application of plasma medicine technology has been actively explored over the last several years. Recently, non-thermal plasmas have demonstrated potential as a safe anticancer therapeutic approach that can kill various types of cancer targets [1-4]. There is the urgent need of new human health care’s technology against cancers based on immuno-modulation. Our research work mainly comprises plasma induced activation of immune cells; which find applications for curing various kinds of resistant tumors and other dreadful diseases. Our main objectives are (i) to clarify basic mechanism on plasma induced immuno-modulations (ii) to develop immunomodulation based strategy for the treatment of various dreadful diseases including cancers (iii) to perform pre-clinical study. Recent preliminary study suggests that plasma significantly modulated immune cells and can induce cancer cell death in co-culture condition [5-7]. Recently, we have reported that plasma-modulated cytotoxic macrophages release TNF-α, which blocks cancer cell growth and can have the potential to contribute to reducing tumor growth in patients in the near future.

References

Prevention of Peritoneal Adhesion by Cold Atmospheric Plasma Treatment

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Abdominal adhesions are defined as fibrous tissue bands that span two or more abdominal organs and/or the peritoneal membrane, that form following abdominopelvic surgeries. Despite the rapid development in surgery, and introduction of novel methods for prevention of peritoneal adhesions, peritoneal adhesions still remain as one of the biggest challenges for surgeons and as well as complications for the patients. The prevalence of adhesion related intestinal obstruction in patients that undergo abdominopelvic reoperation is reported as around 40%. Moreover, consequences of adhesions are not limited to intestines. Peritoneal adhesions are one of the most common reason for secondary fertility in women and cause chronic abdominal pelvic pain. The mechanism of the peritoneal adhesion is not well understood, but is believed to involve wounding of the peritoneum that is followed by inflammatory pathways [1]. Previously, use of argon plasma coagulator, instead of electrocautery reduced adhesion formation from about 85% to 50% [2].

In the present study, efficacy of cold atmospheric plasma on prevention of peritoneal adhesion was evaluated on male CD-1 mice. Sixteen male CD-1 mice were randomly divided into two groups. In both groups adhesion models was created on cecum and peritoneum by serosal abrasion and parietal peritoneum excision, respectively. In the control group, no adhesion preventive method was applied. In plasma treated group, cecum and peritoneum was treated with non-thermal atmospheric DBD air plasma for 60 seconds. A custom-made pin electrode with 6 mm diameter was used. Plasma was generated using a microsecond pulsed AC power supply that was operated at 21 kV of voltage and 1 kHz frequency. Ten days after formation of adhesion model and plasma treatment, all animals were sacrificed and adhesion regions were evaluated macroscopically in terms of area of prevalence and intensity of adhesion using Linsky and Knightly classification, respectively (scores given 0 to 4). Moreover, abdominal organs and peritoneum were excised and histopathologic evaluation was carried out using Zühlke classification.

In control group, Linsky, Knightly and Zühlke scores belonging to cecum were determined as 2.1, 2.6, 2.6 respectively whereas in plasma group those were find as 1.1, 0.5, 1.3, respectively. For peritoneal adhesion model, plasma treatment reduced Linsky, Knightly and Zühlke scores from 3.8 to 1.3, 3.3 to 1.6 and 3.4 to 1.5, respectively.

In conclusion, the present study shows that, cold atmospheric plasma could be an effective method for prevention of peritoneal adhesion.

References

**In-vivo investigation of Cholangiocarcinoma treatment using cold atmospheric plasma gun**

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Cholangiocarcinoma (CCA) is a type of liver cancer, i.e. a heterogeneous group of malignancies that can emerge at every point of the biliary tree. To date, the unique effective therapy, surgical resection, is only possible in 20% of cases with a 5-year survival rate of CCA patients that varies among 30-40% [1]. Since innovative treatments are highly expected and plasma medicine has already been successfully applied to other carcinomas [2-3] the current study aims to evaluate the treatment of CCA *in-vivo* using cold atmospheric plasma (CAP). CAP, generated using a Plasma Gun configuration [4], was applied for the treatment CCA in a model of subcutaneous xenograft tumors in immunocompromised (nude) mice.

First, we investigated the short-time effects of plasma treatment on the skin of nude mice (Figure 1). Several plasma powers, times of exposition and distances between the plasma gun and the skin were tested. Influence of these parameters will be described by histomorphological analysis of the skin after H&E staining in order to find the optimum treatment condition without toxicity and/or unpleasant effects for the animals. Second, tumors will be generated by injection of CCA EGI-1 cells under the skin of nude mice and once the tumors have acquired a pre-established size they will be treated with CAP and/or with gemcitabine in order to compare the efficacy of CAP with current CCA therapy. Tumor volume and weight will be measured and histological analysis will be perform to evaluate the effects induced by CAP on CCA.

![Figure 1. Application of CAP on nude mice skin.](image)

**References**

Realization of a high voltage high frequency sinusoidal power supply with on-board power diagnostics controlled by Arduino

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Cold plasmas are nowadays used in a large variety of applications, from the medicine field to the agriculture one, to waste and pollutant treatment [1]. High voltages, high frequencies and limited currents are usually necessary to ignite a non-thermal discharge at atmospheric pressure.

One of the most important control parameters of a plasma treatment is the energy dose. This quantity is usually defined as the product between discharge average power and treatment time. It gives an easy and reliable criterion to evaluate the efficacy of a treatment (i.e. how effective a treatment is for a given energy dose).

In order to improve the consistency of testing procedures, a sinusoidal high voltage, high frequency power supply equipped with on-board power diagnostics has been designed and tested.

In addition to real measurements, frequency response of the system has been simulated with the software LTSpice, showing that experimental results and simulations are in good agreement.

This power supply reaches voltages up to 20 kV peak, with frequencies in the range of 15÷50 kHz and discharge powers up to 150 W, with the possibility to modulate output voltage with on-off cycles. The power supply is controlled by an Arduino DUE. This microcontroller has been chosen because of its low cost, its ease of programming and its ability to sample analog signals with a sampling frequency up to 500kHz.

The power supply is capable of outputting a constant power, while continuously monitoring voltage and charge with embedded probes. In order to preserve power electronic components and increase power supply efficiency, soft-switching (Fig. 1) working frequency is automatically sought when a new load is connected (Fig. 2). Supply voltage, frequency, power and on/off time of the discharge are shown in a digital display as a visual aid to the user.

A feedback control used to compensate power variations due to load alterations is currently under development.

Fig. 1: Mosfet voltage and input current in the zero-current switching mode

Fig. 2: Normalized output voltage as a function of the scan frequency

References

DBD FOR IMMUNOGENIC CELL DEATH: NOT ONLY AN H₂O₂ GENERATOR

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Research Aims: Immune-mediated cancer cell death (ICD) is a cell death pathway that assists the re-establishment of a patient’s specific anti-tumor immune response for immunotherapy of cancers [1]. Treatment of multiple cancer cell lines in vitro with both plasma jets and dielectric barrier discharges (DBDs) resulted in the expression of ICD-associated signals including surface-exposed calreticulin (ecto-CRT) [2, 3]. For DBD, oxygen species produced by plasma were identified as the most responsible component for ICD induction, while electric fields and ultraviolet radiation had negligible effects [2]. However, the chemical species that elicit ICD are not fully delineated. In this study, our aim was to identify which plasma-generated oxygen species are required for ICD.

Methodology: A microsecond-pulsed DBD (17 kV, 500 Hz, 10 seconds, 1 mm distance) was used to directly treat B16F10 murine melanoma cells. Cells were collected at 24 hours and stained for ecto-CRT as a marker of ICD. PBS was treated at the same condition and analyzed for an array of reactive oxygen and nitrogen species (RONS) (Table 1). To determine whether ecto-CRT was elicited by certain species (H₂O₂, NO₂⁻, etc.) cells were treated at measured concentrations of both species. Results: Plasma was able to elicit emission of ecto-CRT. Cells treated with 500 Hz-equivalent H₂O₂ and NO₂⁻ did not induce ecto-CRT (Fig. 1). We showed that O, O₃, OH, and NO were present in the liquid while ¹O₂ and O₂⁻ were not. Conclusions: Our results suggest that H₂O₂, NO₂⁻, ¹O₂, and O₂⁻ may not be the major effectors of DBD-elicited ICD. Ongoing work includes a vaccination study with C57BL/6 mice to further distinguish the role of persistent plasma species for ICD.

This work was supported in part by the Flanders Research Foundation (grant no. 12S9218N) and the European Marie Sklodowska-Curie Individual Fellowship ‘LTPAM’ within Horizon2020 (grant no. 657304). The DBD plasma system was purchased through Drexel University.

References
In this study, we characterized the biomedical plasmatron “APT001 Plasma-Generated Nitric Oxide Device” from the point of view of active species production. We focused on the NO, NO\textsubscript{2} and H\textsubscript{2}O\textsubscript{2} species as the most active and important for biomedical applications, UV-radiation from the plasma and electrical parameters of the plasmatron.

Concentrations of different components measured using MS spectral analysis (a quadruple mass-spectrometer with e-beam ionization (E\textsubscript{i} = 20 eV)) are shown in the Table 1. NO concentration changes from almost 10000 ppm near the plasmatron nozzle exit to 850-900 ppm at 40 mm from the safety applicator outlet.

<table>
<thead>
<tr>
<th></th>
<th>10 mm</th>
<th>84 mm</th>
<th>84 mm w shell</th>
<th>124 mm w shell</th>
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<tr>
<td>N\textsubscript{2}</td>
<td>0.766</td>
<td>0.761</td>
<td>0.765</td>
<td>0.763</td>
</tr>
<tr>
<td>O\textsubscript{2}</td>
<td>0.202</td>
<td>0.204</td>
<td>0.200</td>
<td>0.201</td>
</tr>
<tr>
<td>Ar</td>
<td>0.0330</td>
<td>0.0341</td>
<td>0.0340</td>
<td>0.0342</td>
</tr>
<tr>
<td>NO</td>
<td>9970 ppm</td>
<td>999 ppm</td>
<td>901 ppm</td>
<td>849 ppm</td>
</tr>
<tr>
<td>NO\textsubscript{2}</td>
<td>113 ppm</td>
<td>70 ppm</td>
<td>68 ppm</td>
<td>70 ppm</td>
</tr>
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</table>

It was found that the major product of the plasmatron is nitrogen oxide NO. No significant production of OH, H\textsubscript{2}O\textsubscript{2}, NO\textsubscript{2} has been detected.

Figure 1 shows a relative spectral photon flux distribution in the range 200-400 nm. Spectral step was 10 nm, the signal is averaged over 5 ms (500 000 sampling points per each mark in Figure 1). The UV emission from the plasmatron has a significant component at 250 nm. Discharge stability was estimated using long sequence of synchronous voltage-current measurements. The record length was 25M points per each channel. The plasmatron’s operation envelope was found. The discharge has a minimal operational voltage ~100 V, maximal voltage on the discharge gap was ~210 V. Current demonstrates operational window between 1.25 and 2.25A. Slightly negative current-voltage dependence is explained by the arc nature of the discharge.
Non-thermal Plasma Induced Immunogenic Cell Death in Colorectal and Pancreatic Cancer
Marian Khalili, Abraham Lin, Adam E. Snook, William F. Morano, Vandana Miller, Wilbur B. Bowne

Background: Immunogenic cell death (ICD) is a form of cell death characterized by emission of damage associated molecular patterns (DAMPs) that can facilitate activation of an adaptive immune response against dead-cell antigens. In the case of cancer therapy, tumor cells undergoing immunogenic death can promote cancer-specific immunity. This study investigates the role of plasma as an ICD inducer in colorectal and pancreatic cancer.

Methods: CT26 (murine colorectal cancer) and Panc02 (murine pancreatic ductal adenocarcinoma) cells were assessed for the presence of hallmark DAMPs, ATP and externalized calreticulin, and cell viability after plasma regimen (29 kV, 30 Hz, 1 mm gap distance, 10 seconds). ATP content was detected in media 10 minutes after plasma treatment using a chemiluminescent kit. Fluorescence-activated cell sorting (FACS) analysis of membrane calreticulin was performed 24 hours after plasma treatment. Cell viability was measured 24 hours after plasma treatment using Propidium Iodide. In a vaccination protocol, Balb/c mice (10 per group) were immunized with CT26 cells treated in vitro with 1) plasma at the ICD-inducing regime (29 kV, 30 Hz, 1 mm gap distance, 10 seconds), 2) media only or 3) Cisplatin (50 µM for 24 hours). One week after immunization, mice were challenged with live CT26 cancer cells on the opposite flank and tumor growth was monitored biweekly until day 26.

Results: In vitro, non-thermal atmospheric pressure plasma treatment of CT26 and Panc02 induces release of the classic ICD “danger signals” (ATP and externalized calreticulin) and reduces cell viability. We observed an approximate 50% increase in externalized calreticulin expression in both plasma treated CT26 and Panc02 cell lines compared to untreated cells (p<0.01). There was a dose dependent increase in ATP detection in both cell lines with increasing plasma energy (p<0.01). Cell viability decrease was also dose dependent for both CT26 and Panc02 (p<0.05), as shown below.

To assess induction ICD in vivo, a vaccination assay was performed. Remarkably, mean tumor volume for the plasma-immunized group was significantly smaller compared to that of the media group (414.7±104.3 mm³ vs. 847.4±141.5 mm³; p<0.001) or the Cisplatin group (1041.8±208.3 mm³ p<0.001) at day 26. 90% of the mice in the plasma-immunized group had tumor volumes smaller than the mean tumor volume of the media group (850 mm³), suggesting that these mice were partially protected by vaccination. Moreover, 3 out of 10 mice in the plasma group did not develop subcutaneous tumors on the challenge site supporting the hypothesis that plasma is an authentic ICD inducer.

Conclusion: Our early investigation provides evidence that non-thermal plasma is an inducer of ICD in both murine colorectal and pancreatic cancer cell lines. Moreover, a whole-cell vaccine produced by in vitro treatment of cancer cells with plasma resulted in protective immunity in a murine model of colorectal cancer highlighting its potential for clinical translation in cancer immunotherapy.
The effect and mechanism of non-thermal atmospheric pressure plasma on the proliferation of adipose tissue-derived stem cells

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Summary (100 words or less)
We showed that exposure to non-thermal atmospheric pressure plasma (NTAPP) increased the proliferation of adipose tissue-derived stem cells compared with untreated cells without affecting their stem cell properties. In ASCs exposed to NTAPP, Akt, ERK1/2, and NF-κB were activated, and the proliferating cell nuclear antigen (PCNA) was highly increased. NTAPP exposure also changed the expression of histone deacetylase 1 (HDAC1) and acetylated H3, and increased the mRNA of Oct4, Sox2 and Nanog in ASCs. These results suggest that NTAPP activate the proliferation of ACSs by inducing epigenetic changes. Altogether, NTAPP would be an efficient tool for medical application of ASCs.

Abstract
Non-thermal atmospheric pressure plasma (NTAPP) is described as a quasi-neutral mixture of charged particles and radicals in a partially ionized gas at atmospheric pressure. We showed that exposure to NTAPP generated in a helium-based dielectric barrier discharge (DBD) device increased the proliferation of adipose tissue-derived stem cells (ASCs) by 1.57-fold on an average, compared with untreated cells at 72 h after initial NTAPP exposure. NTAPP-exposed ASCs maintained their stemness, capability to differentiate into adipocytes but did not show cellular senescence. In addition, the mRNA level of well-known pluripotent genes, Oct4, Sox2 and Nanog, was increased in NTAPP-treated ASCs compared with that of the unexposed cells. Also, Akt, ERK1/2, and NF-κB were activated and the proliferating cell nuclear antigen (PCNA) was highly increased at 72 h in NTAPP-exposed ASCs. These results suggest that NTAPP can activate the proliferation of ASCs without affecting their stem cell properties. In NTAPP-exposed ASCs, the increase of histone deacetylase 1 (HDAC1) and the decrease of acetylated H3 were also detected, suggesting that NTAPP induce epigenetic changes for activating the proliferation of ASCs. Studies using the scavengers for nitric oxide (NO) and ROS demonstrated that NO rather than ROS is responsible for the enhanced proliferation of ASCs following NTAPP exposure. Taken together, this study supports that NTAPP would be an efficient tool to activate the proliferation of ASCs for use in the medical application of ASCs both in vitro and in vivo.

References
Investigation on a He-Plasma Gun source for cosmetic purposes:  
the importance of skin microenvironment

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With the increase of human span life people demand to live and look better. In a world where appearance is synonymous of health, the demand of new cosmetic has literally exploded. Among cosmetic treatments, skin care represents a large part of the business of beauty. From 2017 to 2023, world-wide non-surgical skin cosmetic treatments business will have a \textit{compound annual growth rate} between 4.7\% and 5.3\% \cite{1}. The increased demand of non-surgical beauty treatments pushed cosmetic research during the last years. In Europe since 2013 cosmetic tests on animal model are definitively banned. This encouraged researchers to develop new approaches to study skin “\textit{in-vitro}” using either re-constructed tissue structure or attempting to mimic skin microenvironment parameters. Cell culture has been used since a long time to study organ functions in laboratory. In the last decades cell culture has greatly evolved in order to mimic the real tissue structure and microenvironnement. Cells are cultivated in three dimensions and media are adapted to better simulate the extracellular environment. Attempting to get closer to the physiological micro-environment of a cell, scientists underestimated often a key parameter: the oxygen level \cite{2}. While oxygen represents the 21\% of the air gases, in human tissues, its percentage is significantly lower. In skin, it can vary from 7\% to 1\%. To be as close as possible to skin microenvironment, in our lab we grow human skin cells either in classic normoxic condition (18\% O\textsubscript{2}) or in physioxic condition (3\% O\textsubscript{2}). Oxygen has a key role in cell respiration and in reactive oxygen species (ROS) production. We have already demonstrated that skin cells raised in physioxia produce lower quantity of reactive oxygen and nitrogen species (RONS) even when exposed to plasma treatment \cite{3}. In this study we evaluated the effect of a Cold Atmospheric Plasma (CAP) treatment on human skin cell raised either in normoxia or in physioxia. In particular, modulating plasma parameters from our helium Plasma Gun, we investigated the ability of CAP to improve cell viability and extracellular matrix production such as collagen, hyaluronic acid and elastin, macromolecules involved in maintaining the health and beauty of the skin.

References

\cite{1} https://www.reportlinker.com/p05112181


This work was supported by Cosmetosciences, a global training and research program dedicated to the cosmetic industry. Located in the heart of the Cosmetic Valley, this program led by University of Orléans is funded by the Région Centre-Val de Loire, France.
Spatially Uniform Dose Delivery with a kHz-Excited Atmospheric Pressure Plasma Jet with Model-based Feedback Control

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A critical challenge in plasma medicine is ensuring the delivery of a spatially uniform cumulative effect or ‘dose’ over the treated substrate. The sharp spatial gradients observed in temperature, species concentrations and electric field [1] in common plasma medical devices such as atmospheric pressure plasma jets (APPJs) compound this challenge. Moreover, sources of internal variability [2] as well as the effect of external conditions (such as varying properties of substrate [3] and changes in ambient conditions) can drastically affect APPJ operation. Particularly, the hand-held operation of the APPJ, as is the common practice, relies solely on the expertise of the physician to maintain a constant separation distance between the jet and substrate and deliver treatment at a prescribed rate. Considering that medical therapies require stringent safety considerations, the need for strategies involving in-situ diagnostics and regulation, i.e., feedback control become apparent for high-performance (safe, reliable, reproducible) operation of plasma medical devices [4]. To address this issue, we experimentally investigate the effectiveness of model-based feedback control strategies.

The experimental set-up consists of an APPJ based on a powered copper ring wrapped around a quartz tube (ID=3mm) doubling as dielectric barrier and flow channel. An infrared thermal camera is used to obtain spatially resolved temperature of the substrate surface. Plasma electrical characteristics are measured using a high voltage probe and a Pearson coil. The applied voltage, frequency and helium mass flow rate are actuated via a microcontroller (Arduino UNO). A single board computer (Raspberry Pi 3) is used to process measurements and coordinate actuation. Robotic actuators based on stepper motors are used to translate the APPJ over the target substrate. Considering that the plasma generates multiple effects on the substrate, we define two integrating dose metrics describing the plasma thermal and nonthermal effects on the substrate. Using a lumped-parameter modeling strategy, supported by a 2D transport model, we generate a mathematical description of the plasma circuit and thermal dynamics to describe the dose delivery dynamics and inform the design of control strategies. Using the experimental set-up, and the defined dose metrics, we investigate the performance of two model-based control strategies: (a) classical proportional-integral (PI) control and (b) model predictive control (MPC) in terms of spatially uniform dose delivery.

References

Immunostimulatory Effects of Atmospheric Pressure Non-Thermal Plasma Exposure on Murine Macrophages

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Atmospheric pressure non-thermal plasmas (APNTP) exhibit marked biological effects and potentially widespread applications [1,2]. One emerging application of APNTP is immune modulation, which has mainly been described as a potential adjunct to cancer therapy [3]. The main aim of this study was to examine the ability of APNTP to stimulate RAW264.7 murine macrophages and their phagocytosis of foreign bodies. RAW 264.7 cells were exposed to a kHz-driven plasma jet in culture medium (indirect treatment) for different exposure times. Pro-inflammatory cytokine (TNF-α, IL-6) concentrations were measured in plasma treated cell supernatants using ELISA. Plasma-induced phagocytosis was examined using flow cytometric analysis and fluorescent imaging following incubation of the plasma exposed macrophages with fluorescent latex-carboxylate modified polystyrene beads (diameter 0.5μm) and pHrodo™ red bioparticles®. The ELISA results revealed a significant stimulatory effect, as measure by a significant increase in expression of both TNF-alpha and IL-6, evident after 15 sec of macrophage exposure to plasma (Figure 1). However, the optimum stimulatory activity was observed following 60 sec of plasma treatment. Flow cytometric data demonstrated plasma-induced enhancement of macrophage phagocytosis of both types of beads, following 15 second and up to 60 seconds of plasma exposure time. In conclusion, APNTP elicited a promising stimulatory effect in immune cells, which could be used in future for management of bacterial infection.

Figure 1 Plasma exposure of RAW264.7 murine macrophages leads to significant increase in a) TNF alpha secretion, b) IL-6 production.

Acknowledgements: This work has been generously supported by Applied Science Private University, Jordan, and Queen’s University Belfast.

References

Advanced Cold Plasma Device for Cancer Treatment

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Design and main parameters of a cold plasma device are presented together with the results of modeling of the device physics operation. Using Penning gas mixture and high-frequency resonant electrical scheme, cold plasma plume is generated by dielectric barrier discharge at reduced voltages down to 800 V and gas flow down to 0.5 L/s by the plasma gun having miniature size allowing its insertion into common medical endoscopes and safe plasma generation inside the human body. External view of the setup and plasma plume are shown in Fig. 1. The developed cold plasma device is medically safe and was used for plasma treatment of tumors. The results achieved showed up to 80% success rate with several short treatment episodes (see Fig. 2).

Fig. 1. (a): External view of the experimental setup. 1. Gas cylinder. 2. Gas pressure reducer and flow meter. 3. Gas tube. 4. ac power cable; 5. rf generator; 6. rf coaxial cable. 7. Movable plasma gun. 8. Screw to move the plasma gun. 9. Standard BNC rf connector. 10. Plasma target (grounded). (b): Image of the plasma plume for distance between the grounded target and the output of the plasma gun of 2.3 cm. RF voltage is of 1150 V, rf pulse repetition rate is of 178 Hz and rf pulse duration is of 640 μs.

Fig. 2. The effect of high intensity plasma on large S.C.C. tumors of human origin. Follow up was carried out up to 72 hours after treatment.
Immunomodulation induced by the apical application of nspDBD plasma in a polarized model of human skin

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The noninvasive application of uniform, non-equilibrium, nonthermal plasma (NTP) to living epithelial tissue is currently being evaluated for promising roles in a variety of biomedical fields, including regenerative medicine, cancer therapy, and vaccine delivery. While investigations involving NTP often focus on the achievement of specific therapeutic end-points, fewer studies have investigated the pathways and mechanisms through which epithelial cells respond to NTP exposure. Using a chambered transwell system to establish an in vitro epithelia l model comprised of human HaCaT cells, experiments were conducted to investigate the effects of nanosecond-pulsed dielectric barrier discharge (nsDBD) plasma on polarized epithelial cell viability, monolayer permeability, intracellular oxidative stress, the release of adenosine triphosphate (ATP), activation of the transcription factor NF-κB, and the stimulation of chemotaxis. In initial experiments, HaCaT viability and permeability were minimally affected by exposure to nsDBD plasma at 60 Hz or below. However, the application of nsDBD plasma did cause frequency-dependent decreases in intracellular glutathione (indicating induction of oxidative stress by nsDBD plasma) and higher levels of extracellular ATP in the basolateral (subepithelial) media, which are indicators of cellular stress and an NTP-induced inflammatory response. NF-κB activation was evident 24 hours after nsDBD exposure. In addition, peripheral blood mononuclear cell migration was enhanced in the presence of plasma-conditioned basolateral medium. These studies provide new insights into nsDBD plasma-induced mechanisms and effectors of inflammation, as well as local innate immune responses initiated by plasma-exposed polarized epithelial tissues. These results also provide a solid foundation for further investigations that will demonstrate the translational potential of nsDBD plasma.

This work was supported by a Drexel University Pennsylvania Commonwealth Universal Research Enhancement (CURE) grant and by internal funding provided by the Department of Microbiology and Immunology and the Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine.
A low power atmospheric plasma source for accelerated blood coagulation

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The development of a biomedical tool able to locally accelerate blood coagulation (BC), especially in patients following an anticoagulant therapy, is a very attractive and challenging goal. In this contribution we present the main features of the Plasma Coagulation Controller (PCC) device, a cold atmospheric pressure plasma source based on the Dielectric Barrier Discharge (DBD) scheme, specifically designed for accelerating blood coagulation. The device is controlled by a microcontroller and can explore different operational parameters in terms of discharge repetition rate (1-20 kHz) and applied voltage (2-8 kV). Effective current measured on a metallic target is of the order of 1 mA and, thus, suitable for application on human body. Helium is used as a working gas though some tests have been performed in Argon for investigating mechanisms behind plasma-living matter interaction.

The presence in the PCC plasma jet of reactive oxygen and nitrogen species (ROS and RONS) and of metastable excited states is revealed through emission spectroscopic measurements. The analysis of the rotational OH and N₂ spectra allowed also the estimate of their rotational temperatures, ranging around 300 K. Interestingly, ROS and RONS produced by the PCC are able to induce the production of reactive species in cells, as shown in biological tests performed on human fibroblasts, where it was observed a significant increase in the levels of ROS and NO (nitric oxide) compared to the untreated samples. This might give an insight into the molecular mechanism behind the cellular response to the plasma.

The ability of PCC device to accelerate BC has been investigated through in-vitro and in-vivo tests. In-vitro tests have been performed on blood samples from patients following anticoagulant therapy and exposed either to air (control samples) or to direct plasma jet for different time points. PCC exposure strongly stimulated formation of blood clots that have been successively analyzed by histological methods. A Western Blot has been also performed in order to investigate the relative activation of proteins involved in BC, as well as of enzymes involved in the reactive species’ disposal. In vivo tests have been performed on Male Wistar rats; in particular, the bleeding was induced by a deep cut on both hindlimbs at the same time, and only one was treated by the PCC. The BC was accelerated in the treated area compared to the other side.

Finally, the disinfectant effect of the plasma produced with PCC has been tested by treating different bacteria strains (E. coli, S. aureus and P. aeruginosa). Bacteria viability drops under 50% after only 15s of plasma exposure, and keeps decreasing over time until reaching almost 100% of inactivation after 2min.

Taken together, our data suggest that the PCC is able to reduce the BC time possibly through the production of reactive species.
Improve gene stability in plasma gene transfection by autophagy inhibition

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We have clarified that the mechanism of gene transfer by microplasma depends on endocytosis [1, 2]. However, it is unclear by which process the introduced gene is expressed or degraded. To elucidate the superiority of the plasma gene transfection method, it is an important subject to clarify the intracellular migration of the incorporated gene. We focused on autophagy and late endocytosis pathways, and examined intracellular translocation of introduced genes by using three inhibitors as for Nocodazole, Chloroquine and LY-294 002. The action site of inhibitors is shown in Fig. 1.

The results of the experiments are shown in Table 1. Cells treatment with 1µM Nocodazole increased gene expression more than doubled comparing with control cells, and with 30µM Chloroquine gene expression increases up to 1.5 times. However, in the case of combination of Nocodazole and Chloroquine, gene expression was the same as treatment with Nocodazole alone. Treatment with 1.5µM LY-294 002 increases the gene expression to 1.7 times higher than control. These results revealed that the stability of the genes could be achieved by inhibiting each degradation pathway (Fig. 1). As a reason why no synergistic effect of Nocodazole and Chloroquine was observed, Nocodazole may suppresses upstream pathway in which Chloroquine acts. Therefore, we conclude that the molecular transport pathway after the late endosome and after autophagosome formation are almost same. We also propose a new gene transfer route map (Fig. 2). It became clear that the stability of genes in cells is improved by inhibiting gene decomposition along with endocytosis and autophagy. The mechanism of intracellular molecular transport must be cleared.

References


Table 1. Gene transfection efficiency with inhibitors

<table>
<thead>
<tr>
<th>Reagent and irradiation time</th>
<th>Std Smasec</th>
<th>LY 1.5 µM Smasec</th>
<th>Chloro 30 µM Smasec</th>
<th>Nocoo 1 µM Smasec</th>
<th>Chloro 30 µM + Nocoo 1 µM Smasec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene transfection efficiency %</td>
<td>6.6%</td>
<td>9.27%</td>
<td>10.99%</td>
<td>31.19%</td>
<td>30.31%</td>
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<tr>
<td>Standard deviation</td>
<td>1.1</td>
<td>0.49</td>
<td>1.53</td>
<td>5.34</td>
<td>1.63</td>
</tr>
</tbody>
</table>

※ Chloro = Chloroquine  Nocoo = Nocodazole  LY = LY-294 002

Fig. 1 Map of Molecular transport Pathway and inhibitors

Fig. 2 Map of New Molecular transport pathway in a cell
Plasma applications in agriculture – the next new field of research

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The state of knowledge in the field of Plasma Agriculture today is comparable to that of Plasma Medicine (PM) of 2005 to 2009. Therefore, we could expect that the multidisciplinary subject area Plasma Agriculture will develop faster due to the collected experiences in Plasma Medicine. Similar to Plasma Medicine, the success of Plasma Agriculture research depends on the interdisciplinary cooperation of natural science and life sciences.

Topics connected to Plasma Agriculture comprise:
- Plant biology and plasma technology
- Agricultural economics
- Food production chain
- Biotechnology
- Pollutant decomposition [1], [2]

The development dynamic of this new topic depends on: Success of fundamental research accompanied by establishment of research networks, presentation of results at appropriate workshops and conferences, development of suitable plasma sources for agricultural application, followed by feasibility studies for agricultural industry and finally, policy making for establishment of plasma technology in agriculture.

The contribution discusses topics of Plasma Agriculture and food science with showing synergies to Plasma Medicine.

Reduction of Salmonella on Valencia Oranges by Cold Plasma Treatment

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Abstract:

Orange juice has been the source of recurrent food borne illness outbreaks, primarily associated with Salmonella. There is a need for antimicrobial interventions which can effectively eliminate pathogens from fruit surfaces and reduce the risk of cross-contamination during peeling and processing. To evaluate atmospheric pressure cold plasma as a means to inactivate Salmonella on peel-on oranges, Valencia oranges (n=9) were lab-inoculated with Salmonella Anatum on the peel, in the stem scar, or in the blossom end. The inoculated fruit were allowed to air dry for 2 hours to promote adherence before treatment with air-based, atmospheric pressure cold plasma, created with high-voltage electrical discharge. During treatment, the site of inoculation on the oranges was passed in and out of the plasma plume to simulate “tumbling” of oranges on a conveyor belt that are being exposed to cold plasma from above. Cold atmospheric pressure plasma (4 cubic feet/minute) was applied for 0 (control), 1, 3, or 5 minutes to the sites of inoculation on the oranges (stem scar, blossom scar, and peel). Oranges were treated at distances of 0 cm or 7.5 cm from the cold plasma emitter head. All treatments significantly (P<0.001) reduced Salmonella on oranges, on all surfaces tested. The 0 cm treatments yielded log reductions ranging from 0.94 – 2.09 (stem scar), 1.57 – 3.56 (blossom end), and 2.4 – 4.09 (peel), with longer treatment times yielding greater reductions. The 0cm were uniformly more effective than the 7.5 cm treatments, which yielded reductions ranging from 0.15 – 1.57 (stem scar), 1.01 – 1.80 (blossom end), and 0.37 -1.22 (peel). Temperature measurements confirm plasma treatment as a nonthermal process. These results suggest cold plasma could be a waterless, chemical-free sanitation step for peel-on fruits such as Valencia oranges, and could serve as an in-line means to reduce the potential for cross-contamination.

100 word summary Abstract:

Oranges (n=9) were lab-inoculated with Salmonella Anatum on peel, stem scar, or blossom end, and treated with air-based, atmospheric pressure cold plasma for 0 (control), 1, 3, or 5 minutes, at 0 or 7.5 cm from the emitter head. All treatments significantly (P<0.001) reduced Salmonella on all surfaces; longer times yielded greater reductions. Treatments at 0 cm reduced Salmonella by up to 2.09 (stem scar), 3.56 (blossom end) or 4.09 logs (peel), while at 7.5 cm, reductions were up to 1.57 (stem scar), 1.80 (blossom end), or 1.22 logs (peel). Temperature measurements confirm plasma treatment as a nonthermal process.
Non-thermal plasma: uniquely suited to control of aqueous *Legionella*

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Current approaches to eradicate *Legionella* from the water systems of hotels, hospitals, and other facilities are suboptimal as highlighted by the nearly three-fold rise in cases of disease due to this pathogen since 2000 [1]. *Legionella* survival in aqueous sites is enhanced by its ability to exist within aquatic protozoa and be associated with biofilms [2]. The efficacy of non-thermal plasma (NTP) against *Legionella* might be predicted from studies documenting the killing of such intracellular organisms and those within biofilms by this technology [3].

The elderly and those with compromised immune systems are at increased risk for the more severe form of *Legionella* infection, Legionnaire’s disease, making control of this pathogen in healthcare facilities a top priority [1,2,4]. Current methods to eliminate *Legionella* from facility water supply systems include use of chemicals, copper-silver ionization, ultraviolet light, and ozone. Each offers some unique benefits as well as drawbacks, but none are able to eliminate *Legionella* for an extended period [5].

The successful use of NTP for *Legionella* eradication from water *in vitro* has been reported [6,7], and modeling has shown its potential feasibility and cost-effectiveness [8]. Additional study is needed to better define delivery optimization, scalability, and microbiologic efficacy and cost-effectiveness relative to current treatments. Theoretically, NTP may have a unique ability to penetrate the protected niche *Legionella* occupies within its aqueous environment.

References:


Understanding how nitric oxide plasma chemistry can influence seeds dormancy, germination and seedlings early stage growth

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Innate dormancy is a complex mechanism preventing the germination of seeds despite an ideal combination of environmental factors. Under pure natural conditions, dormancy delays the germination of seeds and has the ability to spread it over time, hence increasing the chances of specimens germination and survival in case of unfavorable environmental conditions (e.g. cold weather, seasonal herbivores, competition with other plants for water consumption). However, under optimal conditions as in intensive agriculture, such dormancy is useless and raises economical issues. Since nitric oxide appears as a key radical involved in the dormancy mechanism as well as in signaling pathways regulating plants growth and plants responses to (a)biotic stresses, our research works aim to investigate how NO plasma chemistry could have a positive impact on seeds dormancy and seedlings development.

Various types of seeds (sunflowers, tomatoes, alfalfa, …) are treated following three plasma approaches: dry plasma process, direct and indirect wet plasma processes (Fig. 1). All these treatments are performed in a smart and versatile piston plasma reactor where dielectric barrier discharges operate in a matrix-like configuration to treat seeds with/without large volumes of water. The gaseous composition is tailored for various nitrogen and oxygen mixtures ratio to create a nitric oxygen chemistry promoting dormancy release and seedlings early stage growth. Dormancy delay is evaluated with a particular attention devoted to integumentary inhibitions. The seminal envelopes surrounding the embryo are more or less effective obstacles to the passage of water or oxygen. Since seed coats could significantly delay the germination, we study the influence of the plasma processes on these coats by means of imaging, microscopy and mass permeametry. Furthermore, biological key parameters are monitored like germination & vigor rates, stems lengths and chlorophyll content.

Finally, to keep in mind that cold atmospheric plasma processes must bridge agricultural profitability with lower energy consumption, energetic and eco-efficiency parameters related to the plasma processes are evaluated based on mass spectrometry measurements and electrical diagnostics: conversion rates, specific energy input and energetic efficiency. These process parameters are correlated with the aforementioned biological parameters.

Determinations of the Inactivation and Filtration of a Viral Aerosol within a Packed Bed Non-thermal Plasma Reactor

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Outbreaks of airborne infectious diseases such as measles or sudden acute respiratory syndrome (SARS) can cause significant public alarm. Where ventilation systems facilitate disease transmission to humans or animals, there exists a need for control measures that provide effective protection while imposing minimal pressure differential. In the present study, viral aerosols in an airstream were subjected to non-thermal plasma (NTP) exposure within a packed-bed dielectric barrier discharge reactor.

The reactor consists of two circular perforated brass plate ground electrodes mounted within a 3.5-inch diameter Plexiglas tube. A brass ring (0.035-inch thickness, 1-inch width) positioned around the outer circumference of the tube served as the high-voltage electrode connected to the AC power supply (either 20kV Trek power supply or 30 kV neon sign power supply). Situated in the void formed by these three electrodes is a packed-bed of 300 inert borosilicate glass beads (0.25 inch in diameter). A downstream induced draft fan induces and maintains the desired air flow rate through the reactor and an activated carbon ozone filter is also installed.

MS2 phage, a single-stranded RNA bacteriophage, served as the biological pathogen. MS2 suspended in virus dilution buffer (VDB) solution was sonicated, producing a mist of liquid droplets of the solution which, drying quickly, left MS2 aerosols to be inducted into the NTP reactor. Two impingers sampled the air stream at 1 LPM both upstream and downstream of the NTP reactor. Plaque assays determined the concentrations of viable MS2 (plaque-forming units, pfus) and qPCR analysis determined the concentrations of MS2 gene copies (genCopies).

Plaque assay results show (Fig. 1) that MS2 inactivation exceeded the limit of quantification at 30 kV, corresponding to at least 2.3-log reduction in the viable virus. qPCR analyses found filtration by the packed bed responsible for ~0.35 log reduction in MS2 genome copies. Ozone filters reduced O3 to background levels.

Acknowledgement: This project was funded by a grant from the USDA National Institute for Food and Agriculture (award # 2016-67030-24892). Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the USDA.

Figure 1. Plaque assay and qPCR analysis of samples collected during NTP inactivation of MS2 aerosols at 30kV AC and 170 LPM air flow rate.
Atmospheric pressure plasma decontamination of water polluted by organophosphates used in agriculture

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In biomedicine, atmospheric pressure plasmas (APP) have proved their excellent potential for sterilization and cleaning of both living tissues and inorganic materials from pathogenic microorganisms [1]. Moreover, large number of recent studies are investigating operation of APP in contact with liquids. Results show that chemically reactive gaseous environment produced by plasma sources can influence and modify physical and chemical properties of liquids [2]. As a result of the knowledge acquired in previous studies the investigation of APP now widens to a new emerging research field - plasmas in agriculture, where novel applications for APP appear.

Nowadays, one of the biggest environmental problems is water pollution with contemporary agriculture techniques as one of the main sources causing the pollution of surface waters. Organic micropollutants, originating from immense use of pesticides in agriculture production, require special chemical or biological treatments for water purification, again using environmentally hazardous substances. Our idea is to employ APP for decontamination of water polluted by pesticides, which is by now successfully used is warfare applications [3]. As a first step, we conducted a study on decontamination of water samples polluted with different pesticides, i.e. organophosphate compounds, by using an atmospheric pressure plasma jet (APPJ) operating with He as working gas. The plasma jet was powered by a continuous kHz signal source. Liquid samples placed below the APPJ were treated for different duration times, different sample volumes and different water contamination levels. Optical and electrical characterization of the APPJ was performed in order to obtain information about stability of the treatment conditions and the plasma properties. Before and after the treatment liquid samples were analyzed by spectrophotometric techniques, high performance liquid chromatography (HPLC) and liquid chromatography coupled with mass spectrometry (LC-MS) in order to follow degradation of organophosphates. Significant and efficient degradation of pesticides is noticed in all cases and appearance of degradation products is observed in the liquid sample. Thus, we could also evaluate toxicity of produced by-products. From measurements of parent molecule degradation we established dependence of the decontamination efficiency on treatment time. Having in mind the possibility of reuse the decontaminated water in agriculture, we also investigated the influence of treated water on seed germination.

This work was supported by projects ON171037 and III41011, MESTD, Serbia.

References

Plasma-mediated inactivation of *E. coli*: Influence of protein content on wet surface and in liquid medium

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Application of cold atmospheric-pressure plasma (CAP) for wound treatment but also for decontamination of food or water often includes the presence of proteins. It is well known that biological plasma effects in general are mediated via reactive oxygen and nitrogen species (RONS) and that RONS signaling in biological systems can be mediated via thiol (SH)-based redox sensors [1,2]. Because proteins contain amino acids wearing SH groups, the aim of this study was to investigate the influence of protein and SH group containing amino acid on the antimicrobial properties of plasma-treated saline solution (0.85% NaCl) and on the direct CAP effectivity on solid wet agar plates. Plasma treatment of NaCl solution was realized using an AC-driven pin-to-liquid discharge [3]. After 10 min plasma treatment time, the amino acids L-cysteine (contains SH group) or L-alanine (no SH group) or bovine serum albumin (BSA; with approximately 6% cysteine content) was added together with the test microorganism *Escherichia coli* K-12 (DSM 11250/NCTC 10538) for an exposure time of up to 60 min. The total viable cell count was determined in appropriate time intervals. A concentration-dependent repeal of the antimicrobial efficacy was determined. Thus, 0.0025% had no influence whereas 0.25% of BSA as well as the tested amino acids resulted in no inactivation of *E. coli*. The difference between L-alanine and L-cysteine was negligible suggesting only a minor effect of the presence of SH groups. To test the influence of proteins in direct plasma treatment on wet surfaces (like wounds), *E. coli* was plated together with BSA on soybean casein digest agar surface. Another setup based on agar plates which contained different concentrations of peptone (mixture of peptides and amino acids). The agar plates were punctually treated by the argon-driven CAP jet kINPen Med® (neoplas tools GmbH, Greifswald, Germany). After overnight incubation, inhibition zones were analyzed. The bacterial growth was independent of protein or peptone content for this direct plasma treatment. Summarizing all, the antibacterial effect of plasma-treated solution was strictly limited in the presence of protein. This inhibiting effect was independent of chemical structures containing SH groups. In direct plasma application, none of the tested protein or peptide/amino acid content resulted in a diminished antimicrobial effect of the kINPen Med® when applied in a protein-containing environment.

This work was partially supported by the Erasmus+ program of the European Union.

References

Use of the plasma activated water as plant defense enhancer

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Bacteria and phytoplasmas (insect-transmitted cell wall lacking bacteria), are involved in severe diseases affecting agronomic relevant crops. Management of the diseases due to these agents has mainly focused on the use of copper compound or antibiotics for bacteria, on insect vector chemical control and infected plant rouging for phytoplasmas, but all of these methods presents some drawbacks. Indeed, many efforts are devoted to find effective and environmental friendly control strategies. The use of plasma activated water (PAW), produced by a nanosecond pulsed dielectric barrier discharge, as innovative and alternative possible management tool to control plant diseases due to bacteria and phytoplasmas was experimentally exploited. The exposure of sterile distilled water (SDW) to a non-equilibrium atmospheric pressure plasma induces a reduction of pH and the production of reactive oxygen and nitrogen species (RONS), such as hydrogen peroxide, nitrates and nitrates [1] that might induce plant defense responses, involving both hypersensitive reaction and systemic acquired resistance. The effects of PAW applications were tested on three different pathosystems: tomato plants experimentally inoculated with Xanthomonas vesicatoria (Xv), phytoplasma infected periwinkle micropropagated shoots and grapevine infected plants in vineyards. Quantitative RT-PCR analyses allowed to determine the transcription level of genes involved in the plant defense response (phenylalanine ammonia-lyase, pal) and in the phytoalexin metabolism of PAW treated materials. The number of leaf spots caused by Xv in tomato plants and the number of symptomatic grapevine plants in in vineyards were significantly reduced by the treatments. Moreover, the transcriptomics results highlighted the pal gene and the genes involved in the phytoalexin production increased expression. The PAW ability to enhance some of the plant defense mechanisms improving the health status of the treated plants was experimentally demonstrated.

Fig. 1. Grapevine phytoplasma-infected plants treated with PAW at different times. From the left: April 2015; April 2016; June 2016 and July 2016 [2].

References

Investigations of a CAP-apparatus using circulating gas flow for sterilization of heat sensitive materials with space application

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Cold atmospheric plasma (CAP) technologies have been investigated for various applications, especially for the inactivation of microorganisms under different circumstances. Based on the fact that the plasma treatment can be applied to sensitive surfaces, it is often used in the field of medicine [1]. In a first study, we showed that the CAP technology is a very fast and promising alternative to sterilisation methods such as dry-heat microbial reduction (DHMR) and vapor phase bioburden reduction using hydrogen peroxide (VHP) [1]. These standard sterilization methods are evaluated methods for the sterilization of spacecraft components with the disadvantage of negatively influencing the sensitive materials.

In this study a new developed apparatus using CAP is presented as an alternative for the sterilization of sensitive materials. The apparatus uses the plasma afterglow generated by a surface micro-discharge (SMD) plasma source and functions with a circulating gas flow and variable treatment volumes to investigate the scalability of the setup. Based on previous published data, it is well-known that sterilization due to plasma exposure is more easily achieved at high humidity [2]. Therefore, the developed apparatus was operated using ambient air and high humidity conditions of ~ 90 %.

Bacterial endospores of *Bacillus atrophaeus* inoculated on stainless steel were used as a biological indicator to test the sterilization efficacy of the plasma apparatus. The achieved D-values varied between 2.5 min and 5.0 min for different treatment volumes and indicate that CAP is a useful alternative to common sterilization methods for spacecraft equipment and medical devices.

The plasma afterglow components were analyzed using an UV absorption spectrometer and a Fourier Transform Infrared spectrometer (FTIR). We present the results of the spectroscopic afterglow investigations in dependence of different plasma conditions. We determined O₃, N₂O, NO₂, H₂O₂ and HNO₃ as major afterglow components and observed the decrease of HNO₃ with high humidity conditions. In addition, we plan to present first results of the material compatibility investigations with XPS for stainless steel, copper, PTFE, aluminum and silicon.

This work was supported by Bavarian Ministry of Economics.

Low temperature plasma treatment of bell pepper (*Capsicum annum, cv ‘California Wonder’) seeds to disinfect and enhance seed germination

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Low temperature plasma (LTP) technologies are emerging as chemical-free biocides and surface disinfectants of plant seeds and fresh foods. However, the effect of LTP on plant tissues and related physiological responses have not been fully established. Such knowledge could strengthen agricultural applications of LTP in addressing significant plant health problems, particularly seed-borne diseases. Two separate experiments were conducted using the seeds of bell pepper (*Capsicum annum, cv ‘California Wonder’) to determine the optimal conditions for assessing LTP effects on seeds with or without seed-borne pathogen, (*Xanthomonas campestris pv vesicatoria* [Xcv]). In Expt. 1, the effects of seed sterilization and plasma on the germination of seed infected with or without bacterial pathogen were determined; In Expt. 2, the effects of plasma or chamber conditions on the germination of seed infected with or without bacterial pathogen were determined.

In Expt. 1. Seeds were either surface sterilized with 30% Clorox solution or rinsed in distilled water, and were either inoculated or not inoculated with bacteria. The expt. I treatments were: i) NSNIP: Non sterilized and non-infected with pathogen and plasma treated; ii) NSIP: Non sterilized, infected with pathogen, and plasma treated; iii) SP: Sterilized only, Plasma treated; iv) SIP: Sterilized, infected with pathogen and Plasma treated; C: NSNI: Non-sterilized, not infected and not Plasma treated. Expt. 2: The treatments were as follows: i) NIP: Not infected with bacteria and were exposed to Plasma; ii) IP: Infected with bacteria and exposed to Plasma; ii) ICC: Infected with bacteria and exposed to chamber conditions only; NICC: Not infected and exposed to chamber conditions only; and C: Non-infected, not exposed to chamber conditions. In both experiments, the seeds were exposed to plasma for 15 s at a chamber pressure of 1.2 Torrs, and a power setting of 30W in plasma treatments. Seeds in all treatments were then tested for total percentage and rate of germination on Whatman #1 filter paper discs pre-wetted with distilled water in 100 x 15mm Petri dishes and incubated in a germination chamber at 25°C for germination. Treatments were arranged in a completely randomized design with four replications. Seed germination occurred between 5 - 11 days after incubation. In Expt 1, seed germination in SIP treatment was the lowest (13%) among treatments. The seed germination was higher in treatments NSIP (95%) and was similar to Control treatment (NSNI). Thus, non-sterilized seed infected with bacteria had higher percentage germination when treated with plasma than sterilized seed infected with bacteria and not treated with plasma. In Expt. 2, seeds infected with bacteria and exposed to plasma (IP) had 90% germination whereas infected seeds exposed to chamber pressure without plasma (ICC) had 95% germination. Germination of non-infected seeds exposed to plasma or chamber conditions without plasma was 86% and 95%, respectively. Bacteria-treated seeds not exposed to plasma or chamber conditions germinated better (97%) than seeds in the C treatment (88.1%). The plasma effects were not clear in the two experiments. Studies to determine plasma effects on the seed-borne pathogen rather than seed germination are in progress.

This research was supported by NSF EPSCoR RII Track 1 Grant OIA – 1655280.
Removal of airborne bacteria by non-thermal plasma and electric wind

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Microbiological contamination of indoor air, especially in clinical operating theatres, getting more and more important cause of health hazard [1]. State of the art is removing bacteria mechanically with high collection efficiency filters [2]. The filters gather microorganisms but still not inactivate them, so it is nevertheless a risk.

Another possibility is the treatment of pathogens in air with non-thermal plasmas [3]. Aim of this project is to construct a plasma stage with high antimicrobial activity for cleaning air in operating rooms. Therefore, a dielectric barrier discharge (DBD) generating reactive species was combined with an extraction electrode at high electric field to improve decontamination. Electrical and optical analyses to optimize the plasma source are already completed. That means the production of ions and active species like ozone can be balanced by applied voltage and frequency in dependence on the relative humidity of the treated air. Microbiological tests, like analysing potential decontamination of airborne pathogens with an experimental setup, are already done with Escherichia coli. Cultivated bacteria were suspended in liquid and sprayed into the plasma device. This composite is plasma-treated and picked up with an air sampler for evaluating the decontamination efficacy. As a consequence of the positive results [4] experiments will be continued with other microorganisms like Staphylococcus epidermidis.

References

Abatement of cockroach protease-dependent sensitization using non-thermal plasma

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Environmental aeroallergens contain proteins that activate and disrupt the airway epithelial barrier contributing to allergic sensitization. These proteins are found in allergens such as pollen, dust mites and cockroaches. Proteases within allergens produce an immune response leading to upregulation of the pro-inflammatory chemokine IL-8 and thymic stromal lymphopoietin (TSLP) responsible for modulating T-helper type 2 (Th2) differentiation and allergic sensitization [1-2]. Cockroach extract and fecal remnants typically found on food and surfaces within infected environments contain trypsin-like serine proteinases that activate protease-activated receptor-2 (PAR-2) in epithelial cells and are implicated in the development of asthma [3] with elevated expression of PAR-2 found in the airways of asthmatic patients [4].

Atmospheric pressure non-thermal plasma or simply cold plasma technology is an effective tool for microbial decontamination and surface cleaning, however its effects on proteases found in allergens such as cockroaches has not been investigated. This study aims to investigate the effect of non-thermal plasma treatment on the cockroach Blattella germanica’s (in both aqueous and dry environments) proteinase activity and PAR activation in human airway epithelial cells (A549). Using an in-house designed kHz driven non-thermal plasma jet [5] plasma exposure of cockroach extracts equivalent to 0.1 mg/ml of cockroach protein were exposed to a helium/oxygen (0.5%) plasma plume for up to 15 minutes. Using a fluorogenic trypsin substrate the enzymatic activity of the cockroach protein was measured following plasma exposure. Total protein content of each exposed sample is reported from fluorometric quantification. Calcium release within A549 cells was monitored for PAR-2 activation from serine proteases in the cockroach extract.

It was determined that plasma exposure of cockroach extract in phosphate buffered saline (PBS) and water resulted in approximately 50% and 60% reduction in enzymatic activity respectively and a 50% and 75% reduction in total protein content with 15 minutes’ plasma exposure. Cockroach extract exposed as a dry powder however was not susceptible to plasma exposure indicating the necessity of the liquid phase in mediating plasma effect on proteins. To the best of the authors knowledge this is the first study of this kind to investigate the potential for cold plasma application for the abatement of cockroach protease-dependent allergens.

This work was supported by BBSRC grant “EnvironSafe: Cold Plasma Innovations for Food Safety and Sustainability”.

References
Effects of the pulse width and additive oxygen flow on the generation of reactive species in bipolar dc pulsed atmospheric pressure plasma jets

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Atmospheric pressure helium plasma jets excited by a low-frequency bipolar pulsed dc voltage were characterized. Some characteristic features of plasma jets driven by bipolar pulse are discussed with regard to temporal profiles of discharge current and light emission from the bullet formation. The electrical and optical characteristics of the jet and the ability to produce reactive species exhibited quite a strong dependence on the pulse width and additive oxygen gas flow [1, 2]. We investigated the effects of the pulse width and additive oxygen flow on the generation of reactive species in the gas and liquid phases. The densities of reactive species including OH radicals were obtained at the plasma-liquid surface and inside the plasma-treated liquids using ultraviolet absorption spectroscopy [3, 4] and chemical probe method [5]. Among the chosen pulse widths of 1.5, 3.5, and 5.5 μs, the 3.5 μs case was observed to exhibit higher values in discharge current and optical emission intensity. The change of OH densities estimated at the plasma-liquid interface and inside the plasma-treated liquid with pulse width follows a similar trend to those of discharge current and optical emission with pulse width. At the additive oxygen flow of 10 sccm, the discharge current and wavelength-integrated optical intensity were maximal, but the estimated OH density inside the plasma-treated liquid exhibits a slight rise with increasing additive oxygen flow.

This work was supported by the National Research Foundation of Korea under Contract No. 2015R1D1A1A09056870.

References
Resonant antenna, sub-atmospheric plasma sources for biomedical applications

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The properties of non-equilibrium plasma, such as ambient gas temperature and high electron temperature (\(\sim 10^4\) K) for gaseous chemical reactions, are well suited for biomedical applications. The challenge is to maintain these properties for sub-atmospheric pressures (0.1 - 1 bar) where steady-state plasma tends to thermal equilibrium. Gas temperatures (\(\sim 10^3\) K) are then incompatible with temperature-sensitive applications. Dielectric barrier discharges are often used to produce low power plasma near to the surface of electrodes at atmospheric pressure, such as for the treatment of seeds [1]. In this work, we investigate the use of novel resonant antenna plasma sources for volume treatment of biomedical samples.

Resonant antennas are RF networks of parallel \(L, C\) meshes with resonant frequencies corresponding to normal modes. The high currents generated at each resonance means they can be used as inductively-coupled plasma (ICP) sources. Planar resonant antennas [2] are used for large area (1 m\(^2\)) industrial surface treatment at low pressures (10\(^{-2}\) mbar) typical of ICP sources, and cylindrical (birdcage) resonant antennas are being tested as volume plasma sources with diameters up to 40 cm. In steady-state, pressures up to 0.5 mbar have been reached, which qualifies as a high pressure ICP, although still far short of the pressures required here. For example, a circulating fluidized bed reactor requires about 10 mbar pressure so that fluid dynamic forces can lift granular materials such as wheat grains [3].

A modified birdcage antenna of 8 cm diameter currently operates at 10 mbar, and work is underway to raise this to 100 mbar. This ICP has greater power density than an atmospheric pressure dielectric barrier discharge in the same configuration [4]. Methods to reduce the heat load on granules include closed loop flow in a cyclone with multiple passes through a short length of plasma, and RF power pulsing. Both techniques will be used to achieve sub-atmospheric plasma volume treatment for industrial biomedical applications.

\textbf{Acknowledgement} This work was supported by the Swiss Commission for Technology and Innovation grant no. CTI 19241.2 PFEN-IW.

\textbf{References}


Low Temperature Sterilization of Small Vial Using Electron Cyclotron Resonance Plasma

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1. Introduction

Plasma sterilization techniques have excellent characteristics such as short treatment times, non-toxicity, and low thermal damages to the materials to be sterilized. In our previous study, we reported that the characteristics of surface sterilization using Electron Cyclotron Resonance (ECR) plasma. ECR plasma has high electron temperature and high plasma density compared with the other plasma. In the present study, we investigated the surface sterilization in small vial using pulsed ECR plasma to confirm the influence of microwave power pulsed conditions (pulse width) on plasma sterilization efficiency and the treatment temperature.

2. Experimental Apparatus and Method

The experiment was performed in a stainless-steel cylindrical vacuum chamber which contains a rectangular Sm-Co permanent magnet (79 × 37 × 33 mm³), as shown in Fig. 1. A small glass vial (Φ 30 × 60 mm 20 ml) which was capped by no-woven fabric, was placed on the magnet. The magnetic field used for ECR (875 G) existed at a distance of 1 mm from the inner surface of the vial. The working gas is air. When microwave with frequency of 2.45 GHz was introduced into the vacuum chamber, the ECR plasma was produced around the ECR point and trapped in the magnetic mirrors formed by the permanent magnet. The biological indicator (BI) was put on the inner surface of the vial. After incubation, the spore survival was determined by observing the color change of the tryptic soy broth. The temperature of the vial during the treatment was measured by the thermo label.

3. Experimental Result

We successfully produced the ECR plasma in the small vial with a volume 2 ml by controlling the magnetic field distribution and the electric field. To confirm the influence of microwave power pulsed conditions on plasma sterilization efficiency and the treatment temperature, we performed sterilization tests using a BI which was placed in the small vial. Microwave power pulse width was changed from 500 μsec to 5000 μsec with keeping the peak microwave power, pulsed frequency and plasma exposure integration time at constant 500 W, 18 Hz and 10 sec respectively. The discharge gas was air and gas pressure was maintained at 0.05 Pa. The result is shown in Table 1. Minus signs indicate the success of sterilization. As shown in the table, it is found that the spore forming bacteria were sterilized in all conditions, while the temperature of vial decrease with decreasing the microwave power pulse width. As a result, we succeeded sterilization of 10⁶ spores placed in the small vial with a volume of 20 ml under the low vial temperature of 60-65°C by being exposed to pulsed ECR plasma.

![Fig.1 Schematic diagram of experimental set up.](image-url)

### Table 1 Antibacterial effects of pulsed ECR plasma.

<table>
<thead>
<tr>
<th>Pulse width</th>
<th>Processing time</th>
<th>Temperature</th>
<th>Sterilization result</th>
</tr>
</thead>
<tbody>
<tr>
<td>5000 μsec</td>
<td>111.1 sec</td>
<td>95°C~</td>
<td>−</td>
</tr>
<tr>
<td>1000 μsec</td>
<td>555.5 sec</td>
<td>80~85°C</td>
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<tr>
<td>500 μsec</td>
<td>1111.1 sec</td>
<td>60~65°C</td>
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(+: Failure  -: Success)
Microwave Plasma Torch for Bio-medical Applications

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For some time cold plasma discharges have gained a lot of attention regarding biomedical applications. The large spectrum of observed effects (cancer treatment, bacterial inactivation, wound healing, etc.) has encouraged scientists to create and utilize different plasma sources. The most preferred plasma device to this moment has been the dielectric barrier discharge (DBD). In this work we are presenting a well-known Surfatron type device coupled to a microwave generator at 2.45 GHz. This atmospheric pressure plasma torch can sustain low gas temperature while using relatively low gas flow and power output, which makes it suitable for working with different biomedical models.

Strong dependence between microwave power, torch length and gas temperature is observed. Gas flow and tube specifications (inner diameter, wall thickness and dielectric permittivity) vary the temperature and length of the discharge, too. The purpose of this work is to precisely determine the working conditions in which this plasma source could be used in contact with biological objects.

This work was supported under grand number DM03/3, 2016 of National Science Fund (NSF), Ministry of Education and Science, Bulgaria
Effect of OD radical and D$_2$O$_2$ on ROS resist gene and non-resist gene which are necessary for mycobacteria's survival during Mtb37Rv and Mtb37Rv mutants infection

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A non-thermal plasma jet (NTPJ) generated from a vapor mixture of N2/D2O is applied to tuberculosis infected BMDM, BMDM, and Raw cells. The results show that viability of Raw cells, BMDM, and infected BMDM treated with D2O plasma decreased more than nitrogen plasma after 1 minutes. The inactivation of M.smegmatis treated with N2/D2O plasma decreased more than nitrogen plasma. So, a NTPJ generated from a vapor mixture of N2/D2O is applied to Mtb37Rv and modified Mtb37Rv and its effects are analyzed by means of microarray. The results will expect that are going to screen genes which are powerful or weak against reactive oxygen species (ROS) by treating OD radical and D2O2 which are generated by NTPJ to Mtb37Rv and Mtb37Rv knock out mutants. We will effect of OD radical and D2O2 on ROS resistance gene and non-resistance gene which are necessary for tuberculosis's survival when infected by Mtb37Rv and modified Mtb37Rv.

References


Enhancement of cancerous cells treatment by applying cold atmospheric Plasma

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Cold atmospheric plasma (CAP) emerged as a novel therapeutic field with applications developed for cancer treatment. In this study, in order to verify the potential of CAP as cancer therapy, we examined the effect of CAP on power (kV), treatment time (s) and gas flow (SLM). Cell death was efficiently induced in human HeLa cell lines upon exposure to CAP. Collectively, these results strongly suggest that CAP should be developed as an efficient adjuvant treatment for cancer therapy.

References

Spectrometric characterization of an atmospheric microwave plasma jet.

Influence of the injected power.

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Increasing interest in atmospheric pressure plasma jets (APPJ) can be observed in various fields of applications such as decontamination and sterilization [1] and dermatology [2]. Their ability to propagate in open air and to allow the formation of a rich chemical environment populated by ions, radicals and excited species make them promising versatile tool.

In this work, a surfatron plasma source (S-wave, Sairem) is used to generate a plasma in a dielectric tube by a solid-state microwave generator (200 W maximum, 2.45 GHz). The microwave electric field propagates longitudinally at the dielectric/plasma interface. Then a plasma jet is created and sustained with length varying as a function of the operating gas flow and microwave power. Plasma "ON", 0 W of reflected power can be achieved. The source can be efficiently used for the production of reactive/excited species.

![S-wave (Sairem) source, argon gas and atmospheric microwave plasma jet](image)

Resolved spatial optical emission distribution measurements were performed with an optical spectrometer (HR2000+, Ocean Optics) and with an iCCD camera (PIMAX-2K-RB, Pearson Instruments). The optical measurements were realized with an iCCD camera coupled with filters to observe the spatial distributions of the main species emissions (argon, oxygen, nitrogen). The presence of the ions created by the jet were investigated using a Time-Of-Flight Mass Spectrometer (TOF MS).

In this presentation, the discharge gas will be argon (1.4 sl/min). The influence of the injected power (20W-200W) on the previous characteristics (optical emissions and ions) will be presented and discussed to evaluate the potential of this source in plasma medicine applications.

References

Experimental characterization of an atmospheric piezoelectric plasma source.

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Recent studies in the field of plasma physics, applied to biology and medicine, have highlighted plasma sources as possible alternative to traditional treatments (bactericidal and medical) [1]. The plasma creation principle, the source geometry, the excitation signal, the gas and the target properties are the major parameters influencing the effects and the efficiency of these plasmas.

In this work, the plasma is generated from a piezoelectric transformer. Its operating principle is based on a double conversion of energy, electro-mechanical and mechanico-electrical [2]. This type of transformer offers good performances in terms of voltage, gain, power, efficiency and cost. Moreover its singular dielectric properties (ferroelectric ceramics) contribute to the attractiveness of this technology for the generation of plasma jets.

Fig. 1. Piezoelectric plasma source setup and argon atmospheric plasma jet iCCD imaging

The objective is to characterize this plasma source under fixed operating conditions (resonance mode frequency / about 55kHz, input voltage / 1.5V, gas / argon, gas flow / 2 l.min⁻¹). An optical spectrometer (HR2000+, Ocean Optics) coupled to an optical fiber was used to identify the optical emissions of the reactive species and to study their spatial variation along the jet. An ICCD camera (PIMAX-2K-RB, Pearson Instruments) gave the 2D distribution of argon and nitrogen within the plasma jet. A time-of-flight mass spectrometer (API-TOF, Tofwerk) was used to characterize positive and negative ions produced by the plasma jet. These results will be presented and discussed to evaluate the potential of this source in plasma medicine applications.

References

The use of an FDA Approved Electrosurgical Device for Plasma Medicine

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Plasma medicine has developed dramatically in recent years and has shown benefits in areas as diverse as wound healing, cancer therapy and reducing bacterial load in infected tissue. Much of this work has been carried out using novel devices and there remains a paucity of approved medical devices that can be effectively translated into patient care in markets such as the USA. While there are a wide variety of argon plasma coagulators approved for medical use, these plasma devices have not been considered for plasma medicine applications such as wound healing as it was feared that their high thermal energy would cause further harm. Recent research has cast doubt on that assumption [1].

In this study we explore the potential of commercial plasma coagulation devices to deliver low energy, non-thermal plasma to a wound site. Particular attention is paid to recently launched J-Plasma device from Bovie Medical. This device uses a low temperature helium plasma and has been shown to deliver precise plasma power with minimal depth of thermal spread [2]. In this study, the impact of this device on biologic materials was investigated. The low energy discharge (40 Watts) was shown to have minimal impact on the chemical structure of various proteins and polysaccharides. The potential to use this device to deliver controlled modification of wound surfaces will be discussed and preliminary in vivo data will be provided.

References

Effect of Grounding a Target on Electrical and Energetic Characteristics of “DBD – Plasma Jet” System

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Plasma sources based on a dielectric-barrier discharge (DBD) plasma jet [1] are widely used in researches on plasma medicine. The way to join-up a treated target as an electrical circuit element can influence the results of plasma processing because it affects the discharge parameters. We consider two different ways of inclusion of a Petri dish containing a nutrient solid medium to the electrical circuit at the generation of plasma jet. The first one uses an additional electrode under the Petri dish (Fig. 1(a)), which is grounded; the second one provides processing a target by a “free” plasma jet, i.e. a target is under a floating potential (Fig. 1(b)).

![Equivalent electrical circuits](image)

Fig. 1. Equivalent electrical circuits at grounding a target (a) and without grounding it (b)

The electrical and energetic parameters of the "DBD-plasma jet" system [2] (discharge currents and voltages, plasma jet currents, Volt-Coulomb characteristics) were measured and analyzed. As a source of power, a ~32 kHz high-voltage sinusoidal supply was used. Plasma jets in laminar (Re=155) and turbulent (Re=800) helium and argon flows were considered.

The amount of energy released into the discharge region increases with the transition of laminar regime of gas flow into turbulent one. Grounding a target results in significant change in energy balance in “DBD - plasma jet” system for the case of argon gas: the amount of energy deposited into the discharge increases by an order of magnitude; whereas in the case of helium, the effect of target grounding is much less noticeable. Therefore, grounding a target can serve as a way to control the development of discharges particularly in argon when the discharge tends to be filamented in comparison with diffuse discharges in helium.

This work was supported by Saint Petersburg State University (grant 0.37.218.2016).

References

Eagle Harbor Technologies (EHT), Inc. is producing commercially available Nanosecond Pulsers which allow for independently, user-adjustable output voltage (0 – 30 kV), pulse width (20 – 500 ns), and pulse repetition frequency (0 – 100 kHz). Typical voltage rise-times on DBD discharge are on order of 10s of nanoseconds with faster rise-time available. EHT has several pulser versions allowing different classes of average output power levels and control options. EHT Nanosecond pulsers are currently being used at universities, medical start-ups and government laboratories to conduct research in biomedical, sterilization and decontamination and clean combustion applications. The Nanosecond Pulsers have been used to produce both atmospheric and liquid plasmas in many dielectric barrier and arc discharge configurations. The pulsers allow for single pulse operation which can enable easier measurement of current and voltage waveforms for energy per pulse and output power determination. EHT has used these power systems for internal research programs including surface sterilization, water decontamination and energy deposition for drag reduction. EHT will present nanosecond pulser output waveforms including output voltage, current and power measurements for example applications including DBDs, plasma arcs and resistive loads.
Development of cold atmospheric pressure plasma jet for medical applications

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Over the last few years, many researchers have performed experiments to understand the low temperature atmospheric pressure plasma parameters for medical applications. Still there is a lack of clarity about optimization of the plasma jet parameters for specific medical applications[1]. Possibly this is due to the adopting various experimental conditions. In this work, we have attempted to further understand the plasma plume parameters. 4kVp-p, 33 kHz sinusoidal voltage source has been used to develop the plasma jet. Helium as an active gas with flow rates of up to 11 lpm is used to produce the plume length of 6 cm into the ambient air. Thorough characterization of the plume has been carried out by using optical diagnostics such as emission spectra measurements, ICCD imaging and electrical discharge using voltage and current probes [2-4]. The plasma parameters such as electron excitation temperature, gas temperature are estimated using the emission spectra data. The estimated values are 800 K and 305 K respectively. Further, the plasma density along the length of plume has been assessed by using data obtained from the ICCD images (for plume drift velocity) and plume current measurements. The estimated values are in the range of 0.05-3.2 x 10^12 cm^-3. Furthermore, the discharge ignition and plasma plume dynamics with gas flow rate will be presented.

References

On the untapped potential of the CO molecule in plasma medicine and agriculture

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In plasma medicine and agriculture reactive species produced by non-thermal plasma sources must play an important role. Many studies have been devoted on the effects of reactive species such as O, O₂¹Δg, OH, N, NO and NO₂ species just to name a few. So far, the CO molecule has been overlooked.

Carbon monoxide (CO) is well known for its toxic effects at high doses. The effects of CO can be quantified via the percentage of carboxyhemoglobin (COHb) it forms in the blood. The percentage of COHb in blood strongly depends on, not only the percentage of CO in air, but also on the exposure time. CO is however also naturally produced at cellular level, mostly via the catabolism of heme. At low doses, CO has therapeutic properties. Experimental studies found several positive effects of CO such as anti-inflammatory, vasodilatory and anti-apoptotic effects. It has also an influence on plants and promote the seed germination and increases the roots formation [1].

In this contribution, we will shortly review the reactivity of CO at the molecular level and its effects as a signaling molecule. It will be shown that CO triggers and competes with several cellular receptors which are sensitive to the NO molecule as well.

Many studies have demonstrated over the years that plasmas are able to produced CO from the dissociation of CO₂ over a wide range of concentrations. Using glow discharges, dielectric barrier discharges, nanosecond pulsed discharges or microwave plasmas, CO can be produced from ppm range to several tens %. Plasmas are flexible sources allowing in situ production of CO and alleviate the risks related to its storage. Additionally, plasmas produce other components of biological relevance such as electric fields, heat, ultraviolet radiation, O, NO, NO₂ and many other reactive species. Synergic effects may therefore occur for the treatment of certain afflictions. For instance, it has been demonstrated that high doses of NO have a pro-inflammatory action [2] while exogenous CO can down regulate its effects [3]. Plasmas with CO₂ admixtures as a source of CO seem then promising in applications such as wound healing and seed germination.

References

Comparative studies of atmospheric pressure Ar and He plasmas for enhancing cutaneous delivery of epidermal growth factor

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Non-thermal atmospheric pressure plasma (NTAPP) has been well established for diverse applications in materials processing and biomedical recently. The majority of plasma used in industrial applications are operated in low pressure and almost room temperature. Because heavy species such as ions and neutrals remain almost the same as the room temperature (37 °C), the plasma do not cause thermal damage to heat sensitive objects. This non-equilibrium characteristic enables NTAPP for treatment heat-sensitive materials including biological tissues. To date many strategies have been made to enhance transdermal drug delivery by regulating the skin barrier structure using physical devices including ultrasound [1] and lasers [2].

In this study, we examined the comparison of non-thermal atmospheric pressure plasma (NTAPP) operating with helium and argon gases on the efficiency of drug penetration through the skin. As a result, under the same power density per volume, NTAPP operating with argon gas reduced the expression of E-cadherin leading to the enhanced transdermal delivery of the epidermal growth factor (EGF) (Fig. 1). The electrical parameters, such as applied voltage, total discharge current, electron density, plasma current, and the optical emission spectroscopy (OES) are observed.

Fig. 1. (a) Results of cellular E-cadherin localization after treatment of same plasma power conditions of non-treated (nt), the argon plasma (ArP) at 3 kV and the helium plasma (HeP) at 4.5 kV. (b) Variation of the plasma power corresponding to applied voltage for the ArP (blue) and the HeP (orange).

References
Comparative Study across four ‘COST Reference Microplasma Jets’

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Atmospheric pressure microplasmas have gained interest in the past decades in many fields [1] including biomedical applications, primarily due to their efficient production of chemical reactive species e.g. reactive oxygen and nitrogen species (RONS). Many plasma sources have been developed for these applications. One of the challenges in the field is comparability of species components and concentrations across different plasma sources, as this limits comparison and correlation of results in the literature.

A plasma source that is relatively simple in design for both simulation and experimental purposes was proposed in the frame of a recent network (COST Action MP1101 ‘Biomedical Applications of Atmospheric Pressure Plasmas’) and presented in [2]. The motivation for the COST Reference Microplasma Jet is to have a reference source for atmospheric pressure plasmas. This reference source should boost fundamental understanding of atmospheric pressure microplasmas, and their interactions with substrates, by making results from different laboratories comparable. For this it is imperative that each plasma jet is reproducible with little variability and has the same characteristics with regards to plasma parameters e.g. power, gas temperature and reactive species concentrations. The purpose of this presentation is to compare a number of these identically constructed micro-plasma jets to each other. The COST Reference Microplasma Jet is radio frequency (13.56 MHz) driven, across two parallel stainless-steel electrodes, with a gap of 1 mm and plasma length of 30 mm.

In this study four COST Reference Microplasma Jets are characterised and compared to each other. Plasma parameters measured include electrical power-delivery, gas temperature of the effluent, temperature of a glass substrate, optical emission spectroscopy of the core plasma, ozone, and absolute atomic oxygen densities by means of two-photon absorption laser induced fluorescence (TALIF). In addition, bacterial inactivation, post plasma treatment, is also compared and presented. The challenges of reproducibility and comparing parameters across jets, along with any limitations of the plasmas with regards to biological applications will be presented. The four different plasma jets compare well with each other.

Thanks to N St J Braithwaite (Milton Keynes), S Reuter (Leibniz Institute for Plasma Science and Technology), G Kroesen (Eindhoven University of Technology) and M. Turner Dublin City University for providing the plasma jets for this study.

References

Nitric oxide (NO) is known to aid wound healing through stimulation of proliferation and migration of wound related skin cells. [1] NO can also be used to combat bacterial biofilms as it is a well-known signaling molecule that can cause biofilm-dispersal in low concentrations by lowering c-di-GMP levels. Thus increasing the efficiency of antibiotic treatment for biofilm related ailments. [2]

Reactions initiated by non-thermal atmospheric pressure air plasma can lead to the generation of Reactive Oxygen and Nitrogen Species (RONS) such as NO, N₂, O₃, OH, H₂O₂, and O₂⁻. The ability to generate such species in air under ambient conditions has led to an increasing interest into the possible applications of cold plasma devices, especially in the field of healthcare. [3]

Given that NO is one of the predominant RONS produced in non-thermal air plasma, this contribution focuses on the generation and transportation of NO from a surface barrier discharge. Laser Induced Fluorescence (LIF) was used to excite ground state NO within the vicinity of the discharge to obtain spatially resolved density measurements, shedding light on its generation and transport mechanisms. Particle Imaging Velocimetry (PIV) was also used to quantify the gas velocity of the flow generated by the electro hydrodynamic (EHD) forces created by the discharge.

Comparison of the spatially resolved NO density and gas flow velocity measurements indicated that NO generation, transport and loss mechanisms are all strongly influenced by the flow dynamics created by the plasma. Through optimization of the electrode geometry it was observed that NO could be transported several centimeters beyond the discharge by the generated gas flow. This work demonstrates that low temperature plasma devices could be designed and optimized to enable the delivery of large doses of NO to remote samples.

This work was supported by the UK Engineering and Physical Sciences Research Council [grant EP/N021347/1].

References
Discharge characteristics of nanosecond pulse-driven atmospheric pressure plasma jets

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Atmospheric pressure plasma jets (APPJs) have been widely used for biomedical applications for the last couple of decades. Many of APPJs are operated with the sinusoidal driving voltage at a frequency range of tens of kHz to MHz using an inverter circuit. In this study, we present the properties of monopolar pulse-driven APPJs which show different performances from those with sinusoidal driving voltages. In this experiment, we varied the pulse duration and the duty ratio from hundred nanoseconds to hundreds of microseconds. Experimental investigation of APPJ includes optical emission spectrometry (OES) and the current-voltage characteristics. Also, a numerical calculation using a global model was used to estimate electron temperature, electron density, and electron energy density of helium and argon gas plasma jet. From the measured breakdown values, we found that the increase of the pulse width leads to the decrease of breakdown voltage when the pulse width is less than 1 microsecond (duty ratio 5% at 50 kHz). OES spectrum peak values also increase with the pulse width when the pulse width is less than 1 microsecond (duty ratio 5% at 50 kHz).

Key words: APPJ, pulsed plasma, OES, global model
The atmospheric-pressure plasma jet with DC microdischarge in a vortex gas flow

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It is known a great diversity of living nature. Moreover, their conditions of existence are very limited, which indicates a very high sensitivity of living to external factors. That is why we consider it necessary to use plasmas generators in the widest possible range of modifications for plasma medicine.

Atmospheric pressure plasma of micro discharged which is formed in the vortex gas flow is a stable source of low-power non-isothermal plasma. The physical feature of the vortex discharge is the generation of a plasma with high energy efficiency by limiting the heat-mass exchange of plasma with the surrounding medium in the volume of plasma generation. This means getting as soft as possible interaction of plasma with a living tissue (surface).

Experimental studies of plasma of low-power (up to 30 W) microdischarge in a vortex gas flow of atmospheric pressure, which showed dependence of the electric field on the interelectrode distance. The emission spectra inside and outside the microdischarge system and absorption spectra of processed solutions were recorded using CCD-based spectrometer Solar TII (S-150-2-3648 USB) (operating in the wavelength range of 200-1080 nm). Emission spectra of microplasma inside microdischarge system contain atomic Ar lines and molecular OH bands, spectrum outside the system also contain NO, N2, and the population temperatures of the rotational and vibrational levels of the plasma-forming gas molecules from the power supply have been carried out.

Also this paper presents the results of investigation of the plasma-liquid system with the secondary discharge supported by atmospheric pressure microdischarge in the vortex Ar flow. The plasma treated fluids were aqueous solutions of AgNO3. The microplasma discharge was powered by a DC supply. The plasma channel behaviour was characterized by photo/video recording, also plasma was studied using emission spectroscopy technique. The working liquid and firm products created after the treatments were studied also.
Estimation of plasma density along the plume length in low temperature atmospheric pressure plasma jet

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In the last couple of years, there is a lot of growing interest in the field of low-temperature atmospheric pressure plasmas for medical applications. Depending on the application, the active species requirement may vary which contingent upon on the plume parameters consequently on applied voltage and gas flow [1]. Therefore, it is essential to thoroughly understand the properties of the plasma plume. Several studies have discussed the plume parameters such as electron and plasma Temperatures ($T_e$ & $T_{gas}$), electron density, etc. using optical diagnostics [2]. The optimization of plume parameters for a specific application is still far away from the realization [3]. Hence, there is a need to execute more experiments to understand the plume dynamics for real-time medical applications. In this course, an attempt has been made to understand the plume dynamics such as plume density range along the plume length.

Experiments are carried out using 4 kV (P-P), 33 kHz sinusoidal voltage source with Helium (He) as working gas. Flow rates up to 11 liters per minute is used and the glass tube nozzle inner diameter is 4mm. With these experimental parameters, the plume length of about 40mm into the ambient has been achieved. The density has been estimated using the $I_{plume}/(e.v_{plume}A)$ relation. In this, the accurate measurement of plume velocity ($v_{plume}$) and area ($A$) has been estimated using ICCD images. The plume current is obtained by direct measurement using the current transformer placed around the plume at various locations along the plume length. The estimated density range is $(0.05879-3.26) \times 10^{12} \text{ cm}^{-3}$ at different positions along the plasma plume. Further, the discharge ignition and plasma plume formation dynamics with flow rate, and accurate velocity measurements at various locations of the plume will be discussed in the presentation.

References

Atmospheric Pressure Multijet Plasma Source for Plasma Medicine Applications

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Single plasma jets have allowed significant advances in \textit{in vivo} or directly on human experiments (e.g. [1], [2]). The results are particularly promising, but, ultimately, they could be limited in the future due to the fact that the treatment times are rather long due to the very small surface area treated by the produced plasma. There is a real challenge to develop sources that allow treatment over larger areas while remaining practical and at a reasonable cost. To this end, there are already flexible or rigid surface DBDs but they require an extremely small distance between the reactor and the treated tissues, which limits their use in many situations (particularly when the treated surfaces exhibit large variations in the surface morphology leading to points of attachment of streamers and therefore to a very inhomogeneous treatment). "Ideal" sources are therefore sources with the flexibility of a jet and treatment surfaces comparable to large DBDs. It is in this spirit that we have developed a new generation of applicators based on a single Plasma Gun system and able to generate a multitude of jets (from tens up to few hundreds) from the primary plasma jet, as shown in Fig. 1.

Before proceeding to \textit{in vivo} treatments, we qualified the sources through \textit{in vitro} experiments of decontamination on colonies grown on agar plates in traditional Petri dishes, to check the equivalence of each of the jets generated, as well as on colonies grown on very large agar surfaces scanned with our system, to check large surface treatment feasibility. The results are extremely encouraging and demonstrate the effectiveness of the multijet system, which allows both the processing of large-scale targets as well as the reduction of the processing times that otherwise can be prohibitive with single-jet systems. We will present experimental results obtained on the generation of different types of multijets, as well as results obtained with one multijet plasma source on the decontamination of multi-resistant bacterial colonies grown from hospital in-patient samples.

Acknowledgements
This work was supported by the CNRS PEPS project ACUMULTIPLAS and the ITMO Cancer in the frame of the Plan Cancer, project N°17CP086-00.

References
Inactivation of Airborne Porcine Reproductive and Respiratory Syndrome Virus (PPRSv) by Non-thermal Plasma

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Biosecurity in agriculture is almost entirely directed toward preventing transmission of infectious agents on surfaces. However, PPRSv is one of numerous livestock diseases which can be transmitted in air. Studies have shown that viable PPRSv aerosols can travel multiple kilometers in air [1, 2], leading to transmission of the disease between barns on a single farm or between farms. Physical filtration (e.g., HEPA filters) of ventilation air supplied to livestock presents numerous challenges that translate into high capital and operational costs. Of the two defining characteristics of infectious aerosols - transport and infectivity [3] – physical filtration only addresses transport where NTPs can address both by electrostatic removal of larger aerosols and sterilization of the remaining smaller aerosols.

Previous experiments at Univ. of Michigan using a packed bed non-thermal plasma reactor demonstrated inactivation of aerosolized MS2 phage ranging from less than one-log inactivation at < 20 kV and a few watts power to more than two-log inactivation at 30 kV. The present study examines the effectiveness of the same reactor in inactivating aerosolized PPRSv using virus stocks and wind tunnel facilities provided by the Univ. of Minnesota. An air-jet atomizer aerosolized a solution containing ~10⁵ TCID₅₀/ml of PPRSv, with the resulting droplets drawn into the packed bed NTP reactor. Twin impingers upstream and downstream of the reactor collected pre- and post-treatment samples followed by TCID₅₀ assay and quantitative polymerase chain reaction (qPCR) analyses. Over the range of voltages applied, results (Fig. 1) showed that PPRSv was inactivated to an equal or greater degree as MS2. The results proved that NTPs can reduce transmission of PPRSv in air and that MS2 is a conservative surrogate for PPRSv in NTP design optimization.

![Figure 1](image_url)

**Figure 1.** Comparison of MS2 (circles) and PPRSv (triangles) aerosol inactivation by non-thermal plasma.


*This project was funded by the National Pork Board, project # 16-198. The NTP reactor was developed through a grant from the USDA National Institute for Food and Agriculture (award # 2016-67030-24892). Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture. The authors also thank Eric Lee for his material contributions to this work.*
Plant Growth Enhancement using Reducing Active Species Produced by Water Vapor and Hydrogen Plasmas

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Plant growth enhancement has been observed when plant seeds are irradiated with plasmas. Active species in the plasmas are considered to be stimuli to plants, and induce growth enhancement and antioxidant activity.¹ However the husk of seed may be damaged by energetic particles generated in plasmas. Therefore, lack of agricultural practicality. There is need to find a gas species that causes less damage to the husk of the seed. In this study, the effect of reducing active species in low-pressure water vapor and hydrogen plasmas on plant growth is investigated. In addition, the biological responses of plants treated with water vapor and hydrogen plasma is attempted to be elucidated by gene expression analysis.

A cylindrical vacuum chamber with an inner diameter of 210 mm and a length of 490 mm is used. The RF electrode powered with the frequency of 13.56 MHz is arranged along the inner wall of the vacuum chamber.¹ Radish sprouts and Arabidopsis thaliana seeds are used as sample. When plasma is generated by water vapor, the RF power and pressure are set to 50W and 50Pa, respectively. When plasma is generated by hydrogen, the RF power and pressure are set to 100W and 120Pa, respectively. Reducing active species such as Hα and Hβ and OH radical are generated in water vapor and hydrogen plasmas. After irradiation, the Radish sprout seeds are hydroponically cultivated in the dark condition at 24°C. Then, the total length of radish sprouts is measured after 4 days of cultivation. Arabidopsis thaliana seeds are cultivated in soil under the LED lamp. The total length of Arabidopsis thaliana is measured after 2 months of cultivation. Then, by comparing the growth enhancement effect and changes in gene expression of Arabidopsis thaliana, the mechanism of the plant growth enhancement is attempted to be determined.

Figure 1 shows the average total length of radish sprouts varying irradiation period of water vapor and hydrogen plasmas. The horizontal axis shows plasma irradiation period, the vertical axis shows average total length of treated radish sprouts normalized by that of control. The maximum average length by hydrogen plasma irradiation is about 119% when the irradiation period of 30 minutes. By analyzing the gene expression of Arabidopsis thaliana seeds, the gene concerning the abscisic acid, which is one of plant hormones, is upregulated. Based on this result, the growth enhancement of radish sprouts generated by water vapor and hydrogen plasmas would be attributed to the variation of gene expression of plant hormones.

References

Stimulation of germination and growth of *Arabidopsis thaliana* using low temperature plasmas and plasma activated waters

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The first published works on the plasma/agriculture applications date on the 1990s where for instance a patented glow discharge device at low pressure in O\(_2\) and N\(_2\)-O\(_2\) mixtures has been used to stimulate the germination and the growth of soybean seeds [1]. This was followed by a small flow of literature works until the last years where the interest of the communities of plant biology and plasma (undoubtedly boosted by the success of the researches on Plasma Medicine field) was significantly increased. Indeed, we have seen a jump on published works and 2 international workshops during 2016 [2-3] specifically devoted this promising plasma/agriculture field.

Our contribution in plasma/agriculture had started by previous works on *Arabidopsis thaliana* seeds to fix some important parameters (choice of plasma device, treatment time, etc.) from the analysis of the early events of germination [4]. Then we continued in the present work by the analysis of the effects on the seed germination and the plant growth by using two specific plasma devices powered by a pulsed high voltage generator: (i) a helium plasma jet setup [5] and (ii) a floating electrode dielectric barrier discharge FE-DBD in ambient air [6]. The aim is to better understand the plasma stimulated germination and also the plant growth using plasma activated tap waters.

During the germination stage, we analyzed testa and endospserm ruptures that are faster in the case FE-DBD air plasma. The plasma effects on germination are analyzed using specific stainings for ROS production and peroxidase activity [7]. They are also based on membrane permeability tests and SEM that shown smoother seed surface after plasma exposure.

During the plant development and growth followed until 42 days, we considered for watering four kinds of waters (tap and deionized waters activated or not by plasma). Only the plasma activated tap water (PAW\(_{\text{tap}}\)) has shown a significant growth. The electric and chemical analysis of PAW\(_{\text{tap}}\) has shown higher conductivity and acidity and also higher concentrations of hydrogen peroxide and nitrogen species that probably explain the observed faster plant growth when using PAW\(_{\text{tap}}\).

**References**

Investigation of Growth Enhancement Mechanism of Plants by Using Oxygen Plasma and Karrikin

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1. Introduction

In recent years, the growth enhancement process of plants by using oxygen plasma is focused on as the process of low harmful influence on human body. However, the detail of growth enhancement mechanism of plants by using oxygen plasma is unexplained. On the other hand, it is known that the forest after occurring forest fire is reproduced by rapid growth of plants. As a cause of the plant growth enhancement, the influence of karrikin as plant growth regulator has been reported. Karrikin is a kind of plant hormone, which is produced when the plants are exposed combustion and high temperatures caused by fire, and acts as factor for plant growth enhancement. Currently, from sharing the same oxidation reaction between burning and oxygen plasma and results of DNA-microarray analysis of Arabidopsis thaliana, the relationship of karrikin and the mechanism of plants growth enhancement by the oxygen plasma treatment are suggested. In this study, oxidation reactions of plants using the oxygen plasma are investigated to understand the relationship between the growth enhancement mechanism of plants and the production of Karrikin by exposing the oxygen plasma.

2. Experimental set up

Oxides on seed surface generated by oxygen plasma irradiation and baking were measured. Seeds of Arabidopsis thaliana placed in a 17L stainless-steel chamber were baked using an electric heater. The smoke after the combustion was guided to a gas cell of Fourier transform infrared spectrophotometer (FTIR), and IR spectra were obtained. Also, oxides on seed surface generated by the oxygen plasma were measured. Seeds of Arabidopsis thaliana are irradiated by active oxygen species in the oxygen plasma that is produced by the RF discharge in the low pressure circumstance. The irradiation time above each experiment was varied from 3 to 30 min. Germination rate of seeds, stem length and leaf area are measured to clarify the growth enhancement effects of plants, when the seeds are treated by baking and plasma irradiation. Plants were grown by hydroponic culture or soil cultivation in an artificial climate chamber.

3. Results and discussion

It was showed that a characteristic peaks are observed in the IR spectrum of seed surface irradiated with oxygen plasma. Observed peaks are attributed to oxides generated by oxidation of plant seeds. Also, in case of baking, several peaks were appeared in the IR spectrum. Some peaks on both IR spectra are found to be somewhat similar to the IR spectrum of Terpenoids those are oxidized tissues of plants. Therefore, Karrikin that is one of Terpenoids would be produced on the seed surface by the oxygen plasma irradiation. The production of Karrikin on the seed surface is supported by the gene expression analysis of seeds. Genes of the response to Karrikin is upregulated in the Arabidopsis thaliana seeds irradiated by the oxygen plasma.

Fig. 1 IR spectrum of seed surface irradiated by oxygen plasma.
High energy leverage method on growth enhancement of bio-mass plants using plasma seed treatment

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Low energy consumption and high productivity processes for biomass is an important topic on greenhouse gas reduction. Sorghum (Sorghum bicolor (L.) Moench) is a strong candidate of biomass plants to produce biomass ethanol [1, 2]. We have found that a scalable dielectric barrier discharge (DBD) plasma irradiation to seeds of Arabidopsis thaliana shows 11% reduction in a period between sowing and harvest and 56% improvement in crop yield [3]. Here, we examine growth enhancement of sorghum using the scalable DBD plasmas.

Seeds were treated using the scalable DBD device [3-7]. The discharge voltage, current, and power density were 7.00 kV, 0.2 A and 3.05 W/cm², respectively. 30 seeds of Sorghum were arranged in line on a glass plate with 5 mm intervals at 3 mm below the electrodes. The device provided various dose of reactive oxygen nitrogen species (RONS) depending on the position x, thus we examined effects of RONS dose on the growth enhancement in a combinatorial way. 30 seeds for each condition were cultivated in soil under a 12 hours light/12 hours dark cycle at 24°C.

We measured dependence of the average length of their stalk after the 26 days cultivation on the seed position x. The electrode region is between x=-44 mm and x=0 mm. The average length is the highest at x=-10 mm and it is 10% longer than control. Based on the results, we assume the plasma irradiation leads to 10% improvement of biomass yield. The device offers simultaneous plasma irradiation to 10 g seeds. The corresponding energy consumption is 83.4 J/g for 180 s irradiation. Conventionally, the 4000 kg of the biomass ethanol is produced from 1 ha field. The plasma irradiation enhances ethanol production of 400 kg using 4.2 MJ/ha energy consumption (50 kg/ha seeds). The energy consumption of the cultivation and harvest of sorghum is 3.3 GJ/ha. The energy consumption for plasma irradiation is 1/786 of that for cultivation and harvest, namely the energy consumption of the plasma treatment is negligible. Such quite small extra energy consumption produces extra biomass energy of 10.8 GJ/ha corresponding to 400 kg Sorghum, that is the significant high energy leverage of 2.6 x 10³ times.

This work was supported by JSPS KAKENHI Grant Number JP16H03895.

References

Influence of different plasma compositions on the germination potential of onion seeds: A thermal profile analysis

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Plasma technology has become a versatile and promising treatment technique, which usually leads to positive results when applied for biomedical [1] and agriculture pursuits [2][3][4]. In this study, we evaluated the influence of different plasma compositions on the germination of onion seeds, by taking a careful look on their thermal profiles. For this, commercial onion seed samples were randomly selected. For the irradiation of the samples, a gliding arc reactor was used, with a gas exhaustion in a reverse vortex and plasma generation by a direct current source (10 kV). Plasma was generated by using argon, helium and air. During all treatments, the temperature was carefully monitored (Figure 1), using a micro-controller data acquisition system with a K-type thermocouple. In addition, thermal images were taken using an infrared camera. After irradiation, the samples were then incubated in Petri dishes for 10 days and evaluated daily. This incubation aimed at simulating germination chambers. High temperatures were observed during irradiations containing plasma composed of compressed air, which have negatively influenced the germination viability of the onion seeds. Although the period of exposure of the samples did not exceed 15 min, the effects were permanent. Conversely, plasma treatments using helium and argon showed no effect in the physiological development of the seeds.

Figure 1. Temperature as a function of time during the treatments of the onion seeds using different plasma compositions.

Although the precise mechanism of action of the plasma has still not been elucidated, our set of results points out that plasma treatments at low temperature has been shown not to be harmful to the seeds. These results open a wide range of possibilities for the development of new irradiation protocols and, thus, should be further studied for different sample treatments.

References

Effect of cold plasma treatment in preventing cross contamination and quality of baby carrots during transportation

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In the United States, about 80 percent of all food shipments and 91 percent of all temperature-controlled freight shipments, including about 28.5 million tons of refrigerated fruit and vegetables, are transported by truck. In the complex food transportation system, microbial contamination is a problem which may be introduced at an earlier stage in the supply chain and can spread out to many distributors, retailers and then to consumers. Baby carrots are consumed worldwide because of their nutritional benefits. However, in the past two decades, there has been an increase in foodborne diseases outbreaks caused by microorganisms associated with fresh produce. Cold plasma treatment of fresh produce, an emerging food preservation process, is a fast and environmental-friendly process that disinfects the product at a low temperature (30-40°C). Cold plasma uses an ionized gas comprised of charged ions and free electrons for treatment of the product and kills microorganisms by altering their metabolic pathways. Till date, very little research has been focused on the effect of cold plasma mist (micro meter size water droplets) on the quality and shelf life of fresh produce and its efficacy in transportation. Therefore, the purpose of this study was to evaluate the efficacy of dielectric barrier discharge (DBD) cold plasma treatment on microbial load and quality of baby carrots. Conditions in transportation truck were simulated by installing cold plasma mist system in a 110L volume refrigerator. Effect of treatment time, distance from plasma source and storage temperature on color values (L*a*b*), carotenoids, texture and microbial load of baby carrots was evaluated. Carrots were inoculated with E. coli O157:H7. This research demonstrated the ability of the cold plasma system in complete inactivation of E. coli O157:H7 when baby carrots will be treated with plasma. Initial results indicate that growth inhibition saturates after 10 minutes of treatment with mist and distance from plasma source had an impact on microbial growth but the position did not affect. The results show the potential of cold plasma technology as an efficient disinfection technology which not only kills microorganisms, but may also enhance shelf life of fresh produce.
Effect of cold plasma on inactivation of *Escherichia coli* and quality of baby kale (*Brassica oleracea*)


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Abstract

The efficacy of cold plasma (non-equilibrium atmospheric pressure pulsed dielectric barrier discharge) treated mist in disinfecting baby kale leaves, and its effect on color values and cuticle were evaluated. Baby kale leaves were inoculated with *E. coli* O157:H7, incubated overnight and treated with plasma mist. Treated and untreated leaves were analyzed for change in color values (L*, a*, b*, chroma, hue, and browning index (BI)) and functional groups (alcohols, esters, aldehydes, and ketones) in cuticle. Color stability of treated leaves was also evaluated after refrigerated storage (4 °C) for 12 days. Complete disinfection of kale leaves was achieved after plasma treatment for 300 s with no significant change in color values. Visible change in color (browning or leaf damage) was observed after 600 s of plasma treatment. Further, color stability of plasma treated leaves was enhanced during refrigerated storage (4 °C), indicated by lower BI values of treated leaves compared to untreated leaves after 12 days of storage. Furthermore, plasma treatment of kale did not negatively affect cuticle composition. This study demonstrated that cold plasma mist has the potential to ensure microbial safety and enhance shelf life of baby kale leaves.
Onion germination enhancement with He-Air RF plasma jet

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Onion (Allium cepa L.) is one of the most important plants cultivated in moderate climate zones. Low temperature plasma with its highly reactive components such as electrons, free radicals, and excited molecules was investigated as a potential tool for improvement of seed germination energy and germination capacity [1-5]. In this paper, the experimental results on RF atmospheric pressure plasma jet operating with helium and air gas mixture on the germination of onion (type Wolska) seeds are presented.

RF plasma reactor was powered by a regulated T&C Power Conversion AG 1021 generator via impedance matching network. The operating RMS voltage, frequency and forward power were 420 V, 14.72 MHz and 45 W, respectively. As a working gas, a mixture of helium and air was used in proportions of 60% to 40%, with a total flow of 0.71 m³/h. Temperature of gas on the surface of seeds was measured using insulated type-K thermocouple, did not exceed 45 °C.

Four groups of seeds characterized by a different exposure times (1, 2, 4 and 8 min.) were positioned in 6 cm distance from reactor’s outlet and further observed along with a control (untreated seeds). In current experiment, each combination consisted of 400 seeds. Number of sprouts was determined every 24 hours. Fraction of germinated seeds (number of sprouts) after 6 days of germination was defined as germination energy GEN, while fraction of germinated seeds after 12 days of germination was defined as germination capacity - GC.

The highest germination energy (92.5%) was obtained for the seeds subjected to pre-sowing treatment with plasma with exposure time of 2 min. These parameters were statistically significantly higher than in the case of control seeds - 71.0%. However, a statistically significant differences between the control seeds and the cold plasma treatment in RF reactor (He+Air) for the exposure time 8 min. amounting to 68.75% were not found.

The highest germination capacity of the seeds was registered for seeds after plasma stimulation for 2 min. - 94.75%, while in the control sample this parameter amounted to 91.25%. Stimulation of seeds for 1 and 4 min. had no visible effect on improvement on germination capacity and 8 min. of plasma treatment resulted in decrease of germination capacity.

This work was supported by M-ERA.NET, Inkubator Innowacyjności+ found, networking actions: KONNECT and CEEPUS CIII-AT-0063.

References
Optimizing legume germination and growth by applying cold atmospheric plasma

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Food security and meeting the increasing demand for energy will be critical challenges for mankind in the near future. Producing sustainably is therefore becoming central in agriculture and food industry. Legume crops could play an important role in this context by delivering multiple services in line with sustainability principles. In addition to serving as fundamental, worldwide source of high-quality food and feed, legumes contribute to reduce the emission of greenhouse gases and add to the fertility of soils in agricultural land due to their ability to fix atmospheric nitrogen. An increasing demand in the usage of legumes requires fertile and resistant crops such as alfalfa, red clover, fava, vetch or cowpeas. Here we show that the germination of legume seeds displaying low germination success (e.g. alfalfa, clover) can be enhanced by applying cold atmospheric plasma. Germination rates, shoot and root length, as well as nitrogen and carbon content will be presented. Finally, the transferability of cold atmospheric plasma application to a wider range of agricultural needs will be discussed.
Nitrogen fixation using the “Propeller Arc” discharge in air

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Fixation of nitrogen due to naturally occurring electrical discharges, such as lightning, is well known. Based on the same principle, reactive nitrogen produced from N₂ and O₂ by non-thermal plasma has been recently proposed as an alternative technology for agricultural nitrogen fixation [1-2]. A key metric is electrical energy expended per molecule of fixed nitrogen. In this work, a novel plasma source named “Propeller Arc” (PA) was designed to efficiently fix nitrogen in air. As shown in Fig. 1, the PA device consists of a rotating cathode, driven by a DC motor, with a fixed anode. The device can also be operated using pulse modulation. Using pulse modulation, the plasma is ignited at the narrowest gap (~0.5mm) between cathode and anode, and is then drawn away by the rotating electrode to a length up to ~55mm. In this way, a relatively large plasma volume can be produced while achieving breakdown at a relatively low voltage. PA is similar to the widely used Gliding Arc (GA) [2] which is also ignited at a narrow gap and then extends to a longer length along the electrodes driven by high speed gas flow. However, unlike the GA, PA does not require gas flow and the discharge frequency can be adjusted with rotor angular velocity and pulse modulation frequency. The primary species produced by PA operated in air under atmospheric pressure and room temperature are NO, NO₂, and HONO. The energy cost of NOₓ production (energy expended per unit NOₓ produced) is measured to be lower than ~110 eV/molecule N at a frequency of 30Hz, making PA a promising new device for nitrogen fixation.

Fig. 1 Propeller Arc (PA) in air

This work was supported by National Science Foundation (1606062).

References

Cold air plasma for apple juice shelf-life extension

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Conventional methods for food processing and inactivation of food borne pathogens are based on thermal treatments, typically referred to as pasteurisation. These thermal processes often lead, besides sterilization, to the loss of food quality. A growing customers’ demand of long-lasting fresh products requires “minimal processing”, e.g. non-thermal food technologies. Non-thermal (cold) air plasmas generated by electrical discharges in atmospheric pressure air are sources of various reactive species, free radicals and charged particles. Such plasmas can be generated in contact with water or other aqueous solutions to generate reactive oxygen and nitrogen species (RONS), leading typically to strong antibacterial effects. They represent a great potential for liquid food processing, such as fruit juices, or in agriculture applications [1-3].

The transient spark discharge in air was successfully tested to induce antimicrobial effects in fruit juices. Inactivation of model and natural pathogens (bacteria E. coli and yeast S. cerevisiae) in fresh apple juice and a significant extension of the shelf-life time of the juice up to 26 days were achieved; both in the batch system and in the flowing electrospray system.

We also examined the potential cold plasma treatment effects on chemical, nutrient and sensory properties of the juice, such as changes of pH, conductivity, color, and produced nitrites/nitrates and hydrogen peroxide. The most typical juice components including polyphenols, organic acids and sugars were detected in the natural juice by HPLC coupled with mass spectrometry, UV and refractive index detectors, respectively. These compounds were cold plasma treated separately in aqueous solutions and together in the treated juice, and further examined. Polyphenols were effectively hydroxylated and nitrated when treated alone but remained unmodified in the plasma treated juice. This can be attributed to the fact that although RONS are formed during plasma treatment, they react with many targets in the juice and their effects on each particular component are low. We also detected no significant changes of pH, conductivity and °Brix degree (sugar content) in the plasma treated juice. The peroxidase (POD) enzyme known for the undesirable browning and the juice quality loss [4], was successfully inactivated.

This cold plasma method represents a novel approach for the non-thermal pasteurization of fresh fruit juices with potential applications in non-thermal food processing.

This work was supported by Slovak Grant Agency VEGA 1/0419/18 and Slovak Research and Development Agency APVV-0134-12.

References

Effect of plasma activated water on plant growth

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With increasing population and decreasing food sources there is demand for new approaches in agriculture. Low temperature plasma (LTP) is a promising technology able to enhance productivity and maintain quality of food and has a potential to be used in various agriculture applications. Beside direct plasma treatment of dry seeds for germination improvement [1] the plasma activated water (PAW) for stimulation of seed germination and plant growth has become recently popular, too [2]. LTP generated by electrical discharges in atmospheric pressure air is a source of various reactive species, free radicals and charged particles. When plasma is in the contact with water the reactive particles originated in the gas phase diffuse into the water and produce reactive oxygen and nitrogen species RONS (•OH, O₂•, H₂O₂, NO₂•, NO₃•, ONOO•, ONOOH). The main species in the PAW responsible for the effect on seeds and plants are H₂O₂, NO₂• and NO₃•. They can act like signal molecules, are a potential source of nitrogen or may cause cross resistance against stress in plants [3].

The PAW was produced by a self pulsing transient spark discharge (TS) generated in a system with tap water repetitively flowing through the discharge zone [4]. We monitored the pH and concentration of H₂O₂, NO₂•, NO₃•, in PAW by colorimetric methods via UV/Vis absorption spectrophotometry. The lettuce Lactuca sativa was used as a model plant. The plants were irrigated either with PAW or with H₂O₂ and/or NaNO₃ solutions to compare the individual and combined effect of the dominant species in PAW. After 5 weeks of cultivation in pots with soil in controlled conditions the growth parameters of the plants (number of leaves, fresh and dry weight), photosynthetic pigments (chlorophylls and carotenoids), rate of net photosynthesis, content of soluble proteins, and activity of antioxidant enzymes in leaves and roots of plants were analyzed.

The number of green leaves, dry weight of above-ground part and photosynthetic pigments content increased with increasing H₂O₂ and NO₃• concentrations with more pronounced effect if both are present in the solution. Level of photosynthesis and protein content was found higher for the plants irrigated with PAW compared with the plants irrigated with a corresponding mixture of H₂O₂ and NO₃• while the results on dry weight and pigments were found comparable. Irrigation with PAW did not increase the antioxidant enzymes activities in above-ground part or roots of plants compared to the reference. The mechanism of RONS effect on plant metabolism and growth is complex and the obtained results need to be verified by further studies.

This work was supported by Slovak Research and Development Agency APVV 0134-12 and APVV 16-0216.

References

Enhancing the shelf-life of banana “Musa acuminate” by using microsecond-pulsed dielectric barrier plasma discharge for the removal of ethylene

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Ethylene (C2H4) is a gaseous natural aging or ripening phytohormone of fresh produce commodity which has multitudinous effects on growth, development, sensory properties and storage life of fruits, vegetables, and ornamental crops [1]. Thus, controlling ethylene in post-harvest storage atmosphere may prolong ripening and reduce post-harvest losses. Conventional methods (inhibitors, absorbers, and oxidizers) of controlling ethylene have not proved to be efficient under all conditions. Non-thermal plasma-generated species have shown to be effective for the decontamination and prolonging the shelf-life of minimally processed produce [2]. Therefore, this may enhance shelf life of bananas by decontaminating the surface and by decomposing ethylene. The purpose of this study was to investigate the effectiveness of microsecond-pulsed dielectric barrier discharge plasma on enhancing the shelf life of bananas by removal of ethylene from the atmosphere. Yellow bananas were exposed to plasma-treated air for one week at room temperature, pressure, and humidity. Effectiveness of cold plasma to retard ripening was evaluated by measuring reduction in ethylene concentration using gas chromatography compared to the untreated controls. The effect of plasma treated air and removal of ethylene on the bananas themselves was evaluated by comparing change in weight, color, texture, and sugar content treated and untreated bananas [3, 4]. Although ethylene concentration significantly decreased after treatment, no significant change in weight, color, texture, and sugar content was observed. This study demonstrated that cold plasma has the potential to prolong the shelf life of bananas by decomposing ethylene in post-harvest storage conditions.

Keywords: bananas, ethylene, cold plasma, gas chromatography, color analysis, shelf life

Application of non-thermal plasma for the activation of cell differentiation and protein secretion in *Aspergillus oryzae*

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*Aspergillus oryzae* is a filamentous fungus extensively used in fermentation industry because of its ability to secrete a variety of high value industrial enzymes such as α-amylase, protease, pectinase, β-galactosydase, etc. [1-3]. In this study, we examined the enhancement of spore germination, growth and protein secretion by a fermenting fungus *A. oryzae* after plasma treatment. The fungal spores were treated with a dimming mode micro DBD plasma using nitrogen as feeding gas. After the plasma treatment, spore germination was analyzed by colony plate count method. The production and secretion of amylase and protease were analyzed by SDS-PAGE method. Results showed that spore germination percentage was slightly increased after nitrogen plasma treatment for 2 and 3 min. Production and secretion of alpha-amylase (~54 kD) and protease (~47 kD) by *A. oryzae* was enhanced 3 days after nitrogen plasma treatment. These results suggest that cold plasma can be considered as an alternative method for activating the functionality of beneficial fungi.

Acknowledgement

*This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No. 2016R1D1A1B03934922 and 2016K1A4A3914113) and by National Fusion Research Institute (NFRI).*

References


Anti-cancer synergistic effect of Self Nano Emulsifying Drug Delivery System (SNEDDS) with cold atmospheric plasma

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Till now, melanoma remains one of the prominent skin cancers exhibits the maximum mortality rate worldwide [1]. Recent improvement of micro-dielectric barrier discharge (µ-DBD) cold atmospheric plasma technology developed by PBRC showed a new way of approach in the treatment of melanoma. Apart from the therapeutic activity of plasma, nanotechnology has dramatically influenced drug delivery research for improving therapeutic performance of drugs for the cure of cancer [2,3] and hence nanoemulsion of flavonolignan (silymarin) and called as self nano emulsifying drug delivery system (SNEDDS) were used in this study. We developed a combination strategy of the combination of cold plasma and nanoemulsion could contribute to improving selective permeability of membrane through the disruption of plasma generated ROS and RNS species leading to intracellular diffusion of nanoparticles towards melanoma.

SNEDDS was developed by incorporate the silymarin into oil-in-water (o/w) based nanocarrier. The size and morphology along with increase in cellular uptake of SNEDDS in melanoma cells using cold plasma were checked by using transmission electron microscopy. Cellular viability, growth inhibition and cell death were assessed along with apoptosis estimation. Reactive oxygen species and reactive nitrogen species were also measured. Melanoma specific enzymes showed drastic reduction in its activity along with reduction in PI3K, Akt and gradually increase in tumor suppressor genes strongly confirms decline in melanoma. Inhibition of epithelial mesenchymal transition (EMT) and melanoma stem cells were also observed. In vivo results on nude mice suggested the reduction in animal weight, tumor volume and melanoma level as compared with the control animal on the 21st day of the experiment.

Together these studies provide rationale to use SNEDDS to improve plasma activity with targeted delivery in melanoma treatment approaches and serve as can be use as plausible therapeutic agent for melanoma patients.

References

Effective and safe plasma disinfection on contaminated skin model using porcine skin tissue by the reduced-pH method

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Medical applications of low temperature atmospheric pressure plasma have been studied for various purposes, such as disinfection of human body, promotion of wound healing, cancer therapy, and so on. We have investigated the bactericidal effect of plasma exposure to bacterial suspension and reported “the reduced-pH method” that the acidic pH (lower than 4.8) of the solution enhanced the bactericidal activity of plasma [1, 2]. For clinical application, this plasma treatment with the reduced-pH method was examined for its effectivity in dental treatment and confirmed its availability with caries models using extracted human teeth [3].

In this paper, we validated the reduced-pH method for skin disinfection using contaminated skin model designed for such as surgical infection and bedsore. Compared with the caries models, skin surface is relatively sensitive to heat, so the power range of plasma is limited for safe treatment. In addition, rich organic components on the skin would scavenge bactericidal reactive species supplied from plasma. For effective disinfection, the inhibition of bactericidal effect must be considered. To evaluate these limitations, porcine skin tissue was used for model skin. As the contaminated skin model, Staphylococcus aureus as pathogenic bacterium was inoculated to the skin. And the pH of the skin surface was controlled by spreading pH buffer solution before plasma treatment. A low-frequency (LF) helium plasma jet was applied to the skin and scanned for all over the inoculated area by an electric controlled mechanical stage. The parameters of plasma treatment were adjusted not to generate heat damage on samples. After the plasma treatment, the remaining bacteria were recovered and the number of living cells were determined by colony assay. Finally, the log reduction (LogR) of viable cells was calculated. In this procedure, bacterial recovery process is very important, because insufficient bacterial recovery would bring the overestimate of bactericidal effect. Therefore, first of all, we evaluated various bacterial recovery methods and the suitable method was selected for this experiment. In the results, LogR were 4.2 at pH 3.5 and 1.4 at pH 6.5. Obviously, the bactericidal effect in acidic condition was stronger than in neutral pH, the reduced-pH method was effective on the skin surface. Stronger bactericidal effect would enable shorter treatment time, which is desirable for effective and safe therapy. Moreover, bactericidal effect in pH 3.5 was enough for skin disinfection compared with the standard for antiseptics, LogR > 1~3 (varied in the disinfecting region and the purpose for use).

In this study, we demonstrated that the reduced-pH method worked on the skin tissue with the contaminated skin model using porcine skin tissue, and that the bactericidal activity of the plasma treatment at pH 3.5 was at least equivalent to antiseptic in practice use.

Wound healing properties of cold plasma in dependence on regime of treatment

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Introduction. Previously, we demonstrated, that stimulation of fibroblast proliferation in vitro was dependent on frequency of treatments with microwave argon plasma [1]. Daily treatments, in contrast to treatments every 48 h slowed down cell proliferation [1]. The purpose of this work was to evaluate if the plasma treatment frequency influences infected wound healing in vivo.

Materials and Methods. A previously described plasma microwave generator (2.45 GHz), Plasma 200 (JIHT RAS, Russia), was used (Fig. 1) [1]. Balb/c mice were used to make full-thickness wounds, infected with Staphylococcus aureus ATCC 25923. Starting on the 4th day, wounds were treated with plasma for 2 minutes daily for 5 days or 3 times with 48h interval, the measurements of a wound contraction, a microbial load and histological morphometric analysis were made on 4, 7, 11, 14 and 17 days (Fig. 1).

Results. Two plasma treatments with the 48 h interval or 3 daily treatments accelerated wound contraction on 25% and 31%, respectively, by the 7th day of the experiment. Wound healing acceleration was not due to a bactericidal effect as the difference in bacterial loads between treated and control mice was non-significant. Rather, the wound healing accelerated due to improved growth of the connective tissue that increased up to 32% on cross-sections (Fig. 2; p<0,05) Higher rates of connective tissue growth was accompanied by earlier and faster growth of the epithelial tissue (4 and 7 % for 2 and 3 treatments, respectively, vs 1 % at the 7th day). Further treatments did not increase a wound healing (by 11th day), probably because of a scab formation. Obtained results demonstrated that plasma stimulated growth of both connective and epithelial tissues at early stages of acute wound healing while the frequency of treatments did not play an important role.

Fig. 1 Scheme of treatments. 1- plasma treatment days; O- days when microbiological and histological analyses were performed

Fig. 3. Area of connective tissue constituents in wounds, %

References

Intraoral treatment of Oral Lichen Planus using Cold Atmospheric Plasma: First results and therapeutic potential

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Background
Oral lichen planus (OLP) is one of the common immune-mediated mucocutaneous disease characterized by chronic inflammatory process, in which an immune response attacks the lining epithelium. In long-standing, atrophic and erosive forms, the treatment is usually aimed at relieving pain and may include immunosuppressive agents, especially corticosteroid, topical cyclosporin, or tacrolimus, topical and systemic retinoids. However, the use of these drugs may be accompanied by several side effects. In this laboratory and clinical study, we examined the therapeutic potential of Cold Atmospheric Plasma (CAP) for the treatment of OLP.

Material & Methods
For preclinical in-vitro investigations, tissue samples have been collected from healthy and diseased mucosal regions from 10 patients suffering from OLP. For detection of CAP-related cell death, investigation of DNA fragmentation using the TUNEL-assay has been performed after CAP treatment. Furthermore, T-cell population has been investigated before and after CAP treatment and was compared to healthy tissue samples. Supernatant culture medium of tissue samples were analyzed for 10 different cytokines. In addition, six patients suffering from intraoral OLP of the cheek have undergone an in-vivo treatment using the plasma jet kINPen MED. Intraoral treatments have been performed for a period of 1 min/cm² and with a continuous exhausting of gas and salivary.

Results
No significant enlargement of apoptotic cells could be detected after plasma exposure within the tissue. At the time this abstract was completed no final data concerning changes in cytokines as well as white cell population were available. The in-vivo treatment of OLP tissue revealed a reduction of pain and local inflammation. Especially the treatment of a therapy-resistant erosive OLP of the cheek could achieve pain reduction and a cure of mucosal ulcers.

Conclusion
It has been shown that CAP could be useful in the treatment of intraoral mucosa diseases, especially OLP. Especially, the waiver of local and systemic drugs enables a gentle treatment without side effects. A larger number of cases, investigation of long-term effects, and clinical studies are needed to further qualify plasma for OLP treatment.
Figure shows the right cheek of a multimorbid patient suffering from a therapy-resistant erosive OLP before (left), and after (right) CAP treatment. All volunteers gave written and informed consent to participate in the laboratory and clinical study.
Cold Atmospheric Plasma is a highly efficient platelet activator

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In major visceral surgery, e.g. tumor resections, blood vessel injury is inevitably. An efficient haemostasis is essential to minimize blood loss and keep the track of safe operation procedure. Thermal coagulation causes a superficial layer of necrotic cells and carbonised tissue, providing a basis for inflammation [1]. Increasing prescriptions of new oral anticoagulants compromise efficient bleeding management of emergency patients [2]. Evolution provides physiologic activators in terms of poison, e.g. Convulxin of tropical rattlesnake. Convulxin is a powerful platelet agonist. Maximal platelet aggregation is induced at concentrations as low as 3–10 ng/ml [3]. In a previous work we could show that CAP is able to coagulate murine blood high efficient and furthermore that CAP is able to overtake haemostatic functions in a murine model of liver incision even in rivaroxaban anticoagulated mice but not in clopidogrel anticoagulated mice [4].

Our present work investigated the CAP induced platelet aggregation in human donor blood ex vivo. Dividing one platelet populations into two parts and labelling these with two different CD31 antibodies (CD31 APC-Cy7, CD31 BV605). After CAP treatment we could show a high number of double positive platelets, showing an aggregated population. To have a meaningful reference point we used Convulxin and yielded similar results, verifying that CAP is a powerful platelet activator. Furthermore, CAP activated platelets in heparinized but not EDTA blood elevated the expression of several surface activation markers (CD62P, CD63, CD69, CD41/61); In further experiment we will elucidate which mechanism is essential for CD62p expression on platelets and differentiate between platelet necrosis, apoptosis and aggregation. This differentiation is important due to the fact, that necrotic platelets seem to be procoagulant in circulation, causing thrombus formations [5].

References

Plasma-cancer: simulation of chemicals and heat transport in mouse skin and tumor, comparison with *in-vivo* experiments

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Plasma-skin interaction can expose the skin both to the heat and the production of reactive oxygen and nitrogen species [1]. These species are generally assumed to play a key role in the biological effect observed. Mild-hyperthermia can elicit a strong biological response such as an increase of pO$_2$, the production of heat shock proteins, a slight decrease in pH [2] [3]. Mild hyperthermia can also stimulate the adaptive and innate immune system: in some cases, the systemic response destroys distant metastases by abscopal effects [4]. Most of these responses are also observed in *in-vivo* plasma-medicine experiments. A model for studying heat-propagation and heat-damages has been developed and validated based on recent *in-vivo* experiments performed on mice whose skin temperature was monitored.

The biological effect of mild-hyperthermia depends on the time and the temperature history of the skin and can be modeled by the denaturation reaction of collagen, the major component of skin [3]. This reaction describes the transition of collagen structure from triple helix configuration to a randomly coiled conformation. The damage function

$$\Omega(t, \vec{x}) = \int_0^t A \exp \left(-\frac{E_a}{k_B T(\tau, \vec{x})}\right) d\tau$$

enables the quantification of thermal damages [3]. Where $T(t, \vec{x})$ is the temperature as a function of time and position, $A$ (in s$^{-1}$) is a constant which depends on the species and tissue, $E_a$ is an energy of activation. The simulation gives the volume of tumor subjected to mild hyperthermia or exposed to temperature leading to tissue ablation. Particular attention is paid to the progressive accumulation of damages. The anti-oxidants defenses of the skin have also been modeled using finite element method to evaluate how deep the reactive species can diffuse. It appears that the volume of mouse skin freely accessible to H$_2$O$_2$ is only a fraction of mm, less than previously envisaged [5].

This work was supported by the Institut Universitaire d’Ingénierie en Santé (IUIS), Sorbonne Université, Projet OS CC 2014/2016, labex Plas@Par, Canceropole, CNRS and Ecole Polytechnique.


Microwave Plasma Torch for Wound Treatment

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Cold atmospheric pressure plasma sources have recently been proven to be an effective therapeutic method regarding wound healing. The most preferred and used plasma device to this moment is the well-known dielectric barrier discharge (DBD). In this work we are studying a low temperature plasma torch at atmospheric pressure sustained by a solid state microwave generator coupled to a resonance structure called Surfatron. This plasma source allows variation of: geometrical parameters (length, diameter, cross section), main plasma parameters (wave power, gas temperature, concentration of charged particles and reactive species, UV and microwave radiation) and gas flow velocity. Appropriate combination of the parameters leads to low temperature plasma torch obtaining (gas temperature up to 30 – 37 °C) suitable for in vivo treatment of live ICR mice models.

Preliminary results show effectiveness even for 3 day treatment (15 s per 24 hours) as shown on Fig. 1. Microwave power and gas flow were limited to 15 W and 13 l/min of Ar 5.0.

![Fig. 1 Treated (left) and control (right) wound immediately after incision (a) and on the fourth day after last treatment (b).](image)

The purpose of this research is to precisely study the optimal discharge conditions leading to acceleration of wound healing at short treatment times with relatively low gas flow and microwave power.

This work was supported under grand number DM03/3, 2016 of National Science Fund (NSF), Ministry of Education and Science, Bulgaria
Improved microcirculation as prerequisite for wound healing: Hyperspectral imaging reveals stimulatory effects on oxygen supply by cold plasma in patients with chronic wounds

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Although the mode of action is not fully understood, a multitude of data show that cold atmospheric plasma (CAP) has a supportive effect on wound healing. As oxygen and therewith energy supply is of crucial importance for wound healing it should be measured and monitored in clinical wound management. Unfortunately conventional techniques like Laser Doppler Flowmetry (LDF) lack suitability for routine use. Recently an innovative technique, Hyperspectral Imaging (HSI) was introduced allowing near real time analysis of spatial oxygenation (perfusion). To test the suitability of HSI to quantify and monitor oxygen supply in patients with etiologically different wounds before and after CAP treatment we measured wounds of three male patients by HSI before, directly and up to 2h after CAP treatment (APPJ, kINPenMed, neoplasm tools, Greifswald, Germany) with TIVITA™ system (Diaspective Vision, Pepelow Germany). Spectroscopic analysis in visual and near infrared spectrum provides perfusion data of recorded tissue in less than 10 sec. The grade of blood oxygenation (1-6 mm subcutaneous, StO2 in % and NIR resp.), distribution of hemoglobin (THI) and water content (TWI) are calculated from remitted spectra after broad spectrum radiation and shown as false-colored images on PC screen. Points of interest (POI) were set to calculate the intended values (STO2, NIR, THI, NIR). Results: In all three patients HSI revealed increasing blood oxygen saturation directly after and up to > 30 min after CAP treatment in the treated lesions but also the surrounding area. In a PAOD-patient we measured NIR increasing by 45% (POI arrow, Fig. 1a, b) and STO2 by 50% (POI arrow, Fig. 1c, d). Conclusion: For the first time CAP was shown to exert microcirculatory stimulation in patients with recalcitrant wounds which can be assumed as important healing support. Microcirculatory diagnostics can be realized by Hyperspectral imaging (HSI) as a novel, noninvasive technology with medical applicability in wound medicine to assess and monitor treatment efficacy.

Fig. 1. a) NIR perfusion before CAP treatment, b) NIR perfusion 30 min after CAP treatment (arrow: POI with 45% increased NIR), c) STO2 perfusion before CAP treatment and d) STO2 perfusion 30 min after CAP treatment (generally increased STO2, POI with 50% increased STO2)

References

Development of a Screened Plasma Jet for the Eradication of Bacterial Skin Infection

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All wounds contain bacteria that are normally controlled by our immune defences. In the case of chronic, non-healing wounds, such as diabetic foot ulcers, prolonged exposure to the external environment ultimately results in severe wound infections. Treatment of these infected wounds requires the use of antibiotics; however, overuse has significantly decreased the effectiveness of these antimicrobial agents. Plasma activated hydrogel therapy (PAHT) has been developed to eradicate colonising bacteria and promote wound healing. It involves treatment of a wound with an ionised gas plasma jet through a hydrogel wound dressing. The dressing filters out the more dangerous elements from the plasma jet (such as the carcinogenic hydroxyl radicals) whilst enabling the beneficial components from the plasma jet to be delivered to the wound site. Previous research has shown that hydrogel dressings can be loaded with reactive oxygen and nitrogen species (RONS), which when applied to a diabetic chronic wound cause no harm and may in fact stimulate the wounds to heal quicker. Furthermore, the development of a triggered-release concept, involving the incorporation of a traditional antimicrobial agent into a hydrogel system offers the possibility of using plasma to initiate controlled drug therapy. This aims to prevent any sub-lethal exposure of precious last-line antibiotics and to avoid contributing to the evolution of resistance.

Here we report the initial findings from a recent in vivo study into the ability of both direct plasma treatment and PAHT to decontaminate infected wounds, using a rodent model. Cytotoxicity of both treatment strategies towards human keratinocytes is also reported, alongside the development of a triggered-release antimicrobial hydrogel system. Such a system is reliant upon the generation of plasma-associated reactive species in order to facilitate drug delivery via covalent bond cleavage.

This work was supported by EPSRC funded project no. EP/P003939/1 (Smart Wound Plasma).

References

Potential applications of non-thermal atmospheric pressure plasma for corneal disorders

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Direct application of plasma ionized gases to living tissue offers a) antibacterial and anti-fungal effects b) promotion of wound healing c) causes targeted cancer cell destruction d) accelerates blood coagulation e) leads to collagen cross-linking. Literature is sparse on the use non-thermal atmospheric pressure (NTAP) plasma on cornea. Effects of non-thermal atmospheric pressure plasma (NTAP) application on human cornea are yet to be reported in literature.

Use of NTAP plasma needs to be investigated in following potential corneal indications after safety studies in human cornea

I. Refractory corneal infection like fungal corneal infection: NTAP plasma is found to be effective against 1) various bacteria. 2) Fungi 3) parasites and 4) biofilms have been found to be successfully removed. NTAP plasma can eradicate corneal infection and result in early resolution of infection. In addition to anti-microbial activity, wound healing properties and inhibition of enzymes (fungal and host) causing collagen degradation in cornea can reduce corneal morbidity and improve visual prognosis in corneal infection. This is potentially a new method of treating difficult corneal infections.

2. Corneal collagen cross linking: Corneal cross-linking (CXL) represents the physiological principal of tissue biomechanical alteration. Cross-linking of cornea results in stiffening and strengthening of corneal tissue. Currently for corneal collagen cross-linking, Riboflavin is the standard photoinducer, as its alkylisoalloxazine structure allows for absorption over a wide range of the light spectrum, including an observation peak in UV-A range. Adequate absorption of Riboflavin is required for effective crosslinking. Being a large molecule with a molecular weight of 376 Gms/per mol, corneal epithelial tight junctions limit the penetration of Riboflavin into the corneal stroma. Breaching of epithelium is required and epithelial debridement is required in corneal cross-linking. UV light is the second necessity component for cross-linking. The absorption peak of Riboflavin at 370 nm is ideal for effective crosslinking and protection of other ocular structures. The original standard Dresden protocol, which was found to provide maximum efficacy of tissue stiffening using 3 mW/cm² of energy for 30 minutes corresponds to a total energy dose (fluence) of 5.4J/cm². Indications for collagen cross-linking include progressive keratoconus, pellucid marginal comeal degeneration, post LASIK comeal ectasia, post-RK ectasia, in-combination with PRK and LASIK, corneal infections and melting because of corneal burns and corneal oedema. Complications of current CXL procedure include infection (bacteria, multiple organisms, acanthamoeba and herpetic keratitis), corneal stromal haze, sterile infiltrates, corneal thinning and endothelial toxicity (when the corneal thickness is less than 400microns, the endothelial UVA dose crossed the cytotoxic threshold value). Corneal collagen cross linking using NTAP plasma can be as simpler and safer alternative for currently practised method.

3. NTAP plasma can be of use in treatment of persistent corneal epithelial defects like post — infection, neurotrophic cornea, partial stem cell deficiency, post corneal transplant as NTAP plasma is found to release growth factors in low dose application.

4. NTAP plasma can offer symptomatic relief and enhance healing in various ocular surface and inflammatory corneal thinning disorders because of its anti-inflammatory and enhanced wound healing properties. These include extreme dry eyes, ocular rosacea, shield ulcers of severe ocular allergy, corneal involvement in various collagen vascular disorders and early Mooren's ulcer.

5. Since NTAP plasma is found to cause targeted cell destruction in tumours, NTAP can be of use in ocular surface tumours alone in early lesions and in combination with existing modalities (surgery, cryotherapy and Mitomycin c) in extensive lesions.
Research on Active Species Production Mechanism of an Atmospheric He-Water Plasma Jet and its Application on Porcine Cancellous Bone Surface Treatment

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Abstract: The active species (OH, O, H₂O₂, etc.) in plasma play important role in bacterial killing and wound healing. Low gas temperature of plasma is another requirement while treating heat labile tissue. A DBD structured He/H₂O plasma jet can effectively produce OH and H₂O₂ with low gas temperature. In this paper, spatiotemporal resolved optical emission lines in plasma jet are measured, gas temperature, vibrational temperature, electron density and electron excitation temperature are deduced from these lines. The influence of plasma working mode and water vapor concentration on active species production and their fluxes arriving on the porcine cancellous bone substrate are also investigated. It is demonstrated that the hydrophilicity of the bone surface is improved after plasma treatment.

Keywords: He/H₂O plasma jet, spatiotemporal resolved, porcine cancellous bone, hydrophilicity

Acknowledgments

This work was supported by Training Program for Excellent Young Teachers in Guangdong Province Higher Education Institutions (No.YQ2015123)
The biocompatibility of osteosarcoma cell on porous Al$_2$TiO$_5$-TiO$_2$ plasma electrolytic oxidation coating

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In this study, we successfully prepared a porous Al$_2$TiO$_5$-TiO$_2$ coating on pure titanium via plasma electrolytic oxidation (PEO) in aluminate-phosphate electrolytes [1]. The Al$_2$TiO$_5$-TiO$_2$ PEO coating exhibited better mechanical properties and higher corrosion resistance. The proliferation and viability of human osteosarcoma (MG-63) cells cultured on Al$_2$TiO$_5$-TiO$_2$ PEO coatings were investigated and monitored by spectrophotometric measurement of mitochondrial dehydrogenase activity. The relative cell viability was calculated by comparing its optical density at 490 nm with that of the untreated control cells. As compared to pure titanium, the adhesion and proliferation of MG-63 cells were be improved on porous Al$_2$TiO$_5$-TiO$_2$ PEO coating.

References

Oxygen- and atmospheric pressure He/Ar plasma-enhanced Ag dissolution of Ag-containing thin films and the subsequent anti-bacteria properties

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AgxO, TaN-Ag and TaOxNy-Ag thin films were deposited by reactive co-sputtering with various oxygen flow rates. After deposition and rapid-thermal-annealing at 400 oC for 4 min., the films' structural and mechanical properties were examined. Then, the samples were tested for their antibacterial behaviors against Escherichia coli. It was first found that the dissolution of Ag ion was varied depending on oxygen contents. This happened on both AgxO and TaOxNy-Ag. The Ag ion concentration would reach a maximum value with the increase of oxygen contents, then level off. The antibacterial efficiency of TaOxNy-Ag films against Escherichia coli could be much improved, comparing with that of TaN-Ag films, i.e. the higher oxygen content, the better antibacterial efficiency. Following this results, an atmospheric pressure plasma jet was used to enhance the dissolution rate of Ag on TaN-Ag nanocomposite thin films. It was found the antibacterial efficiency of TaN-Ag can be much improved. The reasons for this were found to be due to the generation of oxidative radicals in buffer solution. These results were proved with ICP-OES by measuring the solubility of Ag ions, as well as with chemical probes.
Biocompatibility and biocorrosion of biodegradable AZ31 magnesium alloys treated by plasma electrolytic oxidation

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The AZ31 magnesium alloy has been considered as a potential biodegradable implant material for orthopaedic application [1]. The plasma electrolytic oxidation (PEO) treatment is an effective method to improve the corrosion resistance of biodegradable Mg alloys [2,3]. In this work, PEO electrolytes containing different concentrations of sodium aluminate, sodium silicate, sodium phosphate and potassium hydroxide were used to grow different oxide layers on AZ31 alloys. The optical emission spectroscopy was also employed to study the characteristics of generated plasma. Effects of different electrolytes on the microstructure, biocorrosion resistance and biocompatibility of PEO treated AZ31 alloys were discussed in this study.

References

Haemo-compatibility of pyrolytic graphite coated with carbon nanowalls by plasma-enhanced chemical vapor deposition

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Carbon nanowalls were deposited onto pyrolytic graphite which is a commonly used material for synthesizing artificial heart valves. The deposition was performed in a low-pressure plasma reactor which allows for high quality vertically oriented carbon nanowalls by taking advantage of the PECVD method as described to details in [1]. The samples were then incubated with human blood in order to monitor hemostatic response. Blood platelets soon activated on the surface of plain pyrolytic graphite whereas the activation was almost negligible on the surface of pyrolytic graphite coated with carbon nanowalls as shown in Figure 1. The concentration of activated blood platelets on the surface of samples coated with nanowalls was almost three orders of magnitude lower than on uncoated substrates, what was explained by a minimal contact area between platelets and the surface of the coated heart valve. The technique represents an interesting alternative to classical technologies for suppressing of haemostatic response.

Fig. 1. Plain pyrolytic graphite (left) and pyrolytic graphite coated with carbon nanowalls (right) after incubation with human blood.

This work was supported by National Grant no. P2-0082.

References

Study of Modified Microtube Array Membrane as Cells Carrier in Encapsulated Cell Therapy

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Cell microencapsulation technology permits the bidirectional diffusion of molecules such as the influx of nutrients and growth factors. The membrane prevents immune cells and antibodies from causing damage the encapsulated cells. Microtube array membrane (MTAM) was a good cells carrier. After modified by plasma treatment and grafting 𝛾-PGA, MTAM was analyzed by cell proliferation assay with MTS, and cells fluorescent staining to observe the growing situation of MG63 inside encapsulated. In conclusion, grafting 𝛾-PGA on MTAM could promote biomedical characteristic effectively to let MTAM has the potential to be the encapsulated cell therapy carriers.

References

Mechanical, antibacterial, and biocompatible properties of F- and Ag- containing TaC$_x$Ny thin films

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TaN thin film coatings are known to have good mechanical properties, impact toughness, as well as good biocompatibility. However, the friction coefficient of these films is sometimes too high, or the hemocompatibility is poor. The purpose of this study was to reduce the friction coefficient and improve hemocompatibility as well as antibacteria behaviors of TaN coatings by introducing CFx and Ag into the nitride coatings. CFx–doped TaN films were deposited on silicon and tool steel substrates by reactive magnetron sputtering. During the deposition process, C$_2$F$_6$ gas with various flow rates was first added. After deposition, these films were then characterized using XRD, XPS, FTIR, FESEM, as well as tribometry. The tribo-tests were carried out with and without argon flow. Surface energies of the films were also analyzed with contact angle measurement system. In the second stage, Ag target was added for doping purpose. According to structural analysis, TaN phase would transform to TaC$_x$Ny with the increase of the fluoride gas flow rate, which would cause the decrease of friction coefficient and surface energy. This would in turn improve the hemocompatibility. With the additional doping of Ag, the prepared films showed enhanced antibacterial behavior with lower friction coefficient.
Biocompatibility and antibacterial behaviors of TaON(porous)/TaN/TaN-Ag/Ta multi-layered thin films

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In this study, a triple-layered thin film structure was designed and fabricated in order to realize porous and tunable TaOxNy thin films with enhanced biocompatibility and antibacterial behavior. In the design of film structure, the top layer was made of porous and tunable TaOxNy. The porous structure was obtained from TaOxNy-Cu (>50 at.\%) thin films deposited by reactive sputtering. After the film was annealed by using RTA (1st annealing), the Cu phase was etched away to form TaOxNy network structure. The bottom layer was TaN-Ag (11 at.\%) which is used as a Ag source layer. It also provided toughness and hardness. A thin TaN film was inserted between porous TaOxNy layer and solid TaN-Ag layer, and used as Ag diffusion control layer. The function of this layer was to withstand the 1st annealing, then, during the 2nd annealing, to let certain amount of Ag diffusive to the porous TaOxNy layer, and formed Ag nanoparticles. The films fabricated based on this design were studied systematically on their mechanical properties, Ag particle formation, as well as pore size and morphology. Finally, antibacterial property and biocompatibility of these films were studied in terms of O/N ratio, dealloying process, and Ag diffusion control. The relationships among O/N ratio, Ag nanoparticle formation, porosity, and bio-reactions will be discussed and reported systematically.
Effect of Cold Atmospheric Plasma in the Surface Modification of Human Dentin and Enamel

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Nowadays, the use of cold atmospheric plasma (CAP) in the medical field has gained great attention, and the odontological field has been no exception. For example, several methods, sources and processes have been designed for applying plasma to different compounds to carry out treatments for dental pieces, as well treating materials commonly used in this field [1].

Today most dental restoration relies on adhesion. Good adhesion is critical for the effectiveness of the interphase; for this reason adhesive material must spread across the entire surface ensuring optimal wettability, which is desired in these procedures [2]. Most common techniques involve the use of phosphoric acid to clean, increase roughness and improve wettability on dentin and enamel, with the disadvantage that it could affect the longevity of the bonding interphase between the tooth and the adhesive system in the long term [3]. In contrast, CAP offers a potential treatment as a substitute or catalyst for conventional acid treatment, conducting to surface modification without affecting bulk properties, and making contact surfaces super hydrophilic, achieving compatibility between the different adherents, due to reactive species capable of etching [4-5].

The aim of this study relapses in comparing the effect of CAP in the surface modification of human dentin and enamel, by contrasting physical and chemical characteristics between control samples and three different treatment samples: plasma, phosphoric acid and plasma after phosphoric acid treatment. The prepared surfaces were treated for 10, 20 and 30 s by UHP argon CAP at 50 W, generated by an AC plasma source at 23.05 kHz, known as DIBA Plasma Jet. Profilometry, goniometry (contact angle measurement) and scanning electrode microscope (SEM) were employed to characterize the surfaces, in addition to chemical composition analysis using Raman spectroscopy and Energy-dispersive X-ray spectroscopy (EDS).

References

Polyesters have been widely employed as substrates for the covalent immobilization of bioactive compounds, mainly due to their excellent biocompatibility and bulk properties; however, many of them do not present significant amounts of functional groups and, often, a surface treatment is needed prior to the conjugation. This work reports on the use of CAP for the introduction of carboxyl groups on polybutylene terephthalate (PBT) fibrous membranes to enable the bioconjugation of anti-CD10 antibody, which can selectively interact with the surface marker CD10+ expressed by Mesenchymal Stromal Cells (MSCs); MSCs captured in this way can later be detached and employed in tissue engineering applications. The suitability of the produced membranes for selectively capturing mesenchymal stromal/stem cells (MSC) from lipoaspirates has been investigated. PBT melt-blown membranes were subjected to CAP functionalization performed with an air Dielectric Barrier Discharge driven by a function generator producing square voltage signals with microsecond rise time; peak voltage, frequency and treatment time were fixed at 13.5 kV, 500 Hz and 60 s, respectively. Carboxyl groups introduced by plasma were activated with 1-ethyl-3 (3-dimetilaminopropil) carbodiimide/ n-hydroxysuccinimide and then a 1,4 diaminobutane (DAB) linker was covalently bonded to the mats; the fluorescent anti-CD10 antibody was finally grafted on the functionalized membranes. The characterization of the membranes was carried out by water contact angle (WCA) measurements, Fourier Transform Infrared spectroscopy (FTIR) and Scanning electron microscopy (SEM); a semi-quantitative determination of the antibody conjugation was performed by fluorescence microscopy. The potential of anti-CD10 functionalized membranes to selectively capture MSCs was investigated by incubating the membranes with MSCs at 37°C using an orbital shaker. WCA measurements revealed a significant increase of wettability after plasma exposure, due to the formation of polar groups on the membranes’ surface. SEM analysis underlined that plasma does not induce any morphological damage. FTIR analysis confirmed the presence of the diaminic linker; furthermore, fluorescence microscopy revealed a homogenous distribution of the antibody. The biological assay revealed that anti-CD10 conjugated membranes doubled the MSCs capture with respect to the unconjugated membranes and did not result in any morphological alteration of the MSCs after their detachment.
Cold atmospheric pressure plasma for the crosslinking of drug-loaded gelatin films for buccal drug delivery

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Gelatin is a water soluble, biocompatible polypeptide employed in a variety of biomedical applications. Beyond traditional methods, the use of cold atmospheric plasma (CAP) to crosslink gelatin in the solid state has been recently reported [1]. In the present work, this approach has been modified to yield crosslinking of gelatin films for buccal drug delivery. Econazole nitrate was selected as model drug. The comparison between the effects of the CAP approach and the ones induced by the conventional route employing genipin is presented.

The plasma-assisted crosslinking of gelatin films containing econazole (GE) was performed with a Dielectric Barrier Discharge plasma source driven by a function generator producing square voltage signals with microsecond rise time; peak voltage and frequency of 15 kV and 500 Hz, respectively, were used for the process [2]. Different treatment times were applied. Genipin crosslinked gelatin films were produced by adding genipin solution of suitable concentrations to the suspension of drug-containing gelatin [2].

X-ray diffraction highlighted that neither genipin addition nor plasma treatment induce structural changes in econazole. Differently from genipin crosslinked films, Fourier transform infrared spectrum of 5 min plasma treated GE displayed an increase of intensity of the bands corresponding to Amide III groups of gelatin, suggesting an increase of hydrogen bonds. Mucoadhesive test, a measure of the ability of the crosslinked films to adhere onto the buccal mucosa, showed that plasma-treated films had an adhesion strength significantly greater than genipin crosslinked samples. This behaviour might be associated to the formation of polar and hydrophilic groups on the films’ surface subjected to the plasma treatment. Regarding the capability of the films to inhibit Candida albicans growth in vitro, clear growth inhibition areas were observed around GE subjected to plasma treatment and genipin crosslinking; as expected, no growth inhibition areas were detected around the unloaded films subjected to the crosslinking. The results demonstrated that the exposure to CAP is a rapid and effective method to stabilize and significantly improve adhesiveness of econazole-loaded gelatin films, making them suitable for buccal drug delivery. Furthermore, the plasma exposure does not affect the efficacy of econazole in inhibiting the growth of Candida albicans.

Reference

Cold atmospheric pressure plasma jet assisted deposition of phosphate rich functional coatings on the surface of 3D polycaprolactone scaffolds.

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Abstract

Development of polymeric biodegradable scaffolds with distinct architecture is an emerging technique for regeneration or restoration of a living tissues which imitate micro as well as nano-scale extra cellular matric properties of the living tissues. However the crucial limitation of the biodegradable materials that used for tissue engineering applications are inherently hydrophobic which leads to hinder the adhesion and proliferation of cells. The major claim of the present work is to deposit phosphate rich functional coatings on the surface polycaprolactone (PCL) -3D scaffolds using cold atmospheric pressure plasma jet assisted polymerization. Subsequently the plasma polymerized PCL scaffolds are further allowed to the immobilization of calcium carbonate (CaCO\textsubscript{3}) nano particles. Various characterization such as X-ray photo electron spectroscopy (XPS), Fourier Transform Infra-red spectroscopy (FTIR), Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are used to evaluate the surface chemistry, functional and morphology of the plasma polymerized PCL scaffolds. The cytotoxicity, cell adhesion and proliferation of the surface coated PCL scaffolds are examined by \textit{in vitro} analysis using human osteoblast cells. Moreover the bioactivity of the PCL films are further examined by \textit{in vitro} SBF analysis.

Keywords: Cold Atmospheric Pressure Plasma Jet, Polymerization, Surface Analysis, Cytocompatibility

References

Bioactivity improvement of Ultra High Molecular Weight Poly Ethylene by Atmospheric Plasma treatments

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Ultra-high-molecular-weight polyethylene (UHMWPE) as a biocompatible polymer has a long successful clinical record in total joint replacement. However its poor bioactivity affects its potential biomedical uses. In this work, the effect of surface modification of UHMWPE, on deposition of hydroxyapatite (HA) from simulated body fluid (SBF), was investigated. Two types of atmospheric discharges were used for the surface treatment of UHMWPE, namely DBD (Dielectric Barrier Discharge) using a He and O₂ mixture and a rotatory arc blown APPJ (Atmospheric Pressure Plasma Jet) in air. FTIR analysis determined more functional groups on the surface when UHMWPE substrates were DBD treated as compared to APPJ treated samples. The consequence of this was that the former lead to a more hydrophilic surface and therefore more nucleation sites available for in vitro apatite deposition. In addition, the ageing of the surface properties were followed by measuring the WCA (Water Contact Angle), which showed an average hydrophobic recovery of the APPJ treated samples which was higher compared to DBD treated ones, therefore more stable surfaces were obtained by DBD treatments. XRD analyses on the HA-covered samples showed the crystalline structure of hexagonal, corresponding to HA. The TF-XRD analysis of the DBD-treated sample showed no change in the crystallinity of the UHMWPE, while the APPJ in air gave rise to surface amorphization due to the higher temperature of the APPJ. The results show faster, thicker and more homogeneous apatite depositions on DBD treated films. These results suggest that the surface treatment of UHMWPE by the DBD configuration, leads to improved bioactivity of the polymer film. Fibroblast cells seeded onto the complete range of investigated samples and the effects of plasma treatment on cell adhesion, viability and morphology were systematically studied. Results showed that cells spread more readily on the DBD-treated substrates as compared to the APPJ treated ones.

![Fig. 1. Hydroxyapatite layer deposition on non-treated (a), APPJ treated (b), DBD treated (c), UHMWPE film after 7 days immersion in SBF](image-url)
Capillary He and He-O₂ plasma jet simulation and experimental data

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Atmospheric pressure plasma jets are promising for applications in the area of material science and biomedicine[1]–[3]. The advantage of the APPJ compared to other atmospheric pressure plasma devices is their ability to deliver in remote locations a wide range of reactive species, charge species, high electric fields and UV photons. Among APPJ devices, helium plasma jets show very encouraging results for biomedical applications where it was observed that a small amount of oxygen in the helium gas increases its effectiveness against cancer cell [4]. Capillaries are of particular interest because they can deliver the plasma jet to previously inaccessible anatomical structures. In this work, the effect of oxygen admixtures on the evolution and interaction of a capillary helium plasma jet device with a dielectric surface is investigated. For the experiments, 4.0 kV, 50 μs duration, 10 kHz pulses are used to excite the plasma discharge through a 20 cm long capillary soda lime glass tube with internal diameter of 0.9 mm. In order to capture the dynamic behaviour of the plasma jet, an Intensified Charged Coupled Device (ICCD) is used. For the simulation model, the gas mixing of the helium with the ambient air is treated through a gas dynamic model [5] that feeds its output to the plasma fluid model [6]. The simulations focus on the effects of the presence of the dielectric target and that of the oxygen admixtures (500ppm–2000ppm) on the plasma bullet shape and speed, the induced electric field, the secondary emission flux of electrons and the dynamic behavior of the chemical reactions (separating also the Penning reaction contributions) responsible for the plasma jet propagation. The numerical and experimental results have good agreement and show that the evolution of the helium plasma jet is highly affected by the introduction of oxygen admixtures.

This project has received funding by the EU Horizon 2020 (MSCA-IF-2015) program under grant agreement 703497.

References

Pattern formations and self-organization of plasmas are phenomena which have been reported for various applications in which plasmas interact with media. These include material synthesis, electric propulsion, and medicine. Patterns vary by plasmas found in different discharge processes such as vacuum or high-pressure arc, low- and high-pressure glow, and streamer discharges. The existing breadth of research in this area shows that pattern formations depend on experimental conditions such as current, pressure, plasma species composition, properties and topology of material interacting with the plasma.

Our current work investigates models that describe interactions of cold atmospheric plasma (CAP) with biological media which occurs when a CAP jet impinges onto the surface of interest. The model solves a coupled system of transient Navier-Stokes and scalar transport equations accounting for energy, reaction, generation and supply for each species of interest on a parallel block-structured adaptive mesh refinement (AMR) grid. Besides identifying species of interest such as charged particles and reactive species, our research aims to understand the principal drivers of non-equilibrium which lead to pattern generation. Additionally, our model examines induced surface electric potential and electric field in order to characterize the interaction of the localized charges with the discharge column.

This work is supported by The National Science Foundation.

References

Effect of Positive Dust on Non-linear Properties of Electron-acoustic Waves

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The nonlinear propagation of the dust-electron-acoustic waves in a dusty plasma consisting of cold and hot electrons, stationary and streaming ions and charge fluctuating stationary dust has been investigated by employing the reduction perturbation method. It has been shown that the dust charge fluctuation is a source of dissipation and is responsible for the formation of the dust-electron-acoustic shock waves in such a dusty plasma. The basic features of such dust-electron-acoustic shock waves have been identified. It has been proposed to design a new laboratory experiment which will be able to identify the basic features of the DA shock waves predicted in this theoretical investigation.
Cold atmospheric plasma (CAP) applications in the biomedical field include bacterial disinfection, wound healing, and cancer therapy. Designing a plasma device for these applications mainly involve species production and controlling the concentration of the species. Numerical modeling of these plasma sources provides the designer with a means to understand the effect of input parameters such as voltage, frequency and flow rates on the production of plasma species. Furthermore, numerical modeling enables the researchers with a better understanding of how a predetermined combination of reactive species and electric field could bring a desired effect in the tissue, for example, apoptosis[1].

In the current presentation, the models that are currently being developed in USim [2] software for simulating the atmospheric plasma sources and nanoparticle formation will be presented. Simulation of the arc discharge synthesis requires modeling of arc formation (source), wall/substrate heating, material evaporation, fluid expansion, species reactions, species transport and nanoparticle growth. USim has equation frameworks for modeling most of these physical processes. For example, the multi-fluid MHD equation framework within USim can be used to simulate the dynamics of multiple species plasmas subject to external magnetic fields. Besides the existing equation sets, a variety of new source terms can be simultaneously computed in the input file and added to the right-hand side of the equations using the built-in differentiation operators. Applicability of these multi-fluid plasma models for simulating CAP sources will be demonstrated.


Dielectric barrier discharge plasma-based dry reforming: determining the discharge characteristics and optimum condition

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Methane dry reforming (MDR) with carbon dioxide has attracted significant consideration over the years [1,2]. A novel and promising technique for hydrocarbon reforming is plasma technology. Therefore, we performed an extensive experimental and simulation studies, aiming to identify the influence of the most important operating parameters of dielectric barrier discharge plasma on the conversion and energy efficiency. In this regard, the effect of the reactant molar ratio (CO₂/CH₄ ratio), input power, the gap distance and flow rate on the reactant conversion, product distribution, conversion and production abilities, as well as energy efficiency and SEI were reported. Meanwhile, pertaining to the effect of CO₂/CH₄ ratio, the correlation between the discharge characteristics (including the reduced electric field, breakdown voltage, dielectric and gas capacitances, electron density, electron energy distribution function and mean electron energy) and CO₂/CH₄ ratio were examined in order to provide deep insight into the activation and conversions of CH₄ and CO₂ besides the reaction pathways in plasma-assisted MDR. The appropriate operation parameters were determined considering the better energy efficiency and reaction performance, as well as lower carbon formation on the reactor wall. It was found that the reactant conversion and syngas yield are significantly enhanced by increasing the CO₂/CH₄ ratio, discharge power and the residence time. Syngas with an arbitrary H₂/CO ratio can be produced depending on the CO₂/CH₄ ratio in the feed. In the present study, CO₂/CH₄ ratio of 1, the flow rate of 50 ml/min, discharge gap of 1 mm, discharge power of 30 W and frequency of 10 kHz have been justified to present acceptable values of reactant conversion and yields of CO and H₂ as well as to maintain the H₂/CO ratio of close to unity (suitable for liquid fuel production) while maximizing the energy efficiency, conversion ability and production ability of H₂ and CO. To benchmark our model forecasts, we also present an overview of reported conversions and energy efficiencies in literature, to show the potential for enhancement in comparison with the state-of-the-art. Finally, the limitations as well as the advantages and future view of plasma technology were determined.

References:

Fig. 1 Erected experimental rig used in this study, b) Lissajous graph for derivation of the electrical characteristics for DBD in CO₂/CH₄ mixture
Modelling reactive species production and delivery in high aspect ratio tubes for endoscopic applications

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In order to develop minimally invasive, endoscopic plasma-based medical therapies, detailed understanding of reactive oxygen and nitrogen species (RONS) delivery via high aspect ratio tubes is required. Such sources may have lengths of 10’s of cm, while to remain minimally invasive their radii must be as small as possible (typically 1 mm or less). During transport over the length of such systems, the most highly reactive RONS tend to recombine into either; comparatively stable, but still reactive RONS or stable, unreactive molecules, changing the reactivity of the gas reaching the treatment site. As a result, quantification and optimization of the reactive species delivered in these systems is required in order to achieve optimal outcomes in applications.

In this work, endoscopic plasma delivery was studied using 0-D plasma-chemical kinetics simulations incorporating a He/H\textsubscript{2}O plasma chemistry [1] implemented in the GlobalKin code [2]. The base case simulation consisted of a 20 cm long tube with an inner diameter of 1 mm and a gas flow of 1 slm He containing 5000 ppm H\textsubscript{2}O. A constant power deposition was applied for the first 3 cm after which the power was switched off. The densities of the dominant neutral reactive species for the base case simulation are plotted as a function of distance from the gas inlet in Fig. 1.

It is found that the reactive species composition of the gas reaching the outlet of the endoscope is strongly influenced by the device radius and length, the gas flow rate, the location of power deposition (start or end of the tube) and whether the power deposition is continuous or pulsed. These changes result from the differing time constants for species production and loss in the gas phase, and the role of surface recombination [1]. Based on the results of these investigations we propose optimum device operation and design parameters for the delivery of different reactive species.

Acknowledgement: This work was part-funded by the Wellcome Trust [ref: 204829] through the Centre for Future Health (CFH) at the University of York. Funding through UK EPSRC Manufacturing Grant (EP/K018388/1) is also gratefully acknowledged

References

The role of non-thermal atmospheric pressure bio-compatible plasma in osteogenic differentiation of primary mesenchymal stem cells

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Non-thermal atmospheric pressure bio-compatible plasma (NBP) as a new medical tool has drawn great attention in recent years. Several studies has confirmed that NBP could induce stem cell to differentiate into osteoclasts, which leading a possible method for bone tissue engineering. However since most studies only focus on one kind of progenitor stem cell line, therefore it is hard to determine the optimal human tissue-derived stem cell source for NBP induced stem cell differentiation. In this study, human bone marrow-derived mesenchymal stem cell (hBMSCs) and human periodontal ligament-derived mesenchymal stem cells (hPDLSCs) was used to investigate the effect of NBP in stem cell osteogenic differentiation, and to find the suitable human tissue resource for NBP induced bone tissue engineering.

Acknowledgement

This work was supported by the Leading Foreign Research Institute Recruitment Program through the National Research Foundation of Korea (NRF- 2016K1A4A3914113) funded by the Ministry of Science, ICT, and Future Planning (MSIP) of the Korean Government for EH. Choi, I. Han. The Korea government, and the Basic Science Research Program through the NRF of Korea, funded by the Ministry of Education (NRF-2015R1C1A2A01054137) for I. Han.

References

The mechanism of plasma jet delivery of reactive species into tissues

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Abstract

There is significant worldwide optimism that non-thermal, atmospheric plasma will provide the basis for a range of “breakthrough” medical technologies. Recent significant attention has been directed towards understanding the intervention of the plasma-generated reactive species in cellular signaling processes important in disease. But it is equally important to understand how the plasma-generated reactive species are delivered into tissue, and particularly how the reactive species cross major barriers within tissue such as skin. In this study, we set out to understand the role of UV photolysis and molecular transport in the argon plasma jet delivery of reactive oxygen and nitrogen species (RONS) into tissue. A simple experimental set-up was utilised to assess the contribution of both processes by UV-Vis spectroscopy for quantitative measurements and with a potassium iodide-starch complex to visualize RONS delivery. Agarose was used as a surrogate for biological tissue. We observed that UV photolysis promotes the rapid delivery of the highly-reactive hydroxyl radical (OH•) into the uppermost surface regions of the tissue model. Whereas, less-reactive RONS are delivered deeper into the tissue model via slower molecular transport. The combined effect of UV photolysis and molecular transport (and possibly other contributions from plasma not investigated in this study such as electric fields) facilitate the delivery of RONS to millimeter depths within tissue. However, the RONS profile is complex. Plasma generated a heterogeneous mixture of RONS within the tissue model that varied in speciation and concentration as a function of tissue depth and time (even after completion of the plasma treatment). The methodology and knowledge provided in this study can potentially be utilised in the future to further improve our understanding of how the plasma physics controls RONS delivery into tissue. This should aid the future development of more effective and safer and plasma medical technologies that are designed to tailor the species and concentrations of RONS at specific depths within tissue.

This work was supported by a National Research Foundation of Korea (NRF) grant (NRF-2016K1A4A3914113, and NRF-2010-0027963), and in part by Kwangwoon University 2016, Korea. EJS, S-HH and RDS wish to thank the Australian Research Council for partially supporting this research through Discovery Project DP16010498 and UniSA through the Vice Chancellor Development Fund.
Helium atmospheric pressure plasma jet aiding chemotherapy for the treatment of breast cancer

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Atmospheric pressure plasma jets (APPJ) show great potential for biomedical applications [1]–[3]. One of the most interesting applications is cancer treatment where APPJ was used effectively in a number of different types of cancer and in some cases it even exhibited selectivity in treating the disease while leaving the healthy tissue intact [4]. One of the newest developments is plasma enhanced chemotherapy (PEC) where CAP is used synergistically with chemotherapy [5]. PEC promises not only to make chemotherapy more effective (achieving the same results with lower drug doses) but also to enable therapy on resistant cancers. Being able to reduce the drug dose and still offer an effective treatment can have significant implications on the patient by limiting the harmful side effects of chemotherapy. In addition, PEC can allow for the treatment of previously untreated cancer.

In this work two healthy cell lines (MCF-12F and MCF-10A) and two cancerous lines (MCF-7F and MDA-MB-231) are treated with various doses of Camptothecin and plasma jet (generated using a capillary glass tube with internal diameter of 4 mm) ignited by at 6.0 kV, 30 μs duration, 15 kHz pulses. The goal is to show how the combination of drugs and APPJ work in synergy (similar to the well-established method of electrochemotherapy) and not just in an additive way. In the synergetic way, it is expected that APPJ causes the cells to be more permeable and more absorptive of chemotherapy drugs. The cell viability is determined through various means including MTT assay and flow cytometry.

This project has received funding by the EU Horizon 2020 (MSCA-IF-2015) program under grant agreement 703497.

References
Diverse Effects of non-thermal Plasma (NTP) on pancreatic cancer cells and fibroblasts *in vivo*

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Pancreatic cancer remains to be one of the most malignant tumor entities in the western hemisphere with a five-year survival rate of about 5%. Inter alia, this is due to microscopically tumor tissue remaining in the patient and causing relapse of disease [1]. During the last decade, non-thermal plasma (NTP) developed to a highly promising candidate in modifying multimodal cancer treatment. The specific mix of reactive oxygen and nitrogen species induces apoptosis selectively in cancer cells [2]. Although underlying mechanism are not fully understood, yet, there is rising focus onto *in vivo* testing.

In this work, the anti-cancer efficacy of non-thermal plasma generated by kINPPen Med was investigated in a 3D *in vivo* tumor model. According to the 3R principles, developed and propagated by Russel and Burch, widely-used artificial nude mice experiments had been replaced by TUM-CAM models employing fertilized eggs [3]. Using this approach, NTP treatment significantly enhanced apoptosis in murine pancreatic cancer spheroids after short treatment duration already, whereas primary murine fibroblasts displayed higher resistance to NTP’s toxicity. After 60s of treatment, the longest chosen treatment duration in this setting, the selectivity was repealed and fibroblasts also significantly experienced apoptosis (35.7 ± 8.1 % (fibroblasts) vs. 38.4 ± 3.3 % (pancreatic cancer cells)). Shorter NTP performance did not affect fibroblasts’ cell viability.

In conclusion, encouraging *in vitro* results had been affirmed by TUM-CAM *in vivo* model: NTP induced apoptosis preferably in cancer cells in a time-dependent manner. Therefore, an additional, intraoperative NTP-treatment of tumor margins following surgical resection of the pancreas might be a promising approach to reduce relapse rate after radical surgery in patients suffering from pancreatic cancer. The NTP selectivity notwithstanding, non-malignant cells are not invulnerable at all. With respect to this finding, more focus on dose-effect relations of different tissues following NTP-treatment is desirable prior wide clinical implementation. Additionally, we like to recommend TUM-CAM *in vivo* model as a simple and highly reproducible alternative to nude mice testing.

References


The role of NO in the differentiation process of neural stem cells induced by He/O\textsubscript{2} plasma jet

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Neuronal cells are of great interest for medical treatment of neurodegenerative diseases and traumatic injuries of central nervous system (CNS), but efforts to produce these cells have been met with only modest success.\textsuperscript{[1]} With the hope of neural stem cells (NSCs) which can differentiate into mature cell types, it is very important to try new approaches to prepare these specific cells.\textsuperscript{[2]} In our previous study, we found atmospheric pressure He/O\textsubscript{2} plasma jet could effectively enhance neural stem cells predominantly into neuronal lineage and regulate \textasciitilde75\% NSCs to differentiate into neurons. And it generally found that nitric oxide (NO) generated by He/O\textsubscript{2} plasma jet is a key factor in the fate choice and differentiation of NSCs followed by axonal growth.\textsuperscript{[3]}

In this study, we looked insight to the mechanism of the plasma induced NSCs differentiation, especially on the role of NO. The differentiation results of NSCs by 60s He/O\textsubscript{2} plasma treatment is similar to the treatment by 100 \textmu M SNP (NO donor) and could be inhibited by 20 \textmu M Hgb treatment (NO-scavenger). It was also found that extracellular NO concentration increased with up-regulation of intracellular iNOS. The plasma treatment inhibited Notch1 expression, and enhanced the expression of Ngn2 and Ascl1, launching the differentiation of neuron and activated the downstream differentiation factor Neuro D, which finally enhance the C17.2-NSCs differentiation to neurons. All these results suggest NO generated by plasma jet plays the key role in the enhancement of neural stem cell differentiation.

This work was supported by Huazhong Scholar program (Grant no. 3004131118) funded by Huazhong University of Science and Technology and National Natural Science Foundation of China (Grant No. 31501099).

References

Combining cold atmospheric plasma with pulsed electrical fields for cancer treatment

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Pulsed electric fields (PEFs) are used in cancer treatment for electrochemotherapy (ECT) and are currently also investigated for their potential to induce apoptosis directly for sub-microsecond pulse durations [1]. Likewise, the anticancer capacity of cold atmospheric plasma (CAP) has been demonstrated [2, 3]. Accordingly, by combining both methods an even better treatment success might be delivered. This is also suggested by recent experiments combining ECT and CAP for the treatment of melanoma in mice [4]. However, mechanisms for combined effects on cells have not been understood, yet. Therefore, the aim of our study was the application of a combination of PEFs and CAP on cancer cells and to determine the underlying interacting mechanisms and biological responses.

Malignant suspension cells were tested to investigate additive or possible synergistic cytotoxic effects of combined treatments. For plasma treatments, the kINPen was chosen with an exposure time of 10 s for Jurkat cells, a human T lymphocyte cell line. PEF strengths of 0.5-1.3 kV/cm for 10 µs and of 0.25-0.85 kV/cm for 100 µs were tested to retrieve sublethal dosage regimens for the application of 8 consecutive pulses. In addition, also PEFs of 100 ns and field strengths of 5-20 kV/cm were investigated. Of particular interest was the sequence of the combination (first CAP then PEF or vice versa). A number of cellular parameters were investigated to study the modes of action. This included oxidation of cytosolic and membrane compartments, total thiol content, mitochondrial depolarization, caspase activation and phosphatidylserine exposure, metabolic activity, cell membrane permeabilization, cell growth and morphology, as well as protection by antioxidants. An increased oxidation at cytosolic and membrane compartments was found in Jurkat cells directly after 20 s of plasma treatment as well as an increased total thiol content after 4 h and 24 h.

References


Human Epithelial and Skin Cells Interactions on LTP Modified Vascular Graft Materials

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By the year 2030 around 40.5% of the US population is expected to be affected by some form of cardiovascular diseases (CVD) [1, 2]. This alarming trend highlights both the current and future needs for research to provide effective, cost-efficient solutions for the treatment of CVD. Prosthetic vascular grafts provide one such avenue of treatment by allowing the bypass of disease-affected arteries. Traditional grafting of native tissues is quickly being supplanted by grafting with modified synthetic tissues. Recent literature indicates the use of materials such as Dacron and ePTFE modified to exhibit mechanical and surface properties used to fabricate synthetic prosthetic vascular tissue grafts [3-4], but only for large-diameter applications and for small diameter grafts (<6 mm), intimal hyperplasia and thrombosis are limiting their patency. In our study we plan to improve the endothelialization of intimal surface of graft by modifying by low temperature plasma (LTP) to increase the cell attachment/viability and proliferation. The feed-gas used was air and the pressure in the plasma chamber was kept at 800 mTorr. The power used was 45W (HI setting) and the time per sample was 30 sec. The unit is a Harris Plasma Cleaner model PDC-001-HP. X-ray photoelectron spectroscopic analyses and contact angle wettability studies confirmed the introduction of oxygenated functionalities on the surface and enhanced hydrophilicity. This due to the improvement in oxygen content ~1 in the graft surface from LTP air plasma. Further cell culture studies followed by microscopic and metabolic assays indicated interesting data when used various cell types (HEp-2 epithelial cells, Fibroblast, and Keratinocytes). Cell growth was observed on scaffolds up to 15 days. Cells observed on the scaffold using bright-field microscope, immunofluorescence microscope and SEM showed more viability and normal functional morphologies cells in plasma treated scaffolds (especially HEP-2 cells) compared to untreated scaffolds MTT results also validated the improved number of metabolically active viable cells.

Acknowledgements: This work is supported by the NSF EPSCoR RII-Track-1 Cooperative Agreement OIA-1655280. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Reference:

Cold plasma-treated buffered saline solution as effective agent against pancreatic cancer cells

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Cold physical plasma has been suggested as a new anticancer tool. However, direct use of plasma is limited to visible tumors and in some clinical situations not feasible. This includes repetitive treatment of peritoneal metastases which commonly occur in gastrointestinal cancer and in pancreatic cancer in particular. In pancreatic cancer as well as in different gastrointestinal cancer entities Hyperthermic Intraperitoneal Intraoperative Chemotherapy (HIPEC) is used as therapeutic approach. Plasma treated solutions may combine their suspected systemic non-toxic characteristics with the anticancer effects of HIPEC. Previous work has provided evidence for an anti-cancer efficacy of plasma treated cell culture medium. The clinical relevance of this approach is low due to its complex formulation and lack of medical accreditation. Therefore, plasma treated phosphate-buffered saline (PBS) which closely resembles medically certified solutions was investigated for its cytotoxic effect on 2D monolayer murine pancreatic cancer cells in vitro. It significantly decreased cancer cell metabolisms and proliferation whereas plasma treated Dulbecco’s Modified Eagle Medium (DMEM) had no effect. Moreover, tumor cell growth attenuation was significantly higher when compared to syngeneic primary murine fibroblasts. Both results were confirmed in a human pancreatic cancer cell line. Finally, plasma treated PBS also decreased tumor sizes of pancreatic tumors in the HET-CAM model in a three-dimensional manner, and induction of apoptosis was found to be responsible for all anticancer effects identified. Altogether, plasma treated PBS inhibited cell growth in 2D and 3D models of cancer. These results may help facilitating the development of new plasma derived anticancer agent with clinical relevance in the future.

This work was supported by grants funded by the German Federal Ministry of Education and Research (BMBF) grant numbers 03Z22DN11.

References

The topical application of non-thermal plasma stimulates skin-rejuvenation by regulating β-catenin-mediated proliferation of keratinocytes

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For recent years, devices that generate non-thermal plasma (NTP) have been introduced into the field of dermatology. Since NTP has demonstrated strong anti-pathogenic activity with safety of use, NTP was first applied to sterilize the skin surface to aid in the healing of various kinds of skin diseases. However, the effect of NTP on skin regeneration has not yet been fully explored. In this study, the effect of NTP on the growth of keratinocytes was tested using the HaCaT human keratinocyte cell line and HRM2 hairless mice. Treatment with NTP allowed confluent keratinocytes to escape from G1 cell cycle arrest and moved to S and G2 phases. In particular, NTP treatment immediately dispersed E-cadherin-mediated cell-to-cell interactions, resulting in the translocation of β-catenin to the nucleus and leading to the enhanced transcription of target genes including c-MYC and cyclin D1. Moreover, repeated topical treatment of NTP on mice skin also stimulated epidermal expansion by activating β-catenin in the epidermal cells. The symptoms of cellular DNA damage nor abnormal keratinocyte differentiation were not detected after NTP treatment. Furthermore, NTP treated mice skin showed enhanced collagen density. Taken together, these results demonstrate that NTP can stimulates the skin rejuvenation by not only regulating the proliferation of keratinocytes, but also enhancing the expression of dermal structure related genes.

Fig.1 The NTP device used in this study (A), main results showing the effects of NTP on mice skin (B) and the proposed model for NTP-mediated skin rejuvenation (C).

This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) & funded by the Korean government (MSIT) (NRF-2016M3A9C6918283).
Cancer Cell Eradication and Suppression of Their Relapse induced by Cold Atmospheric Plasma (CAP) Activated Solutions Through Metabolism in Chronic Myeloid Leukemia

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The currently treatment for cancer, such as surgery, chemotherapy, radiation therapy, targeted therapy or Immunotherapy, are resulting in cancer patients living longer. However, many patients still suffer a fear of risk that the disease may returning at a later stage. This recurrence or relapse has considered as a stumbling blocks for improving survival and need to be plunge into more efforts.

Here, we report that the injection of cold atmospheric plasma (CAP) activated solutions induces simultaneous inhibition of multiple metabolic enzymes and cellular antioxidants, leading a dramatic cell death in human and murine chronic myeloid leukemia cell (AR230 / BCR-ABL1 expressing cell lines), especially Imatinib-resistant cells (AR230R / BCR-ABL1 T315I), as shown in Fig 1.

Moreover, multiple injections with scheduled interval may generate a continuity eradication of AR230R cells (Fig 2), along with inhibition of multiple critical enzymes, which cause a decreased metabolic activity. These fabulous anti-cancer effects are rarely seen in other single-site therapies, and may benefit from chemical diversity of reactive oxygen and nitrogen species (RONS) of CAP.

Our results suggest that CAP may offer a novel strategy to restricting cancer plasticity and overcoming cancer relapse.
Plasma treatment against immunosuppression in melanoma

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In metastatic, therapy-resistant melanoma, a plethora of immunosuppressive mechanisms is at work. An immuno-tolerant microenvironment as well as mechanisms counteracting effective immune responses can be initiated by tumor cells themselves. They can e.g. express inhibitory surface molecules or release immunosuppressive factors, blunting antitumor immune responses [1]. On the other hand, suppressive immune cells upregulate similar receptors on their surface as well [2]. In the tumor microenvironment, they contribute to this immunosuppressive network along with release of anti-inflammatory mediators such as IL10 and TGFβ. This aids in tumor progression, which is why high expression of immunedampening receptors are associated with poor prognosis in patients [3]. Reactive species are known mediators of myeloid immune cell differentiation and the regulation of the tumor environment [4, 5]. Cold physical plasmas expel reactive species of many kinds, which can be utilized for therapeutic purposes. Therefore, we analyzed the impact of an atmospheric pressure plasma jet (kINPen) on the immunosuppressive potential of melanoma as well as immunosuppressive cells. Utilizing multicolor flow cytometry, we screened plasma-treated cells for changes in key immunomodulatory surface markers and immunosuppressive secretion products. Using real-time PCR, a number of immunomodulatory signaling pathways was investigated. In vitro co-culture assays with melanoma and immune cells verified the potential of plasma in modulating local immunosuppression. Moreover, we analyzed culture supernatants of patient-derived skin cancer samples that had received ex vivo treatment with cold physical plasma. Distinct immunomodulatory cytokine profiles were seen in plasma-treated clinical samples compared to the controls (e.g. IL1β, GM-CSF). In addition, multicolor fluorescence imaging of patient-derived melanoma tissue sections validated the presence of immune cells as well as the impact of plasma-derived oxidants on skin cancer cells. This study illustrates the importance and potential of utilizing plasmas to manipulate the redox environment for tumor immune control. In future, this may become for combination melanoma therapy to overcome local immuno-tolerance.

References

Accelerated blood coagulation through the stimulation with a plasma jet.
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Nowadays, the possibility to obtain a quick blood coagulation and wound healing in certain patients’ cohorts, such as those following anti-coagulant therapies, is still challenging, and it arouses a great interest in clinical and surgical practice. Hence, the aim of our study is to test the effect of a cold atmospheric pressure plasma source based on the Dielectric Barrier Discharge (DBD) scheme, called Plasma Coagulation Controller (PCC), on accelerated blood coagulation using helium as working gas. First, the effect of PCC on whole blood withdrawn from patients following anticoagulant therapies has been tested. In particular, 200ul of blood have been transferred to a 24-wells plate, and treated at distinct operational parameters with the plasma jet for different time points (15'', 30'', 1’). The results showed an extensive increase in blood coagulation in treated samples compared to the controls exposed to air. In parallel, in order to investigate specifically which particles were involved in this process, the same settings of experiments has been performed on glass slides, and processed with Ematoxilin/Eosin Staining. The histological analysis showed both platelets aggregation and fibrin polymerization. Blood coagulation has also been tested by Western Blot for specific markers (i.e. thrombin, pro-thrombin, fibrinogen).

Since it is known that plasma treatment induces the production of reactive species of Oxigen and Nitrogen (ROS and RONS), the expression levels of enzymes involved in catabolism of those molecules (i.e. SOD and iNOS) have been analyzed. The results showed an increase in their expression after treatment.

Also, in order to evaluate the apoptosis and necrosis levels, FACS analysis with 7AAD and Annexin V antibodies has been performed on 293 cells following treatment with PCC. Data displayed that after 1’ treatment the percentage of dead cells was comparable with the controls (about 4%), suggesting that the PCC does not make a strong damage to the living tissue.

Finally, in vivo experiments have been performed in male Wistar rats. Briefly, deep cuts in both hindlimbs have been made, causing an iatrogenic hemorrhage. Right after, one side has been treated with the plasma jet, while the other one has been exposed to air as a control (CTRL). It was observed a significant difference in the bleeding time between the two sides; indeed, 2’ treatment with the PCC resulted sufficient to induce blood coagulation on the left side, while the right side (CTRL) was still bleeding.

Taken together, these results demonstrated that the use of a PCC is able to reduce the coagulation time both in vitro and in vivo. Preliminary data suggest that reactive species production could play a crucial role in these processes. Further studies are now ongoing to determine the exact molecular mechanisms of the plasma-induced blood coagulation.
Plasma technology for blood clot formation

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We have developed a hemostatic equipment using atmospheric pressure plasma. Current hemostatic equipment is electronic device such as high frequent electric coagulator. Electronic device stops bleeding with thermal effect against the patients skin, while our equipment could promptly stops bleeding with formed clots without thermal damages [1].

Blood coagulation by LTP treatment involves Red Blood Cells (RBC) and plasma proteins such as albumin, immunoglobulins to form clot [2], and LTP treatment can coagulate each staff only [3] [4]. In our experiments, the input voltage onto the plasma generator and the gas flow rate can be monitored to control, and the value of the current flowing through the conductor A and be defined by measuring system connected to the toroidal coil (Rogowski coil). The formed clots in albumin and RBC solutions are analyzed by either histological, ultrastructural or protein biochemical methods.

Through a series of studies using LTP, we found that albumin aggregation and hemolysis of erythrocytes correlate with current flowing. It is noteworthy that there are thresholds for protein aggregation and hemolysis, and that the threshold for hemolysis is much higher than for aggregation of albumin and hemoglobin [2]. RBC clot formation doesn’t occur without exceeding a current threshold for hemolysis, because erythrocyte membranes boxed hemoglobin and the other proteins in red blood cells not to form aggregation by LTP treatment [2]. To assess whether threshold control is linked with hemostatic effect in LTP treatment, we compared the amount of hemolysis by using sample trays that have variety of conductivity under the condition that the input voltage value of the LTP equipment was constant. Results from a series of experiments demonstrated that the improved conductivity of the sample tray was more effective hemolysis. From the viewpoint of plasma physics and pathology, we consider that these results help to develop more effective hemostatic equipment by applying these results[5].

This work was supported by Grant-in-Aid for Scientific Research on Innovative Areas (MEXT/JSPS KAKENHI Grant Number 24108006), and MEXT/JSPS KAKENHI Grant Number 15K08413.

References
Adaptation of Cold Atmospheric Plasma Discharge Voltage and its Role in Cancer Therapy

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CAP has been reported as a selective treatment method with a higher affinity of inducing cell death in cancer cells while leaving normal cells unharmed [1]. However, the extent of its effectiveness varies significantly per cell type. An understanding of the immediate effect of CAP on cancer and normal cells may enable improvement of treatment outcomes. We demonstrated that instantaneous CAP response can be monitored in real-time with results interpreted as cell viability. This creates the possibility for developing an adaptive CAP platform which could enable real-time modification of plasma composition and minimize the predicted viability of cancer cells [1, 2].

References

Detection of Plasma Induced RONS in Agarose Gels by Atmospheric Pressure Plasma Jet

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The use of cold atmospheric pressure plasmas for biomedical applications has been increasingly popular during the last decade [1,2]. It is generally accepted that reactive oxygen and nitrogen species (RONS) play a key role in the remedial effects in the context of plasma-bio interactions [3]. To gain a better understanding of interaction of plasma with living tissues, it is essential to obtain quantitative data on the RONS produced by plasma at the interface with tissues, their penetration into the tissue and their effect on cell viability.

In this contribution, we will report on experiments characterizing the interaction between a helium-based cold atmospheric pressure plasma jet operating in an air environment and agarose gels. The agarose gel is used as a model for tissue. We have studied the species production and their penetration into the gel by adding colorimetric reagents to the gel including KI starch, Griess Reagent, Crystal Violet, Indigo Carmine and Titanium (IV) Sulfate. This approach allowed us to indirectly detect the distribution and penetration of RONS in the target agarose gels. Different reagents are sensitive to different reactive species and show highly different patterns and penetration depths and allow us to partly assess production of RONS in the tissue. We will show results that our approach can be adapted for detailed parametric studies that will contribute to a better understanding of the role of RONS in atmospheric plasma-induced reactions in tissue.

This work is partially supported by Department of Energy Early Career Research Award (DE-SC0016053).

References


Cancer Cell Killing via ROS Modulation

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In the interdisciplinary research field of plasma medicine, physical atmospheric cold plasma is used for therapeutic application. To this day, a high number of scientific findings were published displaying various profits for medicine-such as increased wound healing or plasma-supported cancer therapy.[1] It is well known, that different oxygen species are important mediators in central cellular processes.[2] A change in redox homeostasis leads to a high number of human diseases including cancer.[3] Regulating the redox balance within the cell, it might be a promising step to fight cancer. The purpose of this study was to determine the role of an antioxidant protein peroxiredoxin (Prdx) in oxidation regulated cell death.[4] In this study, different human melanoma cell lines were subjected to reactive oxygen and nitrogen species expelled by atmospheric pressure argon plasma jet kINPen. By using Prdx over-expression plasmids, we studied its ability to modulate the cellular redox balance and assed its role in metabolism and cell death. Cell death caused by oxidative processes by atmospheric cold plasma was studied via high-content analysis system, Western Blot and Elisa qualitatively and quantitatively. This study was commenced to understand how combination of altered ROS levels and Prdx enzyme expression affects tumor cell metabolism with respect to treating cancer by cold physical plasma.

This work was supported by the German Federal Ministry of Education and Research, grant number 03Z22DN11.

Study of the penetration of active species from non-thermal atmospheric pressure plasma jet thorough a skin model using a UV-VIS spectroscopy method

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Skin therapies using non-thermal atmospheric pressure plasma (NTAPP) have been extensively studied for medical as well as cosmetic purposes. In these applications, it is important to know how the active species generated from NTAPP diffuse into the skin. Oh et al. investigated those properties using artificial skin models such as homogeneous agarose gel [1]. Though they tried to add proteins or lipids in the gel skin model, it is somewhat different from the actual skin. In the case of actual skin, there are several distinctive layers including stratum corneum (SC), epidermis, and dermis. Among them, SC, the outermost shell layer, is the main barrier that protects us from hazardous external substances from the environment. Since it is very hydrophobic and electrically non-conductive, the diffusion of active species from NTAPP is significantly dependent on the configuration of the SC. Therefore, it is important to include the SC in the skin model for studying the NTAPP effect in the skin. In this study, penetration of the active species generated by NTAPP jet through a SC-containing skin model was analyzed in real time by UV - VIS spectroscopy method according to plasma processing time and distance. The amounts of H$_2$O$_2$, NO$_2^-$ and NO$_3^-$ were quantified via curve fitting, and the values were confirmed by chemical analyses. The effects of SC on the penetration depth and concentration of active species were discussed.

This research was supported by Leading Foreign Research Institute Recruitment Program through the National Research Foundation of Korea (NRF) funded by the Korea government (MSIP) (NRF-2016K1A4A3914113).

References

Vaccination approaches in plasma medicine

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Immunization or vaccination is necessary for eliciting a protective immune response against pathogens. Beside active vaccination by injecting pathogens or parts of it, passive immunization via specific antibodies (humoral immunity) or activated lymphocytes (cellular immunity) confers a protection as well. Interestingly, several redox related processes in phagocytosis and antigen presentation are required during an immune response [1, 2]. For example, proteins associated with antigen presentation are digested in the proteasome before binding to major histocompatibility complex (MHC) via redox-mediated processes. The origin of peptides are self or non-self-proteins and illustrates the health of a cell. Antigen modification including oxidation, hydrolysis of peptides, N-linked glycans, or exchanged amino acids complicate immune recognition [3]. Cancer cells present neoantigens [4] originating from genomic mutations, and these cells frequently lack MHC-I to escape immune invasion.

Since cold physical plasmas generate redox oxygen species (ROS) that are able to oxidize cysteine residues, it is possible to modify amino acids of peptides. Here we show the effect of cold physical plasma on peptides and their immunogenicity. Ovalbumin was treated with plasma to analyze the activity of immune cells via cell tracing.

Furthermore, we analyzed the effect of plasma on antigen presentation in melanoma cells to evaluate their immunogenicity. Antigens of plasma-treated B16F10 were characterized by mass spectrometry and plasma-treated cells were co-cultured with dendritic cells to assess their subsequent peptide repertoire.

This work was supported by National Grant no. 03Z22DN11

References

Investigation of the Isolated Ion Interaction with Biological Substrates and Liquids

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As a source of reactive radical species, charged species, photons and electric fields non-equilibrium cold atmospheric pressure Plasmas show an effective interaction with biological systems and are therefore investigated as a possible therapeutical source in plasma medicine. While the underlying processes and mechanisms of the interaction between cold atmospheric pressure plasmas and biological substrates are better understood, the isolated effect of the plasma components is, however, not known yet. It was shown that in many cases the treatment of a biological substrate is more efficient when it is in direct contact with plasma where several authors have proposed the important role of ions next to the electric fields [1, 2]. The ions are expected to have an enhanced effect due to their charge and their internal energy.

To study the isolated interaction of ions with biological substrates an experimental setup has been developed in which photo-ionized ions are drifting towards the substrate due to the applied voltage (the first version of the setup is shown in Fig. 1). The setup is filled with one atmosphere of helium and a small concentration of particles to be ionized. For the ion generation by photo-ionization the helium excimer emission (60-100nm) of a helium driven micro atmospheric pressure plasma (µAPPJ) is used. The performance of the setup is analyzed experimentally (VUV spectra, current measurements) and theoretically with a fluid model for the gas flow and ion convection/diffusion/drift transport. A comparison of the measured and simulated ion currents is in a very good agreement and helps to obtain optimal operation conditions. The results of the experiments with GAPDH-enzymes will be presented.

Fig. 1. Scheme of the setup for ion generation.

References

Effect of low-temperature plasma-derived oxidants in 3-dimensional tumor spheroids

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Cancer is one of the leading causes of mortality [1]. Radio- and chemotherapy are the most common treatments available when surgical resection is not possible. However, these methods have limited efficacy due to development of resistance or present side effects to healthy cells. Resistance is partially due to the tumor microenvironment, which provides a mechanical and functional shield against anticancer agents. In this context, low-temperature plasmas for cancer treatments have been suggested as a valuable tool in antitumor therapy that could overcome this resistance. Low-temperature plasmas have been explored in vitro and in vivo with considerable success against a variety of cancers [2,3]. However, the role of low-temperature plasmas in metastatic processes has not been determined. The aim of this study was to investigate the effect of plasma-derived oxidants on the architecture and metastatic processes of solid tumors. For this purpose, we used a 3-dimensional spheroid tumor model that mimics the growth kinetics, architecture and physiochemical gradients of solid tumors. The effect of plasma-derived oxidants on the spheroid architecture, cell viability and migration of cancer cells outside the spheroid was assessed. This study explores the application of low-temperature plasma treatments as potential cancer therapy.

References
Investigation of inactivation effects of oral cancer cells by plasma treatment

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1. Introduction  Recently, researches on medical application using plasma have been actively conducted. Especially, plasma cancer therapy is attracting attention as the alternative for surgical therapy, chemotherapy and radiation therapy[1]. Purpose of this study is to investigate inactivation effects of active oxygen species generated by torch-type DBD on oral cancer cells. Since most tumors of oral cancer appear epidermis, plasmas are able to irradiate the tumor directly. Furthermore, it is considered that this method can maximize the effect of short-lived active species generated by discharge.

2. Experimental procedure  Electrodes were installed inside and outside the ceramic tube with a length of 130 mm and an inner diameter of 4 mm, and a high voltage (10 kV) with high frequency (10 kHz) was applied between the electrodes to generate a dielectric barrier discharge in the tube. Cells are irradiated with active species generated by discharge in the ceramic tube by the gas flow. Pure oxygen gas is used as the plasma source gas and the gas flow rate is 0.6 L/m. In order to prevent cell inactivation due to drying by gas flow, the source gas is bubbled in pure water to give constant humidly. Periods of Plasma irradiation to cells are 30 sec. HSC3, human oral cancer cell line, is used as a model of cancer cell.

3. Results and discussion  MAPK signal transduction pathways is activated, in general, by stimulations such as ultraviolet rays, oxidation, radiation, growth factors. JNK, p38 and ERK are the MAPK protein located at the most downstream of the MAPK cascades. Phosphorylation of JNK and p38 proteins induce causes apoptosis and cell cycle arrest, and phosphorylation of ERK causes cell proliferation and division. Figure 1 shows the results of the western blotting of MAPK proteins in cells treated with the plasmas. Amounts of phosphorylated JNK (p-JNK) and phosphorylated p38 (p-p38) increases depending on the discharge voltage of the plasma. In contrast, the amount of phosphorylated ERK (p-ERK) is almost constant for each plasma condition. Therefore, it was revealed that the plasma treatment induces apoptosis and cell cycle arrest of oral cancer cells. Figure 2 shows the number of cells with caspase 3 activation is small by 5 hours from the plasma irradiation, and reaches almost 100% after 24 hours. This result implies that there is a kind of rate-controlling process such as gene expression in p53 or caspase pathway.

References
Cell Response to Pulsed Current Modeled after Atmospheric Pressure Plasma


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Plasma medicine is using the atmospheric pressure plasma, and delivery of active species at the molecular level is a current topic in this research field. Although, most research focuses on chemical reactions produced by plasma, the influence of electrical transmission processes into cell responses is not well known completely. In this experiment, we constructed a stimulation system using various power sources that could not only match the same voltage and current as plasma generator, but also minimize chemical species [1].

Figure 1 was the stimulation system, including a cell culture chamber and a salt bridge setup. The pulsed current transferred to ionic current via the Ag/AgCl electrodes and agar bridges in phosphate buffered saline (PBS). For cell stimulation test, we seeded $2 \times 10^5$ cells/well and incubated at 5 % CO$_2$ and 37 °C for 6 hours, used HT-1080 cell (JCRB, human sarcoma cell line). Cell viability and migration were investigated in this study. After the stimulation with pulsed current, preliminary results showed that cells were extended more longer and overlapped to each other, and the migration of cells increased as shown in figure 2. Obviously, the nanosecond pulsed current affected cell responses. More important findings will be presented in the poster.

This study was supported by JSPS KAKENHI Grant Number 16H02311, and the Collaborative Research Project of the Institute of Pulsed Power Science, Kumamoto University.

References
A Preliminary Study on Combined Radiotherapy and Cold Atmospheric Plasma for Localized Cancer Treatment

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Radiotherapy (RT) is often added to surgery to improve the local control of cancer. Despite the technological advances in RT, normal tissues surrounding the targeted volume receive an important dose, which translates into long-term irreversible treatment toxicities. RT induces the formation of reactive oxygen species (ROS) that damage nucleic acids and lead to cell death. Similarly, cold atmospheric plasmas placed in contact with liquids produce reactive oxygen and nitrogen species (RONS). When plasma is placed in direct contact with the liquid-covered cells, the RONS produced in the liquid medium diffuse into cells and interact with intracellular components. Some RONS produced in the liquids have a prolonged lifetime [1], thus opening the door to indirect plasma treatments, in which a previously plasma-activated liquid (PAL) is applied to the target area. In this work, we investigated the benefit of a localized application of various PALs in adjunct to RT, with the goal of safely reducing the RT dose and subsequent side effects.

We used a nanosecond pin-to-liquid discharges in open air [2] to produce PAL from water, phosphate-buffered saline or cell culture medium. Soft-tissue sarcoma cell lines were incubated in different concentrations of PALs. We assessed the cytotoxicity of PAL, either alone or in combination with RT, using flow cytometry and live-cell microscopy. The production of cytoplasmic and intranuclear RONS was measured using fluorescent probes, and cell death was quantified using propidium iodide.

The preliminary results suggest that a short (< 2h) exposure to different concentrations of PAL increases the intracellular RONS level by a factor ~25 in treated cells compared to untreated controls. PALs also induced 10-100% cell death following 24h exposure to PAL, depending on the type of PAL and concentration. The immediate goal of this project is to develop a strategy to combine 2 physical means of producing ROS to selectively kill cancer cells, which are known to be more sensitive to ROS than normal cells.

This work was supported by Natural Science and Engineering Research Council of Canada, the Gerald Hatch Faculty Fellowship, Fonds de la Recherche en Santé du Québec #34612 and the Montreal Cancer Institute.

Plasma Decontamination: Oxidative Damage of Fungal Cells Induced by Plasma without Impact on Antioxidants in Cereals and Nuts

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The low-temperature plasma (LTP) can effectively eliminate the various microorganisms. Its mode of action on microorganisms is based on the generation of biological active species, including ROS, UV radiation, and charged particles. These agents can penetrate into the cell which can lead to severe local damage such as oxidation of macromolecules [1].

Aspergillus flavus, a toxigenic filamentous fungus, is a common contaminant of economically important agricultural commodities including cereals and nuts [2]. There have been only a small number of ecological methods to eliminate fungal contamination in food.

In this paper, the effects of LTP on A. flavus inoculated on artificial (a cellophane membrane, a microscope slide) and natural (maize, wheat, barley, peanut, hazelnut) substrates was studied. The influence of LTP on antioxidants in cereals and nuts was also investigated. Plasma was generated by Diffuse Coplanar Surface Barrier Discharge (DCSBD) at atmospheric pressure in ambient air [3]. Plasma treatment led to significant reduction (CFU/g) of fungal cells on all tested samples in short time. Sample surface was observed by Scanning Electron Microscopy. Oxidative changes of macromolecules in fungal cells after plasma treatment was examined by Attenuated Total Reflectance – Fourier Transform Infrared Spectroscopy (ATR-FTIR). Fragmentation of fungal genomic DNA was visualized on an agarose gel. On the other hand, significant changes in the content of oxidative unstable compounds (polyphenols and flavonoids) in the natural sample were not recorded by spectrophotometric methods and ATR-FTIR after plasma treatment. However, DNA damage of seedlings was determined in dependence on treatment time by Comet assay.

Our results indicated the potential to use DCSBD plasma for decontamination of various types of commodities without alteration of quality parameters in short plasma treatment time.

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-16-0216

Rapid Analysis of DNA Damage in Plasma-Irradiated Cancer Cells

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Cold atmospheric pressure plasmas have been intensively studied due to growing interest in biological and medical applications. Especially the plasma has been considered as a promising tool for cancer therapy. For example, selective induction of apoptosis in cancer cells has been investigated. However, understanding the molecular mechanisms of the antitumor effect is not still enough. One of the proposed molecular mechanism is DNA damage-associated cell death. Therefore DNA is one of the most important biomolecular targets for investigating the effects of exposure to the plasma. We have been working on a rapid evaluation method for detecting DNA strand break formation induced by exposure to the plasma. It has been reported that DNA strand breaks are induced when DNA is exposed to the plasma. However, most of conventional methods to detect plasma-induced DNA damage require long processing time. To cope with this problem, we have proposed the use of a molecular beacon (MB) for rapid detection of DNA strand breaks induced by atmospheric pressure plasma irradiation [1]. MBs are oligonucleotides that adopt a stem-and-loop structure and carry a 5'-fluorescent moiety and a 3'-nonfluorescent quenching moiety. Scission of the molecular beacon by the plasma irradiation leads to separation of the fluorophore-quencher pair, resulting in an increase in fluorescence that directly correlates with the DNA strand breaks. The results showed that the increase in fluorescence intensity is proportional to the exposure time and the rate of fluorescence increase is proportional to the discharge power [1]. Furthermore, we reported that the plasma jet readily induced DNA strand breaks in synthetic models of tissue fluid, tissue and cells, surprisingly without any significant rupture of the phospholipid membrane [2]. In this study, the feasibility of MB-based methodology for detecting intracellular DNA damage was investigated. Here, a human lung cancer cell line A549 was used. The cells were transfected with MBs, cultured for 24 hours, washed with phosphate buffered saline (PBS), trypsinized, and finally suspended in PBS. A helium atmospheric pressure plasma jet was irradiated to the cell suspension. Immediately after the plasma irradiation, intracellular DNA damage was measured by flow cytometry. As a result, the intensity of DNA damage-associated fluorescence increased in A549 cells. In addition, to assess the DNA damage in plasma-irradiated cells, comet assay was performed as a conventional method. The result also indicated that DNA damage was induced by the He plasma jet irradiation. Furthermore, intracellular ROS production was confirmed by using general ROS-reactive fluorescent probes and flow cytometry. Our novel methodology may allow investigations of the effects of atmospheric pressure plasma on DNA damage-associated cell death in plasma treatments.

This work was partly supported by a Grant-in-Aid for Scientific Research (C) (26390096, 17K05095) from the Japan Society for the Promotion of Science (JSPS).

References

CAP has shown its promising application in many branches of medicine, including sterilization, wound healing, as well as cancer treatment. Understanding the interaction between the cold atmospheric plasma (CAP) and cells is a key challenge in plasma medicine. The CAP-originated reactive species including reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been regarded as the major factors for the interaction between CAP and cells. Thus, the cells have been just regarded as a passive role during CAP treatment.

In this study, we systematically investigated the instant response of 8 cancer cell lines and 2 normal cell lines to the 1 min of direct CAP treatment. The optical emission spectrum and chemical assay have been used to quantitively measure the ROS/RNS in gas phase of CAP and in the CAP-treated medium. By comparing the H$_2$O$_2$ generation in the CAP-treated medium without cells and the CAP-treated medium with cells, we first demonstrated that the micromolar level cell-based H$_2$O$_2$ generation commonly existed during the CAP treatment on not only near all cancer cell lines but also on normal cell line (Fig. 1). Such cell-based H$_2$O$_2$ generation also exists when a simple buffered solution was used to replace the medium during CAP treatment. The optical emission spectrum (OES) of CAP indicates that the significant increase of ROS in CAP at the high discharge voltage may trigger the cell-based H$_2$O$_2$ generation. The cell-based H$_2$O$_2$ generation is a new previous unknown basic cellular response to the direct CAP treatment, which will change the common knowledge about the interaction between CAP with cells.

This work was supported by National Science Foundation, grant 1465061. This work was also supported in part by USPI Inc.
Plasma activated media (PAM) are produced by exposing cell culture media to low temperature plasma for a certain length of time. Recently we reported on the killing effects of PAM treatment on an epithelial cancerous cell line, SCaBER. However, much less is known about the effects of PAM on healthy/normal epithelial cells. To shed some light on this issue non-cancerous canine kidney MDCK (Madin-Darby Canine Kidney) epithelial cells were treated by PAM and the outcome was studied using various techniques. Time-lapse microscopy was used to monitor cell proliferation and random migration. It was found that moderate levels of PAM treatment inhibited cell proliferation and reduced cell migration within epithelial islands. Immunofluorescence staining showed a significant change in the nuclear localization of proliferation marker Ki-67, consistent with the time-lapse results. These results have implication about the cytotoxicity of PAM towards healthy cells as well as they provide information related to cellular migration which plays a role in metastasis.
 Plasma Activated Medium (PAM) Effect on Human Colorectal Cancer and Investigation of Cell Death Mechanisms

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In the past few years the use of cold atmospheric pressure plasmas in the biomedical field have received a great interest [1-3] and among others in the application of cancer treatment. Two approaches are being investigated, either direct plasma treatment or with the use of an intermediate plasma activated medium (PAM) which could be prepared and stored in advance [2]. PAM has demonstrated some interesting effect in cancer treatment like a decrease of cell proliferation [4], DNA damage [2;5] and apoptosis [6].

This study investigates the cell death mechanism of HCT116 MultiCellular Tumor Spheroids (MCTS) after PAM treatment. The 3D model that are the MCTS is a closer model to an in vivo one as its reproduces the 3D organization and regionalization of a tumor. A Helium (flow fixed at 3L/min) dielectric barrier discharge plasma jet was used to activate cell culture medium in order to produce PAM.

To investigate cell death mechanisms, different analyses were conducted. First a cell viability test was studied by measuring the ATP (adenosine triphosphate) rate in cells. Then fluorescence analysis allowed the detection of DNA damage, cell permeabilization and caspase activation. Caspase activation is a revealing method of apoptosis. After several hours in PAM cell detachment is noticed on the outer rim of MCTS indicating cell death. This was confirmed with the viability test and emphasized by cell permeabilization and caspase activation around the same time (Fig. 1). These results underlined apoptosis as the major cell death mechanism.

Fig. 1. (On the left) Optical Microscope image (10X) of cell detachment of an MCTS 24h after PAM treatment. (On the right) Fluorescent image of the same MCTS, at the same time, showing caspase activation.

References
Plasma Exposure and Tail Regeneration: Interplay of calcium with mitochondria and peroxisomes.

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Atmospheric pressure plasma treatment has emerged as a new form of regenerative medicine. Its therapeutic applications involve wound healing, tissue regeneration, and cancer therapy. Reactive oxygen species (ROS) signaling is required for wound healing and tail regeneration of the tadpoles and ROS is in higher concentration in plasma treated tadpoles compared to control (Rivie et al., 2017). In this study, we have focused on the role of Calcium (Ca$^{2+}$), mitochondrial permeability transition pore (mPTP) and peroxisomes during wound healing and blastema formation following tail amputation and characterization of the plasma used in the treatment protocol.

Calcium is essential for all living organisms, where Ca$^{2+}$ sequestration and release into and out of the cytoplasm functions as a signal for many cellular processes such as growth and cell death. Calcium signaling pathways also interacts with other cellular signaling systems including ROS. Peroxisomes are also known play a role in Ca$^{2+}$ homeostasis and antioxidant defense. mPTP is a voltage and Ca$^{2+}$-dependent channel and prolonged opening of these mitochondrial pores lead to massive release of matrix Ca$^{2+}$, and swelling of mitochondria.

Tail amputation was carried out by removing 40% of the tail and the amputated region was immediately exposed to Helium plasma for 40 seconds. The discharge source operated with Helium gas at a flow rate of 50sccm that passed through a quartz tube with a single electrode powered by an AC voltage (15kHz) having peak-to-peak voltages of 18kV. The copper electrode was attached to the outer surface of the tube at a point 5.0cm from the end of the tube. The optical emissions from the plasma were analyzed using a high-resolution spectrometer coupled to an CCD detector. The spectra indicated that molecular nitrogen was present in both the neutral and ionized states. Emissions from the radical OH were observed both inside and extending outside of the quartz tube. The emission profile was used to calculate the vibrational temperature and it was found to be 3500±350K. The rotational temperature was determined from a fitting of the Second Positive System transition at 337nm to a Boltzmann distribution and it was found to be 375±50K. The current was measured by monitoring the ground connection from a metal plate that was placed adjacent to the exit aperture of the quartz tube using a current transformer. When the plasma was “on” the signal consisted of an additional component superimposed on the sinusoidal background wave.

Our results show that all the parameters for in situ staining (calcium, mPTP and peroxisomes) were increased in plasma exposed tadpoles compared to control. In conclusion, a) there is an increase in calcium resulting from exocytosis of calcium from its stores (mitochondria and peroxisomes) that leads to cell death of damaged cells, b) increased mitochondrial staining indicates fission required for normal cell metabolism and to prevent damage from mitochondrial ROS, and c) increased mPTP staining is probably associated with mitophagy of damaged mitochondria.

Reference:

Potential genotoxic effect of low-temperature plasma on eukaryotic cells

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The low-temperature plasma (LTP) has become a subject of the significant research effort in recent years. In addition to common areas of plasma applications, such as surface finishing, there are many scientific teams focused on the study of plasma interactions with cells, microorganisms, for example to sterilization or disinfection [1]. Current results demonstrate the possibility of plasma application in medicine, pharmaceutical and food industry, but its potential genotoxic effect is not still fully clarified.

Our work is focused on the potential genotoxic effect of the low-temperature plasma treatment of eukaryotic cells (such as plant cells, sperms, lymphocytes) using the comet assay method and protein analysis. The comet assay is method used for a primary damage detection of DNA in eukaryotic cells [2]. Our aim was also to determine the levels of selected stress proteins by the western blot method [3, 4].

The plasma treatment of eukaryotic cells was performed by a planar source of the low-temperature plasma based on the Diffuse Coplanar Surface Barrier Discharge (DCSBD) working at atmospheric pressure in ambient air, oxygen and nitrogen. The DCSBD generates on an alumina plate a thin uniform large-area layer of a macroscopically homogeneous non-equilibrium plasma [5]. The discharge is powered by 14 kHz sinusoidal voltage with amplitude of approximately 10 kV [5, 6].

We found out that DNA damage of all cell types raised with increasing time of low-temperature plasma treatment. DNA damage of cells was higher after treatment with plasma generated in nitrogen or oxygen than in ambient air. We detected increased levels of some selected stress proteins after plasma treatment.

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-16-0216.

References
Cold atmospheric pressure plasma treatment kills oral squamous cell carcinoma cells through induction of apoptosis

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Abstract:

Cold Atmospheric pressure plasma (CAPP) techniques developed rapidly during the last decades and attracted attention in the field of medicine and widely used in biomedical applications. Plasma is known as ionized gas consisting of electrons, neutral atoms, ions, free radicals and UV photons. CAPP generates various kinds of highly reactive radicals at room temperature which plays very important role in various biological applications such as device sterilization, chronic wound healing, diabetic treatment, dental care, dermatological therapy, blood coagulation, and treatment of various diseases including cancer. CAPP increasingly gained more interest among researchers in the field of cancer in recent years but mechanism of its effect not fully understood. In this study, we developed a CAPP device and plasma emitted radicals quantity were analyzed using optical emission spectroscopy (OES). We collected oral squamous cell carcinoma (OSCC) cells and investigated the selective effect and the anti-cancer mechanism of CAP plasma treatment in terms of cell proliferation, DNA damage, cell cycle arrest and signaling pathway protein expressions were analyzed by WST-1 assay, immunofluorescence, flow cytometry and western blot analysis. We also measured the intracellular reactive oxygen species (ROS) by using CM-H2DCFDA (5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate). Cell proliferation assay showed that CAPP significantly decreases the proliferation of oral squamous cells in vitro. Flow cytometry and western blot analysis showed that CAPP treatment induces the apoptosis pathway in oral squamous cells. These findings showed that CAPP could be alternative adjuvant therapeutic tool for the treatment of OSCC. Further investigation is required.

Keywords: cold plasma, oral squamous cell carcinoma, cell viability, cell cycle arrest, apoptosis
ATR-FTIR reveals biochemical changes on Candida albicans cell wall after cold plasma exposition

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Increasing antifungal resistance, recalcitrant cases, limited number of therapeutic options are the main challenges to the treatment of superficial mycoses, in particular caused by Candida spp. Among the new therapies, the atmospheric pressure cold plasma jet (APPJ) stands out as a promising agent, showing anti-candidal activity and effects on fungal virulence [1]. However, to date, the mechanism of action has not been elucidated. Therefore, the aim of this study was to evaluate the biochemical changes caused by APPJ on Candida albicans. Standardized ATCC 18804 C. albicans suspensions (10⁸ cfu/ml) were exposed to APPJ using the following parameters: tension 12 kV, frequency 31 kHz, jet power ≈ 0.6 W, Helium flux 2 L/min, distance to sample surface 15 mm. Cells were treated for 120 seconds. Subsequently, the biochemical evaluation was performed at ATR-FTIR [2][3]. Spectra were recorded between 800 a 4000 cm⁻¹ by OPUS software at 4 cm⁻¹ resolution. A total of 64 scans per point were registered. The spectra were baseline corrected and vector normalized. Several differences in the polysaccharides region (980-1200 cm⁻¹) were detected, with decreased intensities of α-glucans and β-glucans bands after APPJ exposition. At protein region (1500- 1700 cm⁻¹), the intensities of Amida I and II and also some bands of chitin (1380- 1553 cm⁻¹) were increased. The results suggest that APPJ modifies cell wall composition in Candida albicans.

References

RADIOBIOLOGICAL RESPONSE OF BREAST CANCER CELLS, EXPOSED TO ATMOSPHERIC PRESSURE PLASMAS AND IONIZING RADIATION, AN IN-VITRO ESSAY.

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The purpose of this project is to study the improvement on cell death rate, using a cold plasma treatment in tandem with clinical photon beam therapy on breast cancer cells. This research may lead to implementation of a new in vitro technique for studying new radiobiological approaches for adjuvant methods to radiotherapy techniques.

Characterization of the plasma generator: An in-house developed Plasma Jet (APPJ) will be evaluated in terms of its functionality, geometric and operational features. The characteristics of the plasma generated using an Argon-Helium mixture will be evaluated regarding the interaction with a biological medium, as function of power, voltage level and gas flow rate, exposure time among other. UV-VIS-NIR spectroscopy [1] will be use to characterize the plume.

Biological Evaluations: The cell lines of human breast adenocarcinoma (MCF-7, MDA-MB-231, MDA-MB-468, Hs 578T, T-47D, MDA-N and BT-54), were placed in standard well sample plates with regular flat bottom for the application of ionizing radiation and CAP, using RPMI 1640 cell culture medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B, kept at 37 °C, in an atmosphere humidified with 5% CO₂ and 95% air.

In order to assess the cell viability at different radiation doses, in the presence and absence of treatment with CAP, the samples were analyzed with the DNA binding fluorochromes Hoechst 33342 (slightly compromised cell membrane), and Propidium Iodide (completely compromised cell membrane). Images will be acquired with the multi-modal Cytation™3 microplate reader and analyzed for segmentation and quantification of the cell nuclei for each of the fluorescent components.

Dosimetric measurements: A beam of 6 MV from a Varian CLINAC iX was used. For the determination of dose levels, radio-chromic films (4x4 cm² samples) were irradiated and measured for calibration using a spectrophotometer in the reflection mode [2]. For sample irradiation, its response is normalized by the same sample position in the plate without dose, as shown in Fig 1, where the range between 3.5 Gy and 5 Gy shows the most uniform response.

Expected results: Cellular modifications produced by the combination of cold plasma and ionizing radiation, would generate statistical information that would allows us to determine the overall effect of modification in radiosensitivity by the exposure to the CAP [3]. This enhancement in programmed cell death using CAP, compared to the programmed cell death rate from radiation alone, could by a proof of selectivity, leading to an increase in uniformity of cancer cells response to ionizing radiation.

References

We have previously demonstrated that nanosecond pulsed dielectric barrier discharge (nspDBD) plasma treatment of cancer cells selectively induces tumor cell death via plasma-generated reactive oxygen species (ROS) \(^1\). Furthermore, nspDBD plasma treatment was shown to modulate the anti-tumor functions of macrophages to induce cell death in tumor cells \(^2\). These observations suggest that plasma treatment may modulate the immune responses in an in-vivo tumor model. Our work with \textit{Drosophila melanogaster} showed that micro-second pulsed DBD (mspDBD) plasma treatment increased blood cell differentiation when compared to the untreated controls \(^3\). Blood cell differentiation in the Drosophila model, known to influence immune functions, such as tissue regeneration, occurs in the lymph glands which are sensitive to change in ROS concentrations \(^4\). Therefore, we hypothesize that mspDBD plasma treatment may modulate the immune response for treatment of tumors in \textit{Drosophila melanogaster}. To test this, tumor-bearing Drosophila, both untreated and mspDBD plasma treated, were analyzed using a microarray to assess the differences in genetic responses. Presented will be the genetic and molecular pathways hypothesized to affect the immune response in normal and tumor-bearing flies to mspDBD plasma treatment.

References
Investigation of effect of Plasma Activated Medium (PAM) on FaDu (Head & Neck Cancer) MultiCellular Tumor Spheroids (MCTS)

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Head and Neck Cancer (FaDu) is one of the most resistant cancer and chemotherapy and radiotherapy have a low rate of success [1]. Recently, Activated Medium (PAM) by cold atmospheric plasma has been studied as an alternative to the direct plasma jet treatment in the cancer therapy. Indeed, it shows a high reactivity enabling the creation of RONS [2] which have been proved to be cytotoxic on various cell lines [3,4] and may induce an apoptosis cell death [4]. The main advantage of PAM is its possibility to be prepared in advance and it can be used several weeks after activation by plasma jet when it is stored at the right temperature [3;5].

This study focuses on the effect of PAM on FaDu MCTS and the involvement of hydrogen peroxide. A homemade helium plasma jet was used to produce PAM. Helium plasma jet was generated using dielectric barrier discharge configuration. Helium was injected with flowmeter and the gas flow is fixed at 3L/min.

The effect of PAM treatment on FaDu MCTS showed two stages. The first one is a volume loss one day post treatment associated with cell detachment which we demonstrate to be linked to the presence of hydrogen peroxide. The second one is a rapid re-growth observed in the following days which may be due to the 3D organization of FaDu MCTS and its defense mechanism (Fig. 1). In order to counteract this non-wanted re-growth, successive treatments were investigated and FaDu MCTS were successfully disrupted after 4 successive treatments.

These results demonstrate that each cancer has its own reaction against PAM treatment and that Head & Neck cancer exhibits a defense mechanism unlike colorectal cancer [3] which underline the need to further investigate PAM treatment on different 3D model MCTS.

References
Analyses of factors affecting He plasma plume length generated after the nozzle of an atmospheric pressure plasma jet

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Abstract

Recently, atmospheric plasma technology has been found to have a huge potential and bright future in plasma medicine. In practice, the plasma plume length is critical in many of the bio-medical applications. In this study, silicone tubes were used to extend the length of plume. Silicone was chosen because it is a non-toxic, biocompatible, highly elastic material. An customized atmospheric pressure plasma jet (APPJ) equipped with unipolar pulse power supply was used to generate He plasma for the present study. By applying statistical analysis, the significance of voltage, frequency, gas flow rate, and tube diameter was analyzed. Overall, peak voltage was found to be the most significant factor. The plumes were also analyzed by OES and UV absorption spectrometry in order to realize the concentrations of existing radicals.

References

Radicals and ozone generated in Ar/He and Ar/He/H₂O plasma by using atmospheric pressure plasma jet systems and their use in methylene blue degradation

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Optical emission spectroscopy (OES) and UV absorption spectrometry were first used to gather information about the excited species present near/in the plasma plume generated using Ar/He and Ar/He/H₂O gases with an atmospheric pressure plasma jet (APPJ). Afterward, the APPJ system was used to study its efficiency in degrading methylene blue as a function of radical and ozone density. According to the results, it was found that the degradation of methylene blue was directly related to the ozone concentration and, perhaps, OH radical density. Complete degradation of MB can be achieved in 80 seconds.
Study of transient spark discharge in low frequency Ar atmospheric pressure plasma jet

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Currently, Atmospheric Pressure Plasma Jets (APPJ) are expanded to the industrial and medical industrial, low temperature plasma application became important. Therefore, measurement of plasma is also important works and widely studied all around world. Previously study, APPJ which used high voltage DC power was observed Transient Spark (TS) discharge, self-pulsing dc TS discharge. Although, TS discharge make relatively high current (~ 1 ~ 10 A), their discharge current sustained for a short time (10 ~ 100 ns).[1] Therefore, ambient temperature of plasma could remain a low temperature. In this experiment, we use home made invertor which adapted duty ratio 6 % with 30 kHz (6 ~ 9 kV) sinusoidal wave packet during 60 ms. And used Ar gas to 2 lpm. TS discharge is found not only DC power but also low frequency (LF) APPJ. In the LF APPJ, after TS discharge, glow discharge was maintained instead of self-pulsing and voltage restored. Typically, electrons play an important role in transporting external energy and carrying it to the plasma. and the experimental result of this report showed that TS discharge electron density tendency is close to later glow discharge electron density.

Fig. 1. (a) Voltage and Current of TS discharge in LF Ar APPJ, (b) ICCD image of TS discharge

References

On the mechanism of a helix plasma plume

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Abstracts: To better understand the mechanism of the helix plasma that was found in a sealed quartz tube, a floating helical coil is placed around the outer surface of the quartz tube in our previous report[1,2]. In this paper, to investigate the interaction between the helix plasma and the helical coil on the outer surface of the tube, an extra high voltage pulsed DC power supply is applied to the helical coil. The parameters of the extra power supply including the amplitude, the phase difference, the power width and the pulse frequency are adjusted. It is found that, the length of the helix plasma decreases with the increase of the voltage amplitude of extra power supply. When the voltage potential on the helical coil is adjusted to 1.8kV, the length of helix plasma reverts to the same length as that without the helical coil. However, their propagation speed for the two cases are significant different. In addition, when the pulse width and the repetition frequency of the voltage on the helical coil are adjusted, several interesting behaviors of the plasma plume are observed. Finally, a simple equivalent circuit model for the plasma propulsion process with the extra helical coil is presented. It is believed all results discovered in this paper are helpful for better understanding the mechanism of the helix plasma.

This work was supported by the National Natural Science Foundation of China (Grant Nos. 51277087, 51477066, 51625701, and 51507071)

References

ELECTRIC FIELD IN PLASMA MEDICINE: AN APPROACH TO MEASUREMENT AND EFFECTS IN WOUND TREATMENT

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Besides reactive species and UV-radiation, the electric field is one of the presumed important effective compounds of cold atmospheric argon plasmas in wound healing \cite{1, 2}. Not only are electric fields produced naturally in the body to monitor healing processes, externally applied electric fields also have shown to support these mechanisms \cite{3}. It is essential to fully understand those interrelationships to further optimize plasma sources. Still neither a reliable analysis of which part of the plasma-induced wound healing depends on the electric field nor a measuring method for electric fields inside a plasma is well established. Therefore the possibilities and limits of an electric field probe based on the Pockels effect \cite{4} are explored. Testing measurements (\textbf{Fig. 1}), necessary calibrations and setups close to clinical conditions set up in the laboratory are carried out with the kINPen\textsuperscript{®} Science and will be presented.

These experiments are accompanied by microbiological tests on Escherichia coli bacteria treated with plasma, where the electric field is shielded (\textbf{Fig. 2}), while reactive species and radiation reach the probe compared to unshielded plasma. On the clinical side the connection of in depth effects of plasma in wound healing with the electric field are explored.

The mentioned perspectives together help to understand the healing effects of the electric field component in cold atmospheric argon plasma and possible consequences for plasma sources and treatment options will be discussed.

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Effect of plasma on helium gas flow in an atmospheric pressure plasma jet: absolute air concentration measurements by Rayleigh scattering

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Cold atmospheric pressure plasma jets (CAPPJ) have received increased attention over the past decades due to their large variety of applications, including material processing \cite{1}, sterilization \cite{2}, and biomedical treatment \cite{3}. An example is a helium-based plasma jet that generates guided streamers in its helium rich jet effluent. The plasma-induced chemistry, critical for many applications, is highly dependent on the air concentration in the jet effluent. In many simulations of plasma jets, the gas flow and corresponding gas composition of the jet effluent is calculated assuming that the plasma has a negligible effect on the air concentration distributions in the helium effluent. Nonetheless recent work has shown that the plasma can strongly impact the gas flow dynamics \cite{4}. In this work, we report on a helium CAPPJ impinging on a glass substrate with operating conditions consistent with biomedical treatments. Gas temperature measurements have been performed using optical emission spectroscopy by analyzing the ro-vibrational transitions of OH(A-X) and N\textsubscript{2}(C-B) and the resonance broadening of the helium emission line at 667.8 nm, which demonstrated that the increase in gas temperature is small. We have measured the air concentration distribution in the jet effluent by means of Rayleigh scattering exploiting the large difference in Rayleigh scattering cross sections between air and helium. The obtained results show that the plasma causes a broadening of the helium effluent channel caused by an enhanced mixing with the ambient air. The impact of the plasma on the jet effluent is polarity dependent and is more pronounced in the case of positive voltage pulses. Differences between positive and negative polarity are further investigated by means of Schlieren and ICCD imaging. The optical diameter of the guided streamer correlates with the air density distribution in the effluent. The mechanisms underpinning these observations will be discussed in detail.

This work is partially supported by a Department of Energy Early Career Research Award (DE-SC0016053).

References

Superficial charge distribution induced by the fluid-dynamic action of an annular plasma synthetic jet actuator.

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Indirect plasma treatments are generally used when samples characterized by complex geometries must be treated or when direct contact with plasma filaments must be avoided. Moreover, in this operating condition, plasma reactor can be optimized without considering the target to be treated. Usually in indirect treatments only neutral long-life reactive species are considered.

Annular plasma synthetic jet actuators (PSJAs) had demonstrated their ability to produce an induced tubular flow [1] normal to the surface where the dielectric barrier discharge is ignited (Fig. 1). This induced wind is due to the electro hydrodynamics effect. This typology of reactor enhances the transport of reactive species toward the treated sample [2]. Long life charged particles are generated within the plasma region and then can be advected together with the induced flow.

In this work the potential distribution induced by charges deposited over a dielectric surface positioned at several centimeters from PSJA, has been detected. The surface charge has been calculated by a MatLab based code, utilizing measured induced potential distributions. In Fig. 2 surface potential distribution after 50 ms, 500 ms and 20 s of plasma on time and for a reactor distance of 2 cm is depicted. These measurements, coupled with the MatLab code, allow to the estimation of a charge flow toward the surface of about $10^{10}$ charges/cm².

The biological effect of free charges [3] into induced flow has been tested by treating Candida Albicans inoculated both in agar plate and in water solution. Charges reaching the surface have been blocked by a grounded metallic mesh. When free charges have been allowed to reach the sample, an additional log1 reduction in CFU has been obtained.

References

Effect of storage temperature on pH and conductivity of deionized water treated with atmospheric plasma

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Plasma-activated water (PAW) consists of a water in which a set of chemical reactions begin or continue after exposure to the plasma. It is believed that these reactions involve the presence of reactive species long-lived, oxygen and nitrogen - RONS [for example, (NO), (O), (OH), (ONOONO) and (H₂O₂)], transferred from the plasma environment to liquid [1]. Based on the assumption of continuity of the reactions in water treated with atmospheric plasma, devised an experiment to monitor pH and electrical conductivity in samples of deionized water before and after treatment with a gliding arc discharge operated the mixture of air + humid air, flow 5L/min, in order to evaluate the continuation of these chemical reactions at different storage temperatures, room temperature (~23.9°C) for group 1 and refrigerated temperature (~15.2°C) for group 2 [2]. To obtain the PAW, treated 250 ml of deionized water with 10 (T10), 20 (T20) e 30 minutes (T30) and made periodic monitoring, with the 24h interval between measurements, for a period of 96h. The Figure 1 show the results.

Fig.1. Results achieved during storage

We can conclude that by increasing the treatment time, increasing acidification and electrical conductivity of the water. And that the storage temperature exerts little influence on these physic-chemical properties of the PAW.

Acknowledgement

This work was supported by PROSUC-CAPES-Univap and FAPESP (grant nº 2015/ 10876-6).

References

ELUCIDATING THE DEPENDENCE OF DBD TREATMENT PARAMETERS FOR IMMUNOGENIC CELL DEATH

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Research Aims: Dielectric barrier discharge (DBD) plasma is being developed for several medical and biological applications [1]. The effects of DBD treatment have been linked to the generation of a cocktail of reactive oxygen and nitrogen species (RONS) [2]. Due to the many parameters that can be tuned for the production and delivery of RONS (e.g. applied voltage, pulse frequency, etc.), DBDs may be used as a versatile and precise medical instrument. In this study, we aim to determine the relationship between RONS generation, pulse frequency, and treatment time for the induction of immunogenic cell death (ICD) in cancer cells (Fig. 1). Cancer cells undergoing ICD have the potential to stimulate the patient’s own immune system to respond against cancer, leading to a more specific and systemic resolution of the disease [3].

Methodology: We monitored the formation of RONS induced by a microsecond-pulsed DBD in aqueous solutions. The measurements were performed using electron paramagnetic resonance spectroscopy, UV-VIS spectrophotometry, and mass spectrometry. The analysis of persistent RONS (H₂O₂, NO₃⁻, and NO₂⁻) provided quantitative insight into the total amount produced. EPR analysis does not assess total amount of RONS delivered to the liquid [4], but showed the absence of certain RONS, the presence of others, and the numerical trends of their formation. Results: RONS production increased near linearly with both treatment time and frequency. Normalization of delivered pulses with different frequencies resulted in an equivalent amount of RONS produced. Conclusions/ Ongoing Work: Our results indicate that formation of species depends mostly on the energy deposited by the plasma. The dependence of plasma energy will be tested for ICD induction in cancer cells. Chemical analyses, tied to biological effect, facilitate anti-cancer plasma therapy development by expanding our knowledge of plasma systems and biological interactions.

This work was supported by the Flanders Research Foundation (12S9218N) and the European Marie Sklodowska-Curie Individual Fellowship ‘LTPAM’ within Horizon2020 (657304).

References
Cysteine derivatives MS-analysis as tool to monitor reactive species production varying cold physical plasma jet and relative parameters.

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Several studies confirm the effectiveness of cold physical plasmas (CPPs) in cancer treatment and wound healing, but the underlying mechanisms are still under study. They involve redox signaling processes, with the deposition of reactive oxygen and nitrogen species (RONS) in body fluids and other liquids [1]. A way to clarify the biochemistry of CPPs action is to monitor the reactions between these species and small organic molecules (MW<1kDa). These act as chemical traps able to disentangle key RONS produced using different cold plasma jets and different relative parameters. Here, we focused on the most reactive molecule towards RONS, using cysteine as model compound. Varying the oxidation state of its thiol moiety, cysteine determines protein structure, function and subcellular localization, and such it has an essential biological role [2]. The structure and the kinetic profiles of appearing cysteine oxidation products were determined using a high-resolution time-of-flight mass spectrometer. We found that cysteine product composition reflects different treatment conditions, such as the plasma source, the treatment duration, the presence of shield gas or the feed gas mixture. The observed cysteine products (Fig. 1) show the oxygen dominated modification of the thiol group, with cysteine sulfinic acid (168 m/z) as dominant product. The impact of nitrogen species can be emphasized using nitrogen as shield gas leading to the presence of S-nitrosocysteine. Other potentially bioactive compounds, e.g. oxidized sulfoxides (271.01 m/z, 390.01 m/z) have been detected for moderately oxidative conditions, e.g. humidifying the feed gas. Overall, these observations imply a profound impact on protein thiol groups when treated by plasma in vivo, with subsequent consequences on cellular redox signaling pathways.

Fig.1. MS spectra of cysteine (120.01 m/z) acquired before (A) and after plasma treatment (B). Treatment conditions: 5 minutes, 3 slm Ar/N₂/O₂ feed gas (99-0.5-0.5%).

This work is funded by the German Federal Ministry of Education and Research (BMBF) (Grant No. 03Z22DN12).

References
The scavenging of hydroxyl radical by terephthalic acid and methanol provide insight of HO° concentration in liquid phase during plasma-liquid interaction

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Hydroxyl radical is one of the highly reactive chemical species produced during plasma-liquid interaction. In pure water, its lifetime is limited by the recombination reaction [1]: $HO° + HO° \rightarrow H_2O_2$ ($k = 5.5 \times 10^9 M^{-1}.s^{-1}$) (R1). The reaction between Terephthalic acid (TPA) and the hydroxyl radical can lead to the formation of one fluorescent product (TPA-OH). However, this reaction is the result of several elementary reaction steps that do not have a 100% yield [2]. Assuming the treated solution is saturated with ambient air, the reaction yield is only 35% according to photolysis experiments [2p-3]. However, the steady state concentration of HO° was low (~15 fM) during such experiments [3]. So that the recombination reaction (R1) is totally negligible compared to the reaction between HO° and TPA [4]: $TPA + HO° \rightarrow Precursor of TPA – OH$ ($k = 3.3 \times 10^9 M^{-1}.s^{-1}$) (R2).

It is first shown that the yield of the reaction (R2) depends on TPA concentration and is not total even when the TPA concentration is several mM (against ~10 µM in the photolysis experiments of Page et al. [3]). This can be interpreted by the assumption that plasma liquid interaction produces a local and transient concentration of HO° much higher (~0.7 mM) than that encountered during photolysis experiments. Hence, reaction (R1) cannot be neglected and a lesser fraction of HO° reacts with TPA via R2; this leads to an underestimation of HO°.

The scavenging of HO° by methanol ($MeOH + HO° \rightarrow MeO° + H_2O$, (R3), $k = 5 \times 10^8 M^{-1}.s^{-1}$ [1]) enables to confirm the order of magnitude of HO° concentration in water under plasma exposure. Finally, with the correction of the error caused by TPA concentration, it is now possible to propose measurements of the production rate (in pmol/s) of the hydroxyl radical HO° based on TPA dosimeter.

This work was supported by the Institut Universitaire d’Ingénierie en Santé (IUIS), Sorbonne Université, Projet OSCC 2014/2016, labex Plas@Par, Canceropole, CNRS and Ecole Polytechnique.


Effectiveness of Plasma-Activated Water Using Gliding Arc Discharges on the Inactivation of *Fusarium proliferatum* and *Penicillium purpurogenum*

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Due to their hazard to human life, many efforts in the investigation of pathogenic fungi have focused on finding new decontamination technologies. Non-thermal plasma appears to be a promising alternative to traditional sanitizers applied in the agricultural and food industries, as it has been often observed to inactivate filamentous fungi with different degrees of efficacy, as per their diversity in terms of spore structure and molecular processes [1].

The application of non-thermal plasma to water, generates a solution that contains nitrogen and oxide species, known as plasma activated water (PAW), which retains microbial disinfection efficacy for several days, having also crop protection and fertilizing properties [2]. The use of PAW for microbial inhibition has many advantages; including a less adverse impact on the environment, and no need for transportation and storage of potentially hazardous chemicals, representing a promising cost efficient and sustainable alternative to pesticides [3].

Therefore, the aim of this work is to present the experimental results for the treatment of two different filamentous fungi, *Fusarium proliferatum* and *Penicillium purpurogenum*, with PAW created by applying a gliding arc discharge into distilled water, using humid air at near room temperature and at atmospheric pressure. Optical emission spectra results show the responsible reactive species for inactivation of the spores when applying PAW in the mentioned setup. The levels of inhibition on spore germination and growth in differing time scales and power, for the two filamentous fungi, were observed by comparing the fungal hyphal extension after the PAW treatment.

References


Investigation of the cytotoxicity of Plasma Activated Medium by the quantification of free radical species generated by low-temperature plasma jet in liquid media

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Plasma medicine has attracted a lot of attention this last decade [1-2]. The use of plasma activated medium (PAM) has been used as an alternative method to direct plasma exposure [2]. However, the need to know and control the produced species by the interaction between medium and plasma jet is vital to next understand the effect of PAM. In literature this interaction has shown that cold atmospheric plasmas can generate reactive oxygen species (ROS) and reactive nitrogen species (RNS) [3-4]. Also, the interaction of the plasma with an aqueous medium can induce the production of species either by the solubilization of the plasma species in the liquid or by the formation of other species coming from the reaction between gaseous plasma excited and charged species and liquids components.

This work identifies and quantifies the ROS and RNS produced by the interaction of a helium cold atmospheric plasma jet with Milli-Q water, culture medium with or without serum and investigates the degradation of culture medium in order to understand the cytotoxic effect of PAM [2]. Several techniques have been used depending on the lifetime of the intended targeted species. Reactive radical species, with a short lifetime (< microsecond), were detected by spin-trapping using an electron spin resonance (ESR) technique: a spin-trap reacts with radicals to form a adduct with a longer lifetime. The quantification of more stable species like hydrogen peroxide and nitrite/nitrate was done by fluorometric and colorimetric methods. As for the deterioration of culture medium by the helium plasm jet, the degradation of several amino acids was conducted (Fig.1).

References
Understanding the interaction of plasmas with liquids is important to optimizing chemical and biomedical applications of activated liquids. In biomedical applications, gas-liquid interactions can involve large volumes, such as plasma activation of media, or small volumes such as fluid (exudate or transudate) covering wounds and tissue. The plasma activation of liquid is in part limited by transport of plasma produced species to the surface of the liquid and, once solvated, in the liquid. In this paper, we report on a computational investigation of dielectric barrier discharge (DBD) treatment of a falling water film which is in direct contact with plasma. This configuration addresses both transport scales – enabling direct contact of the plasma with the liquid, and enabling rapid activation of the liquid by virtue of the large surface-to-volume ratio of the film. The AC-driven DBD reactor (see Fig. 1) operates at atmospheric pressure with different feed gases including argon, helium, air, nitrogen, and oxygen with the addition of water vapor and air impurities.

The 0D GlobalKin model was used to simulate the plasma kinetics and plasma activation of the liquid. In this model, we specify power deposition in the plasma as a function of time, while including gas flow, transport of species to the liquid surface, and liquid interactions.[1] An extensive reaction mechanism (98 and 92 gas and liquid species respectively, and over 2100 reactions) was implemented to address the different feedstock gases and resulting chemistry of the liquid and gas interaction. Applied power in the model extends for 1500 pulses at 600 Hz for a plasma exposure time of 2.5 s, after which the system evolves during the afterglow.

Reactive oxygen and nitrogen species are important due to their biological impacts on cells and tissue and several experimental investigations have reported that plasma treatment creates long-lived reactive species such as H$_2$O$_2$, O$_3$, NO$_3^-$, and NO$_2^-$ in liquid.[2] On the other hand, radicals and short-lived species such as OH*, O$_2^*$, NO*, and ONOOH are effective in chemical dynamics of plasma although they are difficult to measure experimentally. In this paper, the consequence of gas mixture and accumulation of long-lived species on the generation and stability of gas and liquid phase reactive species will be presented, with comparison to experimental measurements.[2]

**References**


**Figure 1.** Schematic of the cylindrical DBD plasma reactor used in this model with a thin layer of water.
Understanding the differences between antimicrobial and cytotoxic properties of plasma activated liquids

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The aqueous environment plays an important role in the transmission of cold plasma effects to both prokaryotic and eukaryotic cells. The exposure of liquids to cold atmospheric plasma discharges results in the generation of relatively long-lived secondary reactive species and specifically, H₂O₂ seems to be one of the most important amongst the reactive species contained in plasma-activated liquids (PALs) in causing cytotoxicity [1]. The chemical composition in PALs depends on discharge parameters and properties of each solution. Recently, PALs have been receiving increasing attention for medical applications [2, 3] as they provide advantages over applications of direct plasma discharge.

Detailed understanding of the effects of PALs on prokaryotic and eukaryotic cells are essential in order to harness this new technology. PALs such as water, phosphate buffered saline, saline and phosphate buffer solution were generated using a custom built di-electric barrier discharge atmospheric cold plasma (DBD-ACP) system. The chemical characterisation of the PALs included pH, hydrogen peroxide, nitrite and nitrate concentration measurements. Moreover, the antimicrobial effects of PALs on Gram-positive and Gram-negative bacteria were examined and cytotoxicity assays using an established mammalian cell line were used to elucidate the cytotoxic properties of these PALs.

The research outcomes showed acidification of plasma activated non-buffered solutions, and differences in concentrations of hydrogen peroxide, nitrite and nitrate. The liquids used in this study acting as models for non-complex solutions demonstrated both varied antibacterial activity and cytotoxic effects. These findings indicate that different reactive species may be responsible for the antibacterial and cytotoxic activity of PALs.

Although further details on the molecular signalling pathway are still needed, our preliminary experiments suggest that antimicrobial and cytotoxic effects are distinct from each other, which may offer promising approaches for future targeted applications in medicine.

This work was supported by Science Foundation Ireland, Grant No 15/SIRG/3466.

References

Air plasma gaseous reactive species determine the chemical properties and antimicrobial effects of plasma activated water

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Plasma activated water (PAW), i.e. water and aqueous solutions treated by cold atmospheric plasmas, demonstrates antimicrobial or cytotoxic effects that are of great interest for many applications in biomedicine, food processing and agriculture. Nonequilibrium air plasmas generate reactive oxygen and nitrogen species (RONS: O, N, OH, H₂O₂, NO, NO₂, O₃, O₂⁻). They are transported from the gas phase into the liquid and induce formation of secondary RONS in water, such as H₂O₂, NO₂⁻/NO₃⁻, peroxynitrites/peroxynitrous acid ONOO⁻/ONOOH, superoxide O₂⁻, or OH radical. This is typically accompanied by acidification of the solution and antimicrobial effects that can last for several hours/days after plasma treatment. [1-2]

Water can be activated by cold plasma discharges indirectly by blowing the discharge effluents onto its surface or by generating a discharge directly into the water surface. Even more efficient water activation is achieved with the water electrospray that drives the micrometric droplets directly through the active discharge region, which allows for very efficient mass transfer of plasma-generated reactive gaseous species into water [2-3]. We present self-pulsing DC-driven streamer corona (SC), transient spark (TS) and gliding arc (GA) discharges operated in air as non-thermal plasma sources [2-3]. Their physical properties can be controlled by the applied voltage and electric circuit parameters, which then affect the production of gaseous active species, such as O₃, NO, NO₂ and OH, and consequently the PAW properties. Low power SC generated dominantly O₃ and H₂O₂ similar to ozone-mode of the surface microdischarge (SMD) [4], while TS with higher current pulses, as well as GA, suppressed O₃ and enhanced NOₓ formation, similar to the NOₓ-mode of SMD [4]. The gas flow conditions influenced the gaseous species and the PAW properties: in the TS electrospray in a closed chamber, the NOₓ generation was enhanced with respect to the open air system, resulting in higher NO₂⁻ and NO₃⁻ in the PAW.

Bactericidal effects induced in water activated by the SC, TS and GA air discharges were tested on E. coli bacteria in water and on biofilms on surfaces and correlated with the generation of RONS. NO₂⁻ interacts with H₂O₂ in acidic conditions and leads to peroxynitrites (detected by fluorescence spectroscopy) that were identified as the crucial bactericidal RONS agents in PAW [1-2]. Our preliminary results on antimicrobial effects of PAW show its great potential for some medical therapies of e.g. of periodontal biofilms, urinary tract infections, or open wounds.

This work was supported by Slovak Grant Agency VEGA 1/0419/18 and Slovak Research and Development Agency APVV-0134-12.

References

Degradation of Tattoo Inks by Cold Atmospheric Plasma Treatment

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History of tattoos date back to about the beginning of the humanity. Today, it is estimated that about 25% of young and middle aged Americans have at least one tattoo. However, many of those, eventually come to regret and will for removal of their tattoos. Various methods are used for removal of tattoos including, lasers, abrasion, cryosurgery and excision [1]. Lasers are the most common modern technique for tattoo removal. As different colors of tattoos require specific wavelength of laser, only one type of laser cannot be used for removal of tattoos with different colors [2]. Moreover, removal of some tattoos with green and yellow usually is not satisfactory. In addition, lasers can cause undesirable tissue damage, which might cause scarring, color differentiation, irritation and infection [3]. In the present study, degradation of tattoo inks with DBD plasma treatment was investigated.

Non-thermal atmospheric plasma was generated using a microsecond pulsed power supply at 2.5 kHz and 31.5 kV with 2 mm of discharge gap. 1 ml of six different tattoo ink solutions (blue, green, red, yellow, white and black) with 0.01% concentration were treated with DBD plasma for 15 minutes. Reflectance measurement of each untreated and treated tattoo ink solution was performed. In all suspended tattoo inks, lighter color was observed visually after DBD plasma treatment. Reflectance spectra collected from each tattoo ink showed modified reflectance characteristics for each tattoo ink as indicator of degradation of pigments in the tattoo inks by DBD plasma treatment. Furthermore, agarose gel model was prepared to mimic tattooed skin. Similarly, six different tattoo inks were mixed with agarose gel at 0.01% concentration and poured into petri dishes to obtain 2 mm thick samples. Dyed agarose gels were cut into 1 cm x 1 cm squares and treated with DBD plasma for 15 minutes up to two sessions. On plasma treated tattooed agarose gels, color fading in a plasma treatment time dependent manner was visually observed. In addition, pictures of untreated and plasma treated gels were collected and colors were determined with CIE Lab color system. Plasma treatment dependent color change was determined as ΔE values. Color change of dyed agarose gels for green, red and yellow tattoo inks was determined in terms of ΔE values as 18.8, 28.4 and 37 respectively. Color measurements for gels dyed with blue, black and white tattoo inks are underway. Moreover, measured L, a, b values were normalized to a 2-dimensional diagram of chromaticity in terms of X and Y values. On the diagram of chromaticity, we have observed approximation of color of all tattoo dyes towards white region which is considered as degradation of tattoo inks in the agarose gel skin model.

In conclusion, cold atmospheric plasma treatment could be considered as an alternative method or an assistive method to laser for tattoo removal. Further studies, including the tattoo removal on excised mouse skin fragments are underway.

References

Time-resolved measurement of the electric field induced by a plasma gun device in a conventional electroporation setup

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An organic tissue exposed to plasma experiences a highly reactive environment consisting of ions, electrons, free radicals, UV radiation, neutral molecules and electric field (EF). Of these active agents, EF is one of the least investigated up to these days [1]. Nevertheless, EF can potentially play a major role transiently increasing cell membrane permeability, a phenomenon commonly known as “electroporation” [2] and nowadays effectively used for several applications including gene transfection and electrochemotherapy [3]. Thus, in the present work, we report the time resolved measurement of the EF induced in a liquid medium within a typical electroporation setup by a plasma gun (PG) device. The results are compared with the values estimated for a conventional electroporation device (Cliniporator, IGEA SpA) in the perspective of a possible impact of plasma induced EF on biological targets. The comparison was done adopting a 4 well µ-slide (Ibidi®) armed with two plane parallel electrodes used for in-vitro electroporation test and filled with 500µl of the target liquid, being either SMEM culture medium or high purity water. Three different experimental cases were taken into account (Fig.1, left): 1) the plane electrodes powered by the Cliniporator; 2) the PG plume hitting on one plane electrode; 3) the PG plume hitting on the liquid with (3a) and without (3b) ground electrode. Despite the considerably higher voltage powering the plasma device, preliminary results show an EF on the target lower than the one applied by the electroporation system. This observation goes along with the recorded current that results in general one order of magnitude lower for the plasma system. Furthermore, results show a key role played by the grounding of the liquid medium which can hinder the induction of an EF by the plasma source. While still preliminary, presented results provide new insights on one of the less investigated aspects of plasma and liquid target interaction, the induced EF.

Fig. 1: (left) Photos and schematics of the experimental set up; voltage, current and EF waveform for case 1 (center) and case 3b (right).

This work and A.S. are supported by PLASCANCER project, INCa-PlanCancer-n°17CP087-00

References

Hydra as a model for screening eco-toxicological effects of plasma treated water

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Atmospheric cold plasma (ACP) has been widely researched for generation of functionalized solutions for decontamination of liquids and waste water effluents [1]. The focus of this work was to study antimicrobial properties of plasma treated water (PTW) and eco-toxicological effects of PTW using a free-living brown *Hydra* as an important and prevalent component in freshwater ecosystems. PTW was obtained by subjecting 10 ml of sterile deionized water to high voltage (80 kV) contained ACP treatment [1] for 15 (PTW15) and 25 min (PTW25). The antimicrobial potential of PTW against *E. coli*, *B. atrophaeus* and *P. aeruginosa* was determined using broth microdilution method, which estimated minimum inhibitory and bactericidal concentrations (MIC/MBC). Toxicity measurements were based on the progressive changes in *Hydra* morphology through scoring procedure [2]. It has been established that PTW25 at a concentration of 25% was lethal for Gram-negative *P. aeruginosa* and *E. coli* while MBC of 50% was obtained for Gram-positive *B. atrophaeus* (Fig. 1). Similarly, toxic effects of PTW15 and PTW25 at concentrations above 50% and 25%, respectively, were observed in hydra tests (Fig. 2). The present investigation demonstrated that Hydra can be used as an additional *in vivo* tool to monitor the impact of plasma processed solutions on aquatic environment.

**Fig. 1:** Surviving populations of bacteria after exposure to PTW at different concentrations.

**Fig. 2:** Images of *Hydra* reflecting changes in body morphology due to exposure to PTW\(_{15\text{min}}\) and PTW\(_{25\text{min}}\) of different concentrations recorded after 24 h of incubation (Score).

This work was supported by RCUK/SFI grant 16/BBSRC/3391 and SFI grant 14/IA/2626.

**References**


Rapid inactivation of pandrug-resistant

*P. aeruginosa* and *A. baumannii* with plasma activated solution.

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Gram-negative “superbugs” are resistant to broad-spectrum antibiotics and some are resistant to all existing antibiotics (i.e. pandrug-resistant bacteria). They represent a major threat to public health for which there is no cure. Gas plasmas have been shown to inactivate a wide range of microbes including bacteria, fungi and algae, and recent studies demonstrate that gas plasma activated solutions are also capable of broad-spectrum antimicrobial activities. However, little is studied whether plasma-activated solution may be effective against pandrug-resistant bacteria.

This study presents evidence that Plasma Activated Saline (PAS) elicits an excellent bactericidal effect on clinical isolates of multiple-, extensive- and even pandrug resistant Pseudomonas aeruginosa and Acinetobacter baumannii (see Figure 1). Moreover, PAS stored at 4°C retain its bactericidal activity within 4h of its utility at room temperature. This demonstrates that PAS might be a promising method to control highly antimicrobial-resistant pathogens.

Fig 1. Antibacterial activity of plasma activated saline tested on different resistant patterns *P. aeruginosa* (PA)
AlmaIDEA project: chemo-physical and biological mechanisms behind the antitumor activity of plasma activated liquids for the treatment of peritoneal carcinosis from primitive epithelial ovarian/tubal tumor

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Advanced epithelial ovarian cancer is characterized by the diffusion of the disease as nodules or plaques from the ovary to peritoneal surfaces (carcinosis) (Fig.1). In advanced stages the disease presents poor prognosis (OS Figo stage III-IV: 15-20% within 5 years) [1]. Since multimodal treatments (e.g. surgery and intravenous/intraperitoneal chemotherapy with platinum and taxanes) cannot eradicate the disease, there is a continuous search for novel therapies. The treatment of liquids by means of cold atmospheric pressure plasma enables the production of plasma activated liquids (PALs) containing reactive oxygen and nitrogen species (RONS) having antitumor activity [2-3], thus the direct application of PALs on peritoneal surfaces could represents an alternative treatment of carcinosis. In this context, the aim of this work is to present preliminary results on the chemical composition and the effect of PALs on human ovarian carcinoma cells in vitro.

The research is performed in the frame of the AlmaIDEA project, whose final aim is the development of a novel intraperitoneal therapy for cancer treatment, starting from the investigation of the bio-chemical effect of PALs on cells in vitro. The project team brings together surgeons of gynecologic oncology, geneticists (with a bias on mitochondrial metabolism in solid tumors) and plasma engineers, allowing a multidisciplinary approach to the research.

Figure 1: Peritoneal carcinosis (red arrows) close to ovary (right) and PAL production using microsecond pulsed DBD jet (left).

This work was supported by AlmaIDEA Grant: “Studio dei meccanismi d’azione chimico-fisici e biologici alla base dell’attività antitumorale di liquid attivati con gas plasma per il trattamento della carcinosi peritoneale da tumore epiteliale dell’ovaio/tuba/peritoneo primitivo”.

References:
Denaturation of thermophilic proteins using cold atmospheric plasma:  
An alternative method for the food processing industry

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In recent years, the use of cold atmospheric plasma (CAP) for cancer treatment and sterilization (bacteria killing) has increased drastically. While the mechanism of protein folding has been widely studied in general to understand the stability of proteins, very limited studies are reported on protein folding after plasma treatment. Specially, the effect of CAP on thermophilic proteins, which play a role in the food processing industry, still remains largely unknown from literature. Typically, operation at high temperature is used to minimize the risk of microbial contamination in the food processing industry, but this is not suitable for thermophilic bacteria. For example, the dairy industry depends on the pasteurisation process as a means of product safety assuring. However, thermophilic bacteria, particularly aerobic species capable of forming endospores with a high resistance to heat, are largely unaffected by this level of heat treatment. Therefore, in this work we have used an air-dielectric barrier discharge (DBD) plasma to treat a model thermophilic protein MTH1880, from Methanobacterium thermoautotrophicum, for 10, 15 and 20 mins. After the treatment with plasma, we have analyzed the structural changes of MTH1880 using circular dichroism, fluorescence and NMR spectroscopy. Furthermore, we have also performed chemical and thermal denaturation analysis before and after plasma treatment for MTH1880. Additionally, we have used molecular dynamics simulations to check the stability of MTH1880 in the presence of H₂O₂ and we also compared the stability of MTH1880 control and oxidized MTH1880. This study may help to provide insight in the effect of plasma on thermophilic proteins, of interest for the food processing industry.

This work was supported by the European Marie Skłodowska-Curie Individual Fellowship “Anticancer-PAM” within Horizon2020 (grant number 743546).

References

Surfatron-produced atmospheric-pressure plasma jet applied in *Candida* biofilms

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Fungal biofilms represent a constant and predominant cause of chronic infections and exhibit an increased tolerance for antifungal agents and immunological variations, making treatment with conventional therapeutic agents difficult [1]. The technology of non-thermal plasmas at atmospheric pressure has been increasingly used in studies aimed at the eradication and control of fungal contamination [2]. This study evaluated the action of a plasma jet generated by a surfatron source using three different gas compositions on polyurethane samples contaminated with biofilms of *C. albicans* (A) and *C. parapsilosis* (B). The samples were treated with plasma of 4L / min of Argon + 6L / min of Air in 100W of power (group 1), 4L / min of Argon + 9L / min of Argon with water vapor in 50W (group 2) and 4L / min of Argon + 9L / min of Argon with water vapor in 150W (group 3). The treatments were performed in the post-discharge region (3 cm) during 10 min. The characterization of the plasmas and the samples was performed by optical emission spectroscopy of plasma, substrate surface temperature monitoring by IR camera during the treatment, determination of colony forming units (CFU) and microorganisms morphology by scanning electron microscopy. Results showed that for *C. albicans*, the plasmas of group 1 and 3 reduced the count of CFU / mL in 100% and for *C. parapsilosis*, the best treatments were with group 2 and 3 that reduced 92, 41% and 97.85% of the contamination. Morphological changes were observed in the biofilm cells when analyzed in SEM, such as the presence of volumetric deformation and cells that appear to have been lysed. The results obtained in this study will be compared with previous works of our research group using a gliding arc system [2], [3]. In sum, although the results varied for the studied microorganisms, the process parameters used are adequate to control the contamination, since they presented a high rate of reduction with a low increase of polyurethane surface temperature (lower than 40°C) during sterilization process.

This work was supported by FAPESP (grant nº 2015/10876-6) and PROSUC-CAPES-Univap.

References


Underwater multiple hole dielectric barrier discharge (MHD) system for mass waste water purification and sterilization

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The underwater plasmas have been highly considered for water treatment technology due to its high chemical reactivity. In recent years, many researchers have developed technology to generate plasmas in water conditions [1-3]. However, developed technology have the disadvantage such as high power consumption, requirement of pulsed power supply [1, 2]. In this study, we propose a MHD system for mass water treatment with low power consumption.

The electrode of MHD system is consists of three components; they are a long stainless steel rod, an inner quartz tube, an outer quartz tube having multiple holes. An inner quartz tube wrapped the stainless steel rod and they are located in center of an outer quartz tube. Once air is introduced through the gap of between an inner and outer quartz tube, and ac high voltage is applied, a discharge is fired in the gap between the quartz tubes. And, the generated plasmas are ejected to liquid through multiple holes. In order to evaluate the efficiency of MHD system for water purification and sterilization, we characterized the property of water after treatment and measured the sterilization rate.

Fig. 1. The photo image of MHD system during discharge(lest) and sterilization results of Vibrio harveyi (right)

This work was supported by basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Minist (NRF-2016R1C1B 2013597)

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Bactericidal effect and characteristics of developed porous-discharge plasma jet in water

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In recent years, water pollution is in progress at a serious level in many parts of the world. Therefore, there is a demand for a technique capable of effectively sterilizing harmful microorganisms in the water. Thus, we developed a micro-discharge plasma jet in previous study [1]. It showed good performance of bacterial disinfection in water with low power and simple structure. Nevertheless, more high density of active radical and plasma jet are required for faster and effective water purification.

In this regard, we have developed a porous-discharge plasma jet that generates higher density and stable plasma jet in water and produces a large amount of active species which has strong chemical disinfection ability [2]. We analyzed voltage and current waveform that the multi current peaks were detected in one cycle. The voltage and current were \( V_{rms} = 1.99 \text{ kV} \) and \( A_{rms} = 244 \text{ mA} \) at 4 L/min, respectively. The porous plasma jet generates higher density plasma jet, but it also showed relatively high power consumption. We confirmed the sterilization results of E.coli which is a typical pathogenic microorganism using porous-discharge plasma jet by air as discharge gas was over 99.9%. In order to analyze the concentration and type of active species generated from the plasma jet, optical emission spectrum, gas analyzer and photometer were used.

We have confirmed that the efficiency of water purification by bacterial inactivation was improved by comparison with the previous plasma jet.

![Fig. 1. Photographs showing the stable porous-discharge plasma jet generated at the flow rate of 2-5 L/min of air gas](image1)

![Fig. 2. Voltage and current waveform of the porous-discharge plasma jet at the flow rate of 2-5 L/min of air gas](image2)

This work was supported by basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Minist (NRF-2016R1C1B 2013597)

References

Investigation of bacteria sublethal injuries in liquid by He/O$_2$ plasma jet

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Plasma decontamination techniques are able to induce in microorganisms a special physiological state known as “viable but non-culturale” (VBNC) state [1]. In this state, the bacterial cells are metabolically active but they (temporally) loose their capacity to produce colonies when cultured on classical media. When bacterial inactivation fails, another outcome is the sublethal injury of the bacterial cells, a transient state in which the bacteria are able to repair and restart growing only if they are found in suitable environmental conditions [2]. Several studies have shown that microorganisms involved in foodborne diseases can suffer sublethal injuries after exposure to preservation treatments such as heating, radiation or oxidative treatments [2]. This study was designed to assess the capacity of He/O$_2$ plasma jet to produce sublethal injuries in bacterial cells.

The experimental setup consists of an asymmetric APPJ. The high-voltage electrode is placed around a 3.7 mm inner diameter tube, and the grounded electrode is placed around the 36 mm outer diameter tube. The feeding gas is a helium-oxygen mixture (0.2% O$_2$) at a 0.5 l.min$^{-1}$ flow. The power supply provides a 6 kV positive voltage pulse with 8 ns rise time, 2.5 µs duration and 20 kHz frequency. Saline solution (0.9% NaCl) was used in this study as target for plasma treatment. The distance between the jet and the liquid target (6 mm) is small enough for the plasma to touch the liquid.

The first part of this work concerns the characterization of the plasma source by measuring the concentration of several RONS such as nitrites, nitrates, hydrogen peroxides and by measuring the pH of the liquid target. Colorimetric assays were used to determine the concentrations of RONS in the liquid phase. In the second part of the study, the ability of *Escherichia coli* ATCC 25922 and *Bacillus subtilis* ATCC 6633 bacterial cells to undergo sublethal injury following plasma treatment was assessed. Stationary phase cultures were plated on TSA plates containing various NaCl concentrations in order to determine the highest concentration that does not impair the growth of the untreated cells. Thereafter plasma treated samples were used to inoculate on TSA plates (complete media) and TSA containing NaCl plates (selective media). Sublethal injury was detected based on the increased sensitivity to NaCl compared to untreated samples. Furthermore, LIVE/DEAD BacLight (Invitrogen Molecular Probes) bacterial viability kit is used to differentiate bacteria with damaged membranes and those with intact membranes.

This work was supported by Occitanie region.

References


Study on degradation of pharmaceutical residues by underwater discharge plasma

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In recent, water pollution caused by pharmaceutical residues are being considered as a serious environmental problem. Especially, the contamination with pharmaceutical compounds have seriously detrimental effects on human health and aquatic ecosystem even at infinitesimal concentrations. With lack of awareness of the pollution caused by pharmaceutical and of the severity, however, the need for purification of this pollution is not widely known. Conventional water treatment methods is ineffectual to remove pharmaceutical residues in water. For that reason, recent studies on remediation of water pollution by pharmaceutical have focused on the ozonation and advanced oxidation processes (AOP), and there are other methods using Fenton’s reaction, photocatalysis, and non-thermal plasma. However, there are pharmaceutical residues that cannot be decomposed by this methods. In this sense, we investigated the degradation of recalcitrant pharmaceuticals (diatrizoate, diclofenac, ibuprofen, and carbamazepine) which are difficult to decompose, by an underwater plasma discharge. The underwater plasma generator with a coaxial geometry was specially designed to generate large volume non-thermal plasma in water. Through this method, the ability to decompose and purify pharmaceutical residues has been confirmed, showing the degradation rates from 70 to 95% for the treatment times of 10 – 60 min.

Acknowledgement

This work was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science, ICT & Future Planning (PG1714)

References

Decomposition Characteristics of Spore Substance Using RF Oxygen Plasma
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1. Introduction
Recently, plasma sterilization method at low-temperature suppressing harmful gas has attracted much attention. Detailed mechanism of spore forming bacteria inactivation owing to plasma irradiation has not been clarified. One of the inactivation mechanism of bacterial spore is speculated to be decomposition of depicolinic acid (DPA) that is a major substance of the spore. DPA occupies 10-15% of the dry weight of spores. DPA forms Ca-DPA chelate during sporulation, and there is relationship between the dissociation of Ca-DPA and the loss of heat resistance of the spore [1]. In this study, the decomposition charasteristics of DPA is investigated to clarify the inactivation mechanism of spore.

2. Experimental procedure
Dimension of the vacuum vessel is 200 mm in inner diameter, 500 mm in length and 17 L internal volume. Inside the vessel, distance between RF discharge electrode and object to be sterilized is 47 mm. After evacuation, oxygen gas is introduced and radio-frequency power (13.56 MHz) is supplied to the electrode to generate capacitively coupled plasma (CCP). Increase of the temperature due to heating of the electrode is suppressed by the water circulation system inside the electrode.

Dipicolinic acid aqueous solution with a concentration of $3.0 \times 10^{-5} \text{ g/cm}^2$ is coated on a CaF$_2$ plate and dried. DPA sample is enclosed in a sterilized bag and irradiated by the oxygen plasma. Irradiation conditions are RF input power of 130 W, irradiation time of 50 minutes and pressure ranged from 50 to 400 Pa. The decomposition rate is estimated from the largest C═O bond peak of dipicolinic acid at around 1700 cm$^{-1}$ in IR spectra those are measured by fourier transform infrared spectrometer (FTIR).

3. Results and discussion
Figure 1 shows IR spectra of DPA after plasma irradiation according with non-treated DPA. No significant change in DPA composition is observed before and after plasma irradiation, and formation of by-products is not observed in IR spectra. Figure 2 shows decomposition rate of dipicolinic acid changing oxygen pressure. The C═O bond in dipicolinic acid is decomposed with pressure decrease. Maximum decomposition rate is 3.5 % that obtained at 50 Pa, lowest pressure in this experiment. Decomposition of C=O is probably owing to dissociation of the C=O double bond by collision with high energy particles. Another inactivation mechanisms of spore have been also investigated as well as decomposition of dipicolinic acid. Possible factors of inactivation mechanism of spores include destruction of spore due to collision with high energy particles, denaturation of proteins constituting bacterial spore and DNA damage due to active oxygen species and UV light.

Reference
The duration of anti-microbial activity of a solution treated with the non-thermal atmospheric-pressure N$_2$ plasma

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This paper presents the anti-microbial activity of the Phosphate buffer solution (PBS) treated with non-thermal atmospheric-pressure plasma. The plasma-jet micro-nozzles were fabricated by micro-machining. The PBS of 1 ml was treated with plasma for 5 minutes on the surface. The concentration of ozone dissolved in the PBS treated with N$_2$ plasma is about 4 times that of air plasma. The dissolved ozone concentration decreases gradually with the time lapse. The outstanding anti-microbial activity of the N$_2$ plasma-treated PBS to \textit{Pseudomonas aeruginosa} and \textit{Staphylococcus aureus} lasts for 24 hours at least.

![Figure 1](image1.png)

(a) The schematic view of the experimental setup for plasma treatment of PBS. (b) The photographs and the light intensity profiles of the plasma discharge for N$_2$ and air

![Figure 2](image2.png)

Figure 2. The concentration change of the dissolved ozone in PBS after the plasma treatment

![Figure 3](image3.png)

(a) The duration of anti-microbial activity of the plasma-treated PBS to (a) \textit{Pseudomonas aeruginosa} (b) \textit{Staphylococcus aureus}

Reference
Assessing effect of Atmospheric cold plasma on stress responses of Listeria monocytogenes mutants

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Listeria monocytogenes EGD-e strain is a persistent strain of the 1/2a serotype which commonly associated with multiple food-borne outbreaks. It is critical to understand how foodborne pathogens like Listeria monocytogenes contaminate fresh produces and survives the disinfection processes. Different environmental factors and food intrinsic factors are known to influence the resistance of the cells towards the treatment. Since environmental conditions exert a great influence on bacterial cell physiology, it was found that the exposure to different physiological stresses may directly affect the virulence of foodborne pathogens like L. monocytogenes. Atmospheric cold plasma (ACP) has proven to be great potential as food disinfection technology. Therefore, in this study we investigated the influence of ACP treatment (80kV, 50Hz) on L. monocytogenes (EGD-e) and its knockout mutants of sigB, rsbR, prfA, gadD and lmo0799 genes. Further, to ascertain if sub-lethal stress exposure could influence L. monocytogenes behavior, ACP resistance was evaluated for the cultures exposed to cold (4°C) or acid (pH 4) stress for 1h.

The wild type and mutants showed similar results with regards to reduction levels, after plasma exposure of 1min and post treatment storage time of 1h. All L. monocytogenes strains exposed to acid/cold stress were hypersensitive to ACP treatment and were significantly reduced or inactivated within 1 min of treatment (p<0.05). While non-stress bacterial strains showed significant reduction of 2.0 Log_{10} CFU/ml after 1 min of ACP treatment (p<0.05). By comparing the response of mutants under ACP exposure to key processing parameters, the mechanism of microbial inactivation was partly revealed with further insight into biological mechanisms of alterations in the cell induced by ACP treatment.

This work was supported by Food Institutional Research Measure (FIRM) administered by Department of Agriculture, Food and the Marine, Ireland (DAFM 13/F/444).
For reusing catheter and endoscopes at medical settings, sterilization treatment under low temperature circumstance is required. Recently, plasma sterilization methods for non-heat resistive narrow tubes with clean source gases has been studied. [1, 2] In this study, we have investigated sterilization characteristics of tubular medical devices by using low pressure oxygen plasma to determine sterilization factors and period required.

A cylindrical stainless chamber is used for the medical devices sterilization. The size of the chamber is 210 mm of inner diameter and 500 mm of length. The chamber is evacuated by a vacuum pump and oxygen gas is filled to designated pressure. RF power (13.56 MHz, 80W) is applied to the RF electrode to generate capacitively coupled plasma. The process challenge devices (PCD) is placed on the 130 mm below the top of electrode. PCD consists of a capsule connected to a silicone rubber tube with 100 mm to 500 mm length, 1 and 4 mm diameter. Sterility assurance was confirmed by a biological indicator (BI) that consists of thermophilic spore of *G. stearothermophilus* with population of $10^6$. Amount of active oxygen species reaching the BI in the capsule is measured by the color of chemical indicator (CI). Since ions in a plasma may damage medical devices, PCD is enclosed in a sterilization bag for preventing the ion impact during plasma treatment.

The largest color change of the CI, which indicates the maximum production of active oxygen, is observed at the oxygen gas pressure of 20 Pa when pressure varies from 20 Pa to 140 Pa. Since the pressure dependence on the sterilization rate of BI has similar tendency to the CI color variation, active oxygen species could be one of the sterilization factor for tubular devices sterilization. As shown in Table 1, sterilization of BIs are successful for 60 to 120 min, when BIs are placed at 100 mm and 200 mm in the tubes with diameter of 4 mm. It is necessary to be irradiated for 180 min to sterilize BI in the 1 mm diameter tube. As the result of the CI color change in 1 mm diameter tube, active oxygen species cannot reach the PCD even for 180 mm treatment. Therefore, one of candidate sterilization factor in 1 mm catheter would be the active species without the interaction with CI. Effects of heat and ultraviolet radiation have also been investigated. BI test results indicate that the heat treatment as the same temperature of plasma sterilization and the ultraviolet lamp irradiation cannot sterilize the tubular medical device.

### References


Dose-Dependent Modulation of Plasma Activated Solution on Minimum Inhibitory Concentration of Antibiotics

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Chronic exposure of a microbe to a sublethal antibiotic is known to elevate progressively the minimum inhibitory concentration (MIC) of the antibiotic against the microbe, ultimately leading to drug resistance. Sublethal exposure to one antibiotic can also confer resistance to a microbe to another antibiotic. Gas plasmas and plasma-activated solutions (PAS) have been extensively shown to rapidly eradicate a diverse range of microbes, and are therefore increasingly used as a novel antimicrobial agent in the healthcare arena. This increased utility raises important questions, for example whether microbes would develop resistance to PAS, and whether PAS-treated microbes would accelerate their acquisition of antibiotic resistance. To begin to answer these questions, we investigated whether E. coli pretreated with sublethal PAS may evolve in their minimum inhibitory concentration of PAS itself and also of three different classes of antibiotics. PAS pretreatment of E. coli in our study was administrated daily for 5 days first and then confirmed with long-term experiments for 20 days. Our data show that it took longer for the MIC of PAS to rise than any of the three antibiotics tested. When both PAS and antibiotics were used, PAS pretreatment increased the MIC of antibiotics in most cases. However, at some sublethal doses of PAS, MIC of ribosome-targeting antibiotics were found to fall. Results of our study are presented and possible mechanisms are discussed.
Biofilm Sterilization of Otorhinolaryngological Endoscopes by Synergistic Treatment of Atmospheric Pressure Plasma and Chelating Agents

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In this study, we investigate the bacteria reduction and surface defecton by synergistic treatment of atmospheric pressure plasma (APP) and chelating agents. Conventional sterilization methods of otorhinolaryngological endoscopes, e.g. tele laryngoscope, are usually moisture methods, mostly autoclave. The main drawbacks of moisture methods are time consumption and material damage. APP and chelating agents had been shown to be effective in bacteria biofilm inactivation [1, 2]. While chelating agents disrupt the biofilm formation through bonding metal ions, APP sterilizes bacteria mainly through reactive species. In our study, we treated Escherichia coli, Enterococcus faecalis, and Staphylococcus capitis on stainless steel and glass surfaces with chelators of trisodium citrate (TSC), ethylenediaminetetraacetic acid (EDTA), egtazic acid (EGTA), and alizarine in combination with APP. The plasma source is a cylindrical shape surface plasma discharge chamber which is designed to contain otorhinolaryngological endoscopes and driven by 2 kHz and 9 kV. The electrical characteristics were examined and ozone density were measured by absorption method. Surface defects were investigated by X-ray photoelectron spectroscopy (XPS) after treatment of both plasma and chelating agents. Results indicates combination of chelating agents and APP increased reduction of bacteria biofilm by 0.5 to 1 log especially for TSC. While single treatment of TSC revealed no significant inactivation of biofilm, synergistic treatment resulted in around 1 log higher sterilization rate.

References

Experimental study of the influence of the target conductivity on the biocidal efficiency of a plasma jet

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In recent years, cold atmospheric plasma technologies attracted a lot of interest for decontamination due to their capacity to produce biocidal agents such as reactive species and UV emission while keeping the temperature relatively low. Working gas, power supply and exposure time are well-known parameters influencing the biocidal efficiency of these device. Recently it was reported that the type of surface placed in front of plasma source affects the properties and the biocidal capacity of the plasma [1-2]. The aim of this study is to investigate the effect of target conductivity on the biocidal efficiency of a plasma jet.

The plasma source used for this work has an asymmetric geometry that is composed of two parts - a cylindrical dielectric chamber and a dielectric tube with a smaller diameter. The source is operated with a helium gas flow of 2.0 L/min and powered by 20 kHz square voltage of 2 kV. In order to evaluate the influence of the target conductivity on the biocidal effect of the plasma, Bacillus atrophaeus endospores were used as biological indicator. Endospores were inoculated on the surface of a dielectric (glass plate) and a conductive (copper plate) targets and exposed to the plasma jet at different exposure time. The survival curves show a higher biocidal efficiency of the plasma jet when the conductive target is used (fig. 1). The mathematical modeling of the dynamics of survival revealed biphasic inactivation kinetics for both conductive and dielectric targets. Additional results regarding the impact of target conductivity on jet capacity to generate sub-lethal injuries as well as an assessment of the spores morphological changes following plasma exposure will be presented and discussed.

![Survival curves of Bacillus atrophaeus treated with the atmospheric pressure plasma jet device](image)

This work was supported by Occitanie region.

References

Development of non-thermal plasma applications to the treatment of poly- and perfluoroalkyl substances (PFASs), an emerging class of water contaminants

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Poly- and perfluoroalkyl substances (PFASs) are ionic organofluorine (C-F) surfactants that exhibit unique physical and chemical properties, such as hydrophobicity and oleophobicity, which contribute to its use in a variety of industrial and consumer applications. Due to growing evidence that PFASs are persistent in the environment, bioaccumulative, and toxic, many state, federal, and international governing bodies have begun regulating some compounds, such as perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). One of the major sources of these contaminants in soils, sediments, surface waters, and groundwater are due to the use of aqueous film forming form (AFFF), which can be used to extinguish fuel-based fires. Although PFASs can be removed from water by adsorptive process (i.e., using activated carbon or resins), they are recalcitrant to degradation by many conventional forms of water treatment and advanced oxidation processes. Only a small number of technologies have shown promise of degrading PFASs (1-5), many that produce conditions that promote reductive degradation of PFOS and/or PFOA. One of these promising technologies includes the use of laminar corona jet plasma with bubbling (1). Here we present preliminary results demonstrating the application of dielectric barrier discharge (DBD) plasma technology to the treatment of PFAS contaminated water. Optimal plasma regimes and gases that achieve significant mineralization of PFASs to F- and CO2 are described.

References

Effect of liquid contaminants on sterilization by CO₂ plasma bubbled-up water

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Atmospheric cold plasma indicates high bactericidal effect, and the bactericidal factor is considered as reactive species. The afterglow of plasma can introduce reactive species into water by bubbling. This water contained reactive species shows bactericidal effect, and we called it Plasma Bubbled-up Water (PBW). In our laboratory, bactericidal effect of PBW generated by various gas species has been investigated. It was found that CO₂ PBW showed a high bactericidal effect. In sterilization using CO₂ PBW, survival bacteria were decreased quickly after mixing PBW and bacterial suspensions. The bactericidal factors of CO₂ PBW are expected to be the reactive species having lifetime less than 1 hour, and it is difficult to directly detect them. In this study, the bactericidal effect of CO₂ PBW mixed some liquid contaminants was investigated due to reveal the bactericidal factors.

A setup of PBW generation is shown in Fig.1. Atmospheric plasma jet is placed above a container including 50 mL of pure water. CO₂ PBW was generated with CO₂ plasma bubbling at 3 L/min gas flow rate through a porous filter for 2 min. As investigation of bactericidal effect, 890 µL of CO₂ PBW, 100 µL of pure water including liquid contaminant, and 10 µL of bacterial suspension including *Escherichia coli* ATCC25922 (*E.coli*) at ca.10⁷ CFU/mL were mixed. L-Histidine that is reported deactivating effect of O₂ was used as liquid contaminants, and dissolved in pure water so that the concentration after mixing is 0-10 mM. After 10 min from the mixing, the number of survival bacteria in the solution was examined by colony-counting methods.

The number of surviving *E.coli* was decreased less than detection limit without L-Histidine as shown in Fig.2. Decreasing of bactericidal effects was not confirmed less than 10 µM of L-histidine. When L-histidine is 100 µM, decreasing of bactericidal effects was confirmed. No bactericidal effect was confirmed above 1 mM of the L-Histidine. These results indicate that the reactive species of bactericidal factor was inactivated by L-Histidine. In the presentation, influences of other liquid contaminants on bactericidal effect of CO₂ PBW will be reported.

![Fig. 1 Setup of plasma bubbling method](image1)

![Fig. 2 Effect of L-histidine on the bactericidal effect](image2)
Surface Sterilization with a Pin-to-Hole Spark Discharge (PHD) Array

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There are many standard sterilization techniques (i.e. – thermal (wet and dry), chemical, or ionizing radiation) that offer high reliability, but also require dangerous handling conditions, indiscriminately treat the entire object, or damage the substrate in some applications [1,2]. If a sample collection vessel required sterilization of the exterior surfaces for safe transportation, but the sensitive samples it contained must remain unaffected, a more selective method is required. Non-thermal plasma sources have been shown to provide the oxidizing species and UV radiation for decontamination of bacteria and spores without adversely affecting the substrate [1,3]. Typically, these discharges operate at room temperature and atmospheric pressure. This work sought the optimal spacing and necessary time for treatment for an arrayed Pin-to-Hole discharge (PHD) plume to decontaminate a surface inoculated with *Escherichia coli* and sporulated *Bacillus atrophaeus* without damaging the surface. The spark reaches thousands of Kelvin, but the pulsed nature of the discharge and subatmospheric gas flow prevent the produced plumes from reaching much higher than ambient temperatures [4]. Zones of decontamination can be overlapped for selective treatment without damaging the surface and can be applicable to spores.

Fig. 1: Picture of the complete cylindrical array of Pin-to-Hole spark discharge (PHD) testing chamber. A maximum of 10 inches in diameter could be treated within. The chamber was evacuated down to approximately 10 Torr. The discharges displayed here were in air flowing at a combined flow rate of 0.1 SLPM.

This work was supported by Jet Propulsion Laboratory subcontract # 1571756.

References

Eliciting the Mode of Bactericidal Action of Cold Atmospheric Plasma

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The field of plasma medicine is rapidly expanding, with a wide scope of potential applications throughout the medical field. One application of Cold Atmospheric Plasma (CAP), is its’ antimicrobial properties. CAP jets are known to produce a wide range of Reactive Oxygen and Nitrogen Species (RONS) including hydroxyl radicals (OH) and free oxygen radicals (O). Whilst it is widely accepted within the plasma community that CAP jets kill bacteria, the precise mechanism of kill is still hypothesised. A leading theory is that killing is primarily mediated by Hydrogen Peroxide ($\text{H}_2\text{O}_2$), which is produced as a result of the RONS.

Here we report that CAP jet bacterial killing is predominantly the result of $\text{H}_2\text{O}_2$ production. $\text{H}_2\text{O}_2$-resistant mutants were compared to non-$\text{H}_2\text{O}_2$-resistant bacteria to assess susceptibility to CAP jet killing. The difference in killing was assessed using reduction in CFU/ml of bacteria in planktonic and biofilm.

The body’s endogenous immune response to invading pathogens involves the production of $\text{H}_2\text{O}_2$ to cause oxidative stress and results in bacterial death. Bacteria have evolved a mechanism to resist oxidative stress from the host immune system. The enzyme catalase causes oxidation of $\text{H}_2\text{O}_2$ into harmless $\text{H}_2\text{O}$ and $\text{O}_2$. Catalase negative bacteria, for example Streptococcus species are more susceptible to CAP Jet killing due to their inability to mediate the Oxidative stress from CAP jet produced $\text{H}_2\text{O}_2$. This provides further evidence that CAP jet killing is primarily the result of $\text{H}_2\text{O}_2$ production.

Going forwards, we intend to investigate the effects of serial passaging of CAP jet treatment on bacteria, looking predominantly at Pseudomonas aeruginosa and Staphylococcus aureus species, to examine how long it would take bacteria to mount resistance to the CAP jet treatment, and how this compares to antibiotics and $\text{H}_2\text{O}_2$ alone. Once we have generated CAP resistant mutants, whole genome sequencing can be carried out to understand where in the bacterial genome mutations have occurred, which again could help us further understand the mechanism of bacterial killing by the CAP jet.

This work was supported by EPSRC funded project no. EP/P003939/1 (Smart Wound Plasma). I myself am funded by The James Tudor Foundation and University of Bath Alumni-Alistar Watson

An atmospheric cold plasma jet was designed to treat the antibiotic resistant bacteria using a low-temperature plasma-based sterilization process. In this work, enterococcus faecalis bacteria were plasma sterilized through the Taguchi method to determine the optimum sterilization parameters. After plasma treatments, the result of colony-forming unit showed that the efficiency of the sterilization process is dependent on the applied power, exposure time, and selected gases. Optical emission measurements indicated that the reactive species such as O and OH played significant roles in the plasma sterilization process.

References


Plasma Activated Water Obtained by a Low-cost Gliding Arc Reactor for the Inactivation of *Escherichia coli*

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Foodborne illness caused by the consumption of contaminated products has increased during recent years [1]. On these products surfaces, pathogens that are able to survive for long periods of time, such as *E. coli* and *Salmonella* spp, have been identified [2]. The traditional way to combat this issue in the food industry is to use chemicals to sanitize and disinfect the contact surfaces of the products, which can be dangerous for the human health and the environment. Therefore, it is important to find better ways to disinfect and eliminate these pathogens avoiding the use of dangerous substances.

Gliding arc discharge leads to the formation of regions of thermal and non-thermal plasma and the formation of radicals, ions, and other reactive species, making this feature extremely important to study, as it combines the advantages of both types of plasma [3-4]. Due to these radicals, the gliding arc discharge can be used in biodecontamination and microbial inactivation. A practical way to do this, is by using a liquid treated with plasma as a medium [5]. In the proposed scenario, the use of water treated with plasma, known as Plasma Activated Water (PAW), is a promising solution to disinfect product threaten by the above-mentioned pathogens [6].

The aim of this work is to present the implementation and characterization of a low-cost gliding arc plasma reactor and the effectiveness of plasma activated water in food safety related to bacterial population. Distilled water was exposed to plasma using humid air at near room temperature and at atmospheric pressure, then used as a disinfectant on the surface of previously inoculated tomato with *E. coli*. The inactivation of *E. coli* and the generation of reactive species, as well as acidification, are discussed.

References

Correlation between the concentration of reactive species produced by a Surface Barrier Discharge plasma source and its antimicrobial effects

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In recent years the food-packaging industry has been looking for innovative disinfection and sterilization technologies. The conventional methods currently used for this purpose, such as vapor phase hydrogen peroxide and liquid chemical sterilants (e.g. peracetic acid), still present drawbacks such as the formation of toxic by-products, whose traces could be found on the decontaminated surfaces. Several studies have shown that atmospheric pressure plasmas exert an antimicrobial activity thanks to the reactive oxygen and nitrogen species (RONS) generated in air [1] without the involvement of heat and chemical reagents. In this work, a Surface Barrier Discharge (SBD) is used for the decontamination of polypropylene (PP) strips, a thermo-sensitive material commonly used for food packaging. The antimicrobial potential has been tested against Bacillus atrophaeus and Aspergillus niger endospores using different power densities (W/cm²). Several studies report that SBDs operating in air at low power densities (<0.2 W/cm²) allow to produce an ozone enriched atmosphere, while at higher power densities the ozone enriched atmosphere changes in time into a NOx enriched one due to the so-called discharge poisoning (also known as ozone quenching). Following these considerations, by varying the applied power density, two different operating modes of the SBD have been identified (O₃ mode at power density of 0.14 W/cm² and NOₓ mode at power density of 3.2 W/cm²). SBD operation has been investigated in terms of O₃ and NO₂ kinetics; these were analyzed by means of optical absorption spectroscopy (OAS) [2], a non-invasive optical technique, free of calibration procedures and well suited for the on-line monitoring of the gas phase concentration of RONS. The antimicrobial effects of the plasma treatment have been evaluated for both operating modes at different treatment times; higher inactivation levels were obtained in NOₓ mode than in O₃ mode. Spores’ morphology was evaluated by means of scanning electron microscopy. The achieved results have shown the correlation between the RONS concentration and the antimicrobial activity of the SBD, eventually emphasizing OAS potentialities as a technique for monitoring a possible industrial decontamination process.

Reference

Characterization of horseradish peroxidase after discharge plasma treatment

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Peroxidases are a class of heme-employing enzymes that scavenge hydrogen peroxide to oxidize a variety of substrates. Horseradish peroxidase (HRP) is the most intensively studied peroxidase with regard to its structure and catalytic cycle [1, 2]. HRP is often used in diagnostics and biotechnology because of its extended lifetime while retaining high catalytic activity, as HRP is naturally secreted to the extracellular space, warranting remarkable stability [3]. We therefore investigated whether HRP stays active after treatment with a DBD plasma device that was designed for use in dermatology [4].

A buffered solution of HRP was plasma-treated and then added to a chromogenic substrate and H₂O₂ to allow for activity measurements. Enzyme activity decreased significantly with increasing treatment times, while no activity was detectable after 10 min (Fig. 1A). In order to investigate the origin of activity loss, circular dichroism (CD) spectroscopy was employed to check for protein unfolding. Even after 10 min of plasma treatment, no protein unfolding was detectable, indicating that the apoprotein stayed intact (Fig. 1B). Consequently, heme damage was studied by UV-VIS spectroscopy. Heme shows a specific absorption at ~405 nm (“soret band”) that was diminished by increasing plasma treatment times (Fig 1C). As heme damage occurred substantially before protein unfolding, we conclude that loss of HRP activity is mainly due to cofactor degradation.

This work was supported by CRC 1316.

References

Investigation of Chemical Components in Plasma-exposed Organic Buffer for Drug Transfer Application

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Many medical applications of non-equilibrium atmospheric pressure plasma (APP) have been reported and one of them is a drug transfer tool. We have ever demonstrated membrane-permeabilizing effects of APP exposure and clarified the underlying mechanism based on the interaction of reactive species and charged particles with the cell membrane and the molecule permeation dynamics through the cell membrane [1-3]. Our previous results indicated that short-lived reactive species in APP-exposed organic-buffered saline could induce calcium ion influx and uptake of a middle-size membrane-impermeable molecule. However, the key chemical component remains unclear due to extremely complicated chain reaction in the saline. Therefore, we have investigated the composition change in the APP-exposed saline for identification of the key chemical component.

APP was generated using low frequency (LF) (frequency: 8 - 10 kHz, voltage: 5 - 12 kV) with Helium gas flow, which was exposed to the saline. The plasma-irradiated solution was put on a hot plate (37°C) for a retention time $t_r$, and added to mouse fibroblast cells 3T3-L1 (indirect plasma irradiation) or added to peroxynitrite(ONOO$^-$) detection probe NiSPY-3. Real-time changes in the intracellular amount of calcium ions ([Ca$^{2+}$]$i$) and YOYO-1 ([YOYO]$i$) were obtained using a calcium indicator fluo-4 and the drug-mimicked fluorescent dye YOYO-1.

Figure 1(a) shows the ability of the plasma-exposed saline to generate ONOO$^-$ as a function of the retention time $t_r$. ONOO$^-$ was significantly generated even 60 s after completion of the plasma exposure process although the half-life of ONOO$^-$ should be about 1.9 sec at 37°C and pH 7.4 [4], which indicates that ONOO$^-$ was released after the plasma exposure. In addition, the ONOO$^-$ generation ability decreased over time. As shown in Fig. 1(b), either [Ca$^{2+}$]$i$ or [YOYO]-1 decreased with increasing $t_r$, suggesting the key component decrease over time. Therefore, the observed ONOO$^-$ generation could be related to the chemical reaction with the key component. In the presentation, I will show other evidences that some radical chain reactions deactivated over time.

High-throughput combinatorial screening identifies mitochondrial targeting drugs that show synergistic effect with cold physical plasma

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Cutaneous melanoma is a highly aggressive malignancy and has rapidly increased over the past several decades [1]. Despite advances in melanoma therapy the success in drug treatment of disseminated disease remains limited, being often used only as a palliative method [2]. The use of combination therapies is an emerging field and may be a strategy to overcome resistance and decrease toxicity of melanoma treatment. Cold physical plasma effluent generates reactive oxygen and nitrogen species [3] in cell culture media [4] and selectively kills cancer cells \textit{in vitro}. Previously, we demonstrated that inhibiting mitochondria cytochrome c oxidase (CcO) sensitizes melanoma cells towards cold plasma induced cell death. Hence, we sought out to identify potential therapeutic targets in the mitochondria, which can show synergistic or additive effects towards melanoma toxicity. Herein we describe the results of a high-throughput combination screen of cold plasma versus a library of 48 FDA approved compounds targeting mitochondria. We generated dose response curves of the 48 compounds in murine B16F10 and human SK-Mel 28 melanoma cell lines to identify compounds with higher sensitivity towards melanoma cell lines. We then treated the cells with IC\textsubscript{20} of the shortlisted compounds for 24h followed by 30 secs exposure of cold physical plasma effluent of an atmospheric pressure argon plasma jet (kINPen). After 6 and 24 hours incubation, the combination treatment induced a progressive increase in cytotoxicity and decrease in proliferation that was accompanied by loss of mitochondrial membrane potential and reduction in the metabolic activity in both cell lines. Transwell migration assay was performed with SK-Mel 28 supernatant on THP1 monocytes, revealing increased migration and differentiation of these cells. qRT PCR revealed pro-inflammatory cytokines (TNF-a, CXCL10 and IL6) and secretion of damage associated molecular patterns (ATP, CART and HMGB1) were significantly elevated in combination therapy versus monotherapy suggestive of immunogenic cell death. In this study, we demonstrate that targeting mitochondrial pathway in conjunction with cold plasma derived oxidants is a potential therapeutic strategy in treatment of malignant melanoma.

This work was supported by grants funded by the German Federal Ministry of Education and Research (BMBF), grant number 03Z22DN11.

References


Klebsiella pneumoniae is a Gram-negative bacterium to cause a range of diseases. This pathogen is often resistant to multiple antibiotics, which becomes a great problem in hospitals worldwide. The atmospheric pressure plasma (APP) has been used as an alternative infection control approach to treat the multidrug-resistant pathogens [1,2,3]. The bactericidal evaluation of an APP jet against Klebsiella pneumoniae was studied in this work. Various helium/argon gas combinations, different voltage and frequencies of APP jet were used to study their effects on the treatment of Klebsiella pneumoniae. The relationships between the working parameters, generated plasma species and bactericidal behavior were discussed.

References

Improved fermentation efficiency of *S. cerevisiae* by changing glycolytic metabolic pathways with plasma agitation

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Production of ethanol by the yeast *Saccharomyces cerevisiae* is a process of global importance. In these processes, productivities and yields are pushed to their maximum possible values leading to cellular stress. Transient and lasting enhancements in tolerance and performance have been obtained by genetic engineering, forced evolution, and exposure to moderate levels of chemical and/or physical stimuli, yet the drawbacks of these methods include cost, and multi-step, complex and lengthy treatment protocols. Here, plasma agitation is shown to rapidly induce desirable phenotypic changes in *S. cerevisiae* after a single treatment, resulting in improved conversion of glucose to ethanol. With a complex environment rich in energetic electrons, highly-reactive chemical species, photons, and gas flow effects, plasma treatment simultaneously mimics exposure to multiple environmental stressors. A single treatment of up to 10 minutes performed using an atmospheric pressure plasma jet was sufficient to induce changes in cell membrane structure, and increased hexokinase 2 activity and secondary metabolite production. These results suggest that plasma treatment is a promising strategy that can contribute to improving metabolic activity in industrial microbial strains, and thus the practicality and economics of industrial fermentations.

![Fig. 1: Strategy for plasma induced changes and metabolic pathway analysis in yeast.](image-url)
Production of nano-materials with different nature, like carbon, non-metallic or metallic nanoparticles in plasma systems is one of the perspective directions of plasma technology. The noble metal nanoparticles (silver especially) has wide application in medicine: sterilization, healing acceleration and stabilization of bactericidal ointments. One of the effective methods to produce such particles is the processing of these metals salt colloidal solutions using electric discharge. The plasma-liquid systems with rotating gliding discharge submerged in liquid and with secondary discharge that supported by rotating gliding discharge are can be used for this task. The interest to systems with rotating gliding discharge is caused because such discharge allows to obtain non-equilibrium atmospheric pressure plasma with large cross section. This provides a large contact area between the plasma and the treated liquid. The plasma-liquid system with secondary discharge that supported by rotating gliding discharge was used in this work for production of nanoparticles of noble metals. A potential jump created by a secondary discharge above the liquid surface provides more efficient penetration of active particles into the liquid.

The scheme of plasma generator is secondary or non-self-sustained discharge (NSSD) is maintained between the liquid surface and the channel of self-sustained discharge (SSD). Rotating gliding discharge (RGD) is used in this system as the SSD. The plasma-forming gas was supplied in the discharge chamber through two supply channels tangential to the inner cylindrical wall of the reaction chamber. The rotation of the SSD occurs due to the rotation of the gas stream. The working liquid was placed below RGD in a glass vessel. The electrical potential is transferred to the liquid through the electrode at the bottom of the vessel. For the generation of discharges, DC and AC power supplies were used. Solution of AgNO₃ with addition of different concentrations of surface-active substances was used as a working liquid.

Parameters of liquids after plasma treatment were investigated by absorption spectroscopy method. The absorption spectrum of processed solutions was recorded using CCD-based spectrometer Solar TII (S-150-2-3648 USB) (operating in the wavelength range 200…1100 nm).

The Atomic Force Microscope (AFM) and Dynamic Light Scattering (DLS) measurements were used to determine the particle sizes obtained during the processing.

The sizes of nanoparticles measured a few days after processing were from tens to hundreds of nanometers.

This work was partially supported by Ministry of Education and Science of Ukraine, National Academy of Sciences of Ukraine, Taras Shevchenko National University of Kyiv.