SYMPOSIUM BM06

Plasma Processing and Monitoring for Bioengineering and Biomedical Engineering
November 26 - November 27, 2018

Symposium Organizers
David Graves, University of California, Berkeley
Emilio Martines, Consorzio RFX
Deborah O'Connell, University of York
Hajime Sakakita, National Institute of Advanced Industrial Science and Technology

* Invited Paper

SESSION BM06.01: Plasma Interaction with Living Systems and Liquids
Session Chairs: David Graves and Hajime Sakakita
Monday Morning, November 26, 2018
Sheraton, 2nd Floor, Independence West

8:45 AM *BM06.01.01
Plasma-Bio Interactions—Linking Plasma-Induced Liquid Phase Chemistry with the Biological Impact of Plasma Peter Bruggeman; University of Minnesota, Minneapolis, Minnesota, United States.

Non-equilibrium atmospheric pressure plasmas interacting with biological matter offer a unique source of highly reactive chemistry beneficial for many applications including food decontamination, wound healing and cancer treatment. Many of these applications require controlled and selective interactions. For example, in decontamination processes, the inactivation of bacteria on healthy tissue or food samples needs to occur with minimized off target effects. Controlling selectivity requires a detailed understanding of the underlying plasma-bio-interaction mechanisms. As the majority of these plasma-bio interactions occur in an aqueous environment, linking plasma-produced reactive species in this environment with biological responses is of key importance to develop controlled and selective applications of plasmas in biomedical applications.

My group, in collaboration with microbiologists, has investigated the reactive species responsible for plasma-induced liquid phase processes such as the inactivation of bacteria and virus and the interaction of plasma with mammalian cells using a well-characterized atmospheric pressure plasma jet with a known composition of the reactive species in the gas phase. As in several other reports we found the importance of long-lived species such as H_2O_2 and OCl^-. However many results indicated the direct or indirect importance of short-lived species. We will discuss these results in detail illustrating the unique character of the plasma treatment and also outline the strong possible sensitivity of plasma-bio interactions on treatment modalities.

Acknowledgements
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9:15 AM *BM06.01.02
Investigation of Damage to Nucleic Acids Induced by Plasma Irradiation Hirofumi Kurita, Tomoko Nakajima, Kaori Sano, Saki Miyachika, Yoshito Uchihashi, Natsuki Haruta, Hachiro Yasuda, Kazunori Takashima and Akira Mizuno; Toyohashi University of Tech, Toyohashi, Japan.

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. 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. Over the last decade, many studies have attempted to characterize DNA damage and the associated cellular responses induced by plasma irradiation. In the early stage of the investigation, most of the reports used isolated plasmid DNA molecules in liquids and the analysis was based on gel electrophoresis. For example, it has been reported that oxidative damage is induced by exposure to the plasma, resulting in single-strand breaks (SSBs) and double-strand breaks (DSBs) separated by conventional agarose gel electrophoresis [1]. In recent years, the analysis of genomic DNA in the plasma-irradiated cells were reported. For example, the single cell gel electrophoresis assay, also known as the comet assay, is a versatile method for measuring DNA damage. Although gel electrophoresis is relatively inexpensive and easy to perform, it requires long run times. Therefore we have developed non-electrophoretic methodologies. Our first investigation is a single-molecule-based method for evaluating strand breaks in large linear DNA molecules that allows the length of individual DNA molecules to be measured [2]. In this investigation plasma-induced DNA breakages have been kinetically analyzed. However, the single-molecule method requires the acquisition and processing of many fluorescence images for reliable analysis. We have also investigated rapid detection of DNA strand breaks induced by plasma irradiation using a molecular beacon (MB) [3]. 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 stem by plasma irradiation leads to separation of the fluorophore-quencher pair, resulting in an increase in fluorescence that directly correlates with the extent of DNA strand breaks. In addition, we reported that a plasma jet readily induced DNA strand breaks in synthetic models of tissue and cells, surprisingly without any significant rupture of the phospholipid membrane [4]. Furthermore, the feasibility of MB-based methodology for detecting intracellular DNA damage was investigated. Our novel methodology may allow investigations of the effects of atmospheric pressure plasma on DNA damage-associated cell death in plasma treatments.

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9:45 AM BM06.01.03
Effects and Mechanism of Electric Stimulation Through Carbon Nanowalls Scaffold on Proliferation and Differentiation of Cultured Cells Hiroki Kondo[1], Tomonori Ichikawa[1], Kenji Ishikawa[1], Hiromasa Tanaka[1], Takayoshi Tsutsumi[1], Keigo Takeda[2], Makoto Sekine[1], Masaru Hori[1] and Mineo Hiramatsu[2]; [1]Nagoya Univ, Nagoya, Japan; [2]Meijo University, Nagoya, Japan.

In recent years, nanomaterials, such as carbon nanotubes, graphene nanoflakes, and so forth, have attracted much attention as a cell culturing scaffold. It has been reported that an introduction of nanometer level fine structures affects cell differentiation induction. Very recently, it has also been reported that an electric stimulation through conductive scaffold of resin containing carbon nanotubes can affect proliferation and differentiation of cultured cells. Based on these backgrounds, we have focused on carbon nanowalls (CNWs), in which multiple layers of graphene sheets are vertically grown on a substrate and form randomly intricate wall structures like maze. Due to their unique morphology, the CNWs have very high aspect ratio over 100 and high specific surface area. In addition, they also have high conductivity and robustness against chemical treatments. Therefore, the CNWs are promising as electrically conductive cell culturing scaffold with nanometer level fine structures. We have reported that the wall density and the chemical termination of CNWs influence the proliferation rate and morphology of HeLa cell. Furthermore, electric stimulation through the CNWs scaffold increased proliferation rate of human osteoblast-like cells (Saos-2) and suppress their ossification only if frequency of electrical stimulation was 10 Hz. In this study, the effects and mechanisms of such the electrical stimulation through the CNWs scaffold with the different wall densities were investigated. Proliferation rates and intra- and extra-cellarer calcium amounts were measured for the Saos-2 cells cultured on the CNWs scaffold with the electric stimulation.
The CNWs were grown on Ti substrates by a radical injection type plasma excited chemical vapor deposition (RI-PECVD) system. In order to change the wall densities, the total pressures during the growth were controlled from 1 to 3 Pa, which realizes density controls of hydrogen and methyl radicals. According to scanning electron microscopy (SEM) images, distances between neighboring walls were 208 and 341 nm, when the total pressures were 1 and 3 Pa, respectively. Saos-2 cells were cultured on these CNWs scaffolds in an environment at 37°C and with CO2 concentration of 5%. Electric stimulation with a frequency of 10 Hz, square wave shape and a peak-to-bottom voltage of 226 mV was supplied for 24 hours after seeding the cells. Then, after culturing for a total of 100 hours, the number of cells and morphology were observed. A 58% increase in proliferation rate was observed on the CNWs with a wall density of 341 nm, while that on the CNWs with a wall density of 208 nm hardly changed. At the same time, it was confirmed that aggregates of cells were formed only on the on the CNWs with a wall density of 341 nm. This suggested that an intercellular adhesion can be controlled by the electrical stimulation on the CNWs scaffold. These results could open the way of novel cell control system.

10:00 AM BREAK

SESSION BM06.02: Plasma Treatment toward Therapy and Pharmacology I
Session Chairs: Hirofumi Kurita and Deborah O'Connell
Monday Morning, November 26, 2018
Sheraton, 2nd Floor, Independence West

10:30 AM *BM06.02.01
Non-Thermal Atmospheric Pressure Plasma as a Tool to Control the Proliferation of Various Adult Stem Cells Kiwon Song[1], Jeongyeon Park[1], Hyunyoung Lee[2] and Hae June Lee[2]; [1]Yonsei University, Seoul, Korea (the Republic of); [2]Pusan National University, Pusan, Korea (the Republic of).

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. Recently, many researches attempted to take advantage of the low temperature of NTAPP for biomedical applications thanks to the controllability of plasma chemistry and kinetics. Adult stem cells can differentiate into various mature cell types within tissues or organs at specific conditions. Adipose-derived stem cell (ASC) is a kind of mesenchymal stem cell, which is able to self-renew and differentiate into adipocytes, chondrocytes, osteoblasts and neurons. In this study, we exposed ASCs to NTAPP generated in a helium-based dielectric barrier discharge (DBD) device 10 times, for 50 sec each time every hour, and incubated the cells till 72 h. NTAPP exposure increased the proliferation of ASCs by 1.57-fold on an average, compared with unexposed cells. NTAPP-exposed ASCs maintained their stemness, capability to differentiate into adipocytes but did not undergo the cellular senescence. In addition, the mRNA level of well-known pluripotent genes, *Oct4*, *Sox2* and *Nanog,* was increased in NTAPP-exposed ASCs compared with that of the unexposed cells. Also, signaling pathways that activate the cell proliferation such as 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. Studies using the scavengers for nitric oxide (NO) and reactive oxygen species (ROS) demonstrated that NO rather than ROS is responsible for the enhanced proliferation of ASCs following NTAPP exposure. Moreover, NTAPP induced the increased proliferation of bone marrow-derived stem cells (BM-MSCs) and hematopoietic stem cells (HSCs) by 80 % and 100 %, respectively. These results suggest that NTAPP can activate the proliferation of ASCs without affecting their stem cell properties. Taken together, this study supports that NTAPP would be an efficient tool to activate the proliferation of various adult stem cells for the medical application of stem cells both *in vitro* and *in vivo*. Currently, we are investigating the whole genome expression profile of NTAPP-exposed ASCs to understand the molecular mechanism of the activation of adult stem cell proliferation by NTAPP.

11:00 AM *BM06.02.02
Effectiveness and Safety of Plasma Activated Medium Hiromasa Tanaka, Masaaki Mizuno, Kenji Ishikawa, Shinya Toyokuni, Hiroaki Kajiyama, Fumitaka Kikkawa and Masaru Hori; Nagoya Univ, Nagoya, Japan.

It is important to investigate effectiveness and safety for clinical applications of new therapeutic methods. Non-thermal plasma is pretty new technology which is expected to be applied for various medical applications such as blood coagulation [1], wound healing [2], and cancer treatments [3]. We have previously developed plasma activated medium (PAM) for cancer treatments [4]. We have also developed plasma activated Ringer's lactate solution (PAL) for cancer treatments [5]. We would like to discuss effectiveness and safety of these solutions based on our accumulated knowledge through numerous studies.

PAM selectively killed glioblastoma brain tumor cells against astrocyte normal cells [4]. Selective killing of cancer cells by PAM have been reported in not only glioblastoma, but also ovarian, pancreatic, and other cancer cells. We have reported that PAL also selectively killed glioblastoma against normal keratinocyte cells and mammary epithelial cells. These *in vitro*experiments are good evidence to use PAM/PAL safely.
Various *in vivo* experiments also demonstrated safety of PAM/PAL. For example, PAM inhibited ovarian cancer cell metastasis, resulting in prolonged survival in a mouse model, while PAM intraperitoneal injection exerted little influence on body weight *[6]. Intravitreal injection of PAM suppressed laser-*

induced choroidal neovascularization, while PAM injection had no effect on regular retinal vessels, nor did it show retinal toxicity [7]. These *in vivo* experimental results are important evidences to use PAM effectively and safely.

To establish plasma medical science, understanding molecular mechanisms and effectiveness and safety tests are necessary. Since PAM was developed, many *in vitro* and *in vivo* experiments have demonstrated its effectiveness and safety especially in cancer treatments. Selective killing of cancer cells by PAM are good evidences for effectiveness and safety of PAM. These results suggest PAM therapy may be a promising treatment option.

Acknowledgements
This work was partly supported by a Grant-in-Aid for Scientific Research on Innovative Areas "Plasma Medical Innovation" Grant No. 24108002 and 24108008.

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11:30 AM BM06.02.03
Helium Plasma as a Tool for Interacting with Cells and Pathogens Emilio Martines; Consorzio RFX, Padova, Italy.

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
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11:45 AM BM06.02.04
High Throughput Toxicity Assay on Coordinately Ordered Single Cells with Individual Identity Qingxuan Li and Ming Su; Northeastern University, Boston, Massachusetts, United States.

Cytotoxicity at single cell level plays an important role in understanding biological mechanisms, detecting diseases, and screening therapeutics. Since cell populations are heterogeneous, differentiating individual cells in large populations become very important. This abstract reports a single cell array where the identity of each individual cell can be determined from coordinators assigned to the cell. Cytotoxicity upon X-ray radiation exposure and drug treatment are determined through reactive oxygen species (ROS) signal in cells and quantified by fluorescent intensity with MATLAB. This method can provide toxicities of over thousands of cells with superior statistics power after different treatment. The heterogeneity of cells in response to chemical and physical stimuli can be easily obtained to allow deep analysis of obtained toxicity data.

SESSION BM06.03: Plasma Processing of Biocompatible Materials
Session Chairs: Tetsuji Shimizu and Kiwon Song
Monday Afternoon, November 26, 2018
Sheraton, 2nd Floor, Independence West

1:30 PM *BM06.03.01
Plasma Processing of Liquid Media for Biology—An *In Vitro* Study of Chemical Actors and Biological Effects Eloisa Sardella[1], Valeria Veronico[2], Francesco Fracassi[2], Michele Casiello[2], Lucia d'Accolti[2], Loris Grossi[3], Francesco Ciminale[2], Pietro Favia[5], Michael Schmidt[4], K.D. Weltmann[4] and Roberto Gristina[1]; [1]Institute of Nanotechnology, CNR-NANOTEC, Bari, Italy; [2]Department of Chemistry, University of Bari, Bari, Italy; [3]University of Rimini, Rimini, Italy; [4]Leibniz Institute for Plasma Science and Technology, Greifswald, Germany; [5]University of Bari, Bari, Italy.

During the last 10 years atmospheric pressure plasmas have shown great promise for the treatment of wounds and cancer. All reported literature attests that

the synergy between the plasma and liquid is critical to understanding the outcome of plasma treatment and envision targeted breakthrough in medical therapeutic approaches. In this work chemistry of cell culture liquid media was investigated after application of dielectric barrier discharges switched on at 6KHz with different gas feed: Air, Nitrogen, Oxygen and mixtures of them. A DBD closed system and a controlled gas environment were used in order to address important answers to the questions: is the H_2O_2 really involved in promoting certain cell behaviors during in-vitro testing? Has the NO and its derivatives an active role in promoting some cell responses stimulated by a plasma activated biological media? Is there a clear role of biological molecules of cell culture media eventually modified by plasma in stimulating some biological responses? Plasma activated liquid media (PALM) have been analyzed by ESR, LC-MS and spectrophotometric quantification of reactive oxygen and nitrogen species. The chemical composition of such kind of plasma activated media show that [H_2O_2] increases while [NOx] species decreases with the percentage of O_2 in the gas feed. Such results have been correlated to the biological characterization of plasma activated DMEM 10% FBS incubated for 2 hours with an osteoblasts cell line (SAOS2) and primary cells (BMSC). Cell growth of cells incubated with PALM have clearly shown that, the plasma processing with O_2 (6KHz, 13kV, 25%DC, 1min) is more effective than that one carried out with N_2 and air, performed in the same experimental conditions, in promoting a reduced cell adhesion, an absence of cell clusters and contemporary inhibiting cell growth of SAOS2 cell lines. Meanwhile, a different behaviors have been observed for the primary BMSC, with a less detrimental effect on cell adhesion and growth both on 2D and 3D growth. Two-dimensional (2D) cellular monolayers remain the standard for validation of several kind of biomedical and therapeutic approaches, even though 2D monolayers are unable to replicate the complicated environment and mechanisms of a tissue or a solid tumor and its growth. The production of three-dimensional (3D) in vitro models is now established as a much more accurate representation of in vivo conditions when compared to other in vitro models, such as the production of 2D monolayers. For these reasons the co-author of this paper will show the cell responses exposed to PALM both on 2D and 3D environment. The obtained results give important insight on plasma interfaced to biological liquids showng the potentialfor future application of plasma assisted approach both in regenerative medicine and cancer therapy.

2:00 PM *BM06.03.02
Cold Atmospheric Plasma Device for Decontamination of Space Equipment Hubertus M. Thomas[1], Petra Rettberg[4], Gregor Morfill[3], Julia Zimmermann[3], Meike Mueller[1], Markus Thoma[2] and Tetsuji Shimizu[5]; [1]DLR-Institute of Materials Physics in Space, Wessling, Germany; [2]University of Giessen, Giessen, Germany; [3]terraplasma GmbH, Garching, Germany; [4]Institute of Aerospace Medicine, DLR, Cologne, Germany; [5]National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan.

Cold Atmospheric Plasmas (CAP) are commonly used in plasma medicine and plasma hygiene due to its sterilizing conditions. We are presenting a new device based on the circulation of the long-living species of an afterglow air CAP for the decontamination of space equipment. In space exploration the decontamination of equipment for the visit to other planets or moons is very strictly regulated through the planetary protection policies of the Committee on Space Research (COSPAR). Proven methods for the decontamination are using for treatment of the equipment dry heat or H2O2-gas, both having negative side effects in addition to the decontamination efficacy.

In a first project we investigated the use of afterglow plasma produced in a CAP for the decontamination effects on bacterial spores [1]. The afterglow plasma contains only the long-living species like ozone, NO2, etc. which allows the treatment of very sensitive materials at room temperature. In a follow-on project the apparatus was completely redesigned to gain efficacy, stability and reproducibility.

Measurements of the decontamination efficacy combined with physical measurements of the produced reactive components (measured by FTIR and UV absorption spectroscopy) and their effect on treated materials allow a better understanding of the involved processes.

We will give an overview on the status of the plasma decontamination project funded by the Bavarian Ministry of Economics.

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2:30 PM BM06.03.03
Maskless Hydrophilic Patterning of Superhydrophobic Aluminum Surface by Atmospheric Pressure Micro-Plasma Jet for Water Adhesion Controlling Jiyu Liu, Guansong Wang, Jichao Zhang, Faze Chen, Zhuji Jin and Xin Liu; Dalian University of Technology, Dalian, China.

Superhydrophobic surfaces with hydrophilic patterns have great application potential in various fields, such as microfluidic systems and water harvesting. However, many reported preparation methods involve complicated devices and/or masks, making fabrication of these patterned surfaces time-consuming and inefficient. Here, we propose a high-efficient, simple and maskless micro-plasma jet (MPJ) treatment method to prepare hydrophilic patterns like dots, lines and curves on superhydrophobic Al substrates. Contact angles, sliding angles, adhesive forces and droplet impact behavior of the created patterns are investigated and analyzed. The prepared “dot” patterns exhibit great water adhesion, while the “line” patterns show anisotropic adhesion. Additionally, MPJ treatment does not obviously change the surface structures, which makes it possible to achieve repeatable patterning on one substrate. Adhesion behavior of these patterns could be adjusted using MPJs with different diameters. MPJs with larger diameters are efficient for creation of patterns with high water adhesion, which can be potentially used for open channel lab-on-chip systems (e.g., continuous water transportation); while MPJs with smaller diameters are preferable in preparing patterns with low water adhesion for diverse applications in biomedical fields (e.g., loss-less liquid droplets mixing and cell screening).

2:45 PM BM06.03.04
Prevention of Candida Biofilm Formation over Microplates by Plasma Polymerization Technique Gizem Kaleli Can[1], Pinar Yurdakul-Mesutoğlu[1], Ferda H. Ozguzar[1], Elvan Hortaç-Istar[2], Gözde Kabay[1], H. Cenk Mirza[2], Ahmet Basustaoglu[2], Julide Sedef Göçmen[1] and Mehmet Mutlu[1]; [1]TOBB ETU, Ankara, Turkey; [2]Baskent University, Ankara, Turkey.

Candida spp are considered as one of the common microorganisms of health care associated infections, mostly through biofilm associated infections. Biofilms are well known for forming on implanted medical devices such as catheters, pacemakers, prosthetic joints etc. *Candida* biofilms are resistant to many antifungals clinically used, making these infections a significant challenge. Higher doses of antifungals with removal of the colonized device are generally the only options to cure these infections. One of successful approaches to prevent biofilm formation is surface modification by plasma polymerization, coating only surface of device. In this study, biofilm formation on plasma polymerized microplate surfaces were investigated.

Thirty biofilm positive *Candida spp* isolated from blood as well as two ATCC control strains (*C. albicans* ATCC 10231 and *C. parapsilosis*) were included in this study. Biofilm formation was determined as described by quantitative plaque assay method. Suspensions of *Candida spp* isolated from blood cultures were inoculated in triplicates onto microplate wells. Individual strains isolated from patients as well as negative and positive controls were incubated for 48 hours. Biofilm production on plasma modified and non-modified surfaces were evaluated both at 48 hours and two weeks after. Evaluation was done using crystal violet (CV) binding assay. After staining with CV, the optical density (OD) of each well stained with CV was measured

at 570 nm against the OD of negative controls (at 48 hours and at two weeks).

Surface modification of microplates with plasma polymerization technique was achieved by low pressure plasma system. In this particular study, acrylic acid (AA), 2–hydroxyethyl methacrylate (HEMA) and diethyl phosphite (DP) were assessed for their biofilm inhibition efficacy.

On non-coated control surfaces 100% biofilm formation by *Candida spp* was observed. When plasma-modified microplate surfaces were evaluated at different plasma powers (30, 60 and 90W), the most significant inhibition of biofilms was observed on DP coated microplate wells at 90W, for all *Candida spp* tested. For HEMA and AA coated microplates at 90W, biofilm formation was observed for only one Candida isolate. When all three monomers (AA, HEMA and DP) were investigated at 60W and 30W, DP and AA were the most effective monomers, respectively. When readings were re- evaluated after two weeks, only DP seemed to be stable at 90W whereas AA and HEMA in particular seemed to have lost their anti-biofilm effects.

Regardless of the monomers and plasma parameters used, biofilm formation was inhibited for all plasma modified microplate wells ($p<0,000$). Of all the monomers included, the most significant anti-biofilm effect was shown for DP at 90W. In this study, *in-vitro* results indicated a potential for reducing biofilm associated Candida infections on selected plasma modified surfaces.

3:00 PM BREAK

SESSION BM06.04: Monitoring of Plasma for Biomedical Applications
Session Chairs: Vittorio Colombo and Zdenko Machala
Monday Afternoon, November 26, 2018
Sheraton, 2nd Floor, Independence West

3:30 PM *BM06.04.01
Plasma Jet Delivery on Targets Relevant for Skin Treatments—Towards *In Situ* Monitoring and Loop Controlled Plasma Gun Based Device Eric Robert, Azadeh Valinattaj Omran, Giovanni Busco, Sébastien Dozias, Catherine Grillon, Jean Michel Pouvesle and Loick Ridou; CNRS-Univ d'Orleans, Orleans, France.

We will first report on the key role of conductive targets mimicking biological samples (such as those involved during *in vitro*, *ex vivo* and *in vivo* skin or skin cells), on most of the plasma characteristics during non-thermal plasma treatments. Drastic mutual influence of plasma jet and targets has been shown to induce critical modifications of: reactive species balance and densities, current amplitude, temperature, gas flow features when comparing with the situation of the so called “free jet expansion” in ambient air. This strong interplay between plasma and targets has to be considered before (gas flow impingement before plasma ignition) and during plasma delivery as a dynamic feature. Both plasma and targets may indeed encounter various continuous evolutions during plasma treatment such as: plasma source feed gas purity, plasma kinetics, target humidity, target electrical conductivity, thermal load, on target charge deposition, cell microenvironment modulation (oxygen level, plasma induced permeabilization) …
When translating results obtained during *in vitro* or preliminary skin sample experiments to clinical situation, one has also to account for the critical role of plasma nozzle to surface gap variation and of the skin specificity of individuals.
The first objective of our work is to try to achieve a safe, reproducible and individual-independent plasma delivery on human skin. This approach includes first the implementation of, as simple and user friendly as possible, diagnostics tools providing signals likely to be correlated with the plasma jet source operating parameters (voltage, repetition rate, gap distance). This requires to select the most sensitive *in situ* signals captured by diagnostics tools and then try to reach a “real time” modulation and loop controlled operation of the plasma source. This first task represents a prerequisite for the development of a safe and reliable plasma device likely to offer new opportunity for anti-aging issues or skin treatments in cosmetics or dermatology applications.
This work is supported by the research project PlasmaCosm-ARD 2020 Cosmetosciences, Région Centre Val de Loire.

4:00 PM *BM06.04.02
Ultrafast Laser Spectroscopy of Plasma Liquid Systems Stephan Reuter[1], Benjamin Goldberg[1], Yibin Zhang[1], Arthur Dogariu[1] and Richard Miles[1, 2]; [1]Mechanical and Aerospace Engineering, Princeton University, Princeton, New Jersey, United States; [2]Department of Aerospace Engineering, Texas A&M University, College Station, Texas, United States.

Non-thermal atmospheric pressure plasmas provide high reactivity at low gas temperatures, ideally suited for sensitive surface treatment. Recent studies have, for example, demonstrated that plasma jets in clinical use provide great potential for novel chronic wound therapies and cancer treatment. Plasma generates reactive oxygen and nitrogen species triggering biological responses that initiate healing processes. In order to target plasma-based therapy to a specific medical application, control over the plasma generated reactive oxygen and nitrogen species composition is required. First concepts in tailoring plasma reactivity for targeted therapies show exciting results. Controlling plasma requires in-depth knowledge of its parameters. Especially atmospheric pressure plasma jets pose a challenge to the diagnostics of reactive species and reaction processes due to small dimensions and high gradients in space and time. As mediator for the plasma interaction effect, liquid interfaces frequently play a major role. In plasma liquid systems, these liquid interfaces need to be taken into account for diagnostic studies. Methods based on laser spectroscopy have proven invaluable to study species generation and transport in atmospheric pressure plasmas. Our work focuses on the diagnostics of accurate flow profile measurements and determination of the electric field initiated by the ionization wave of plasma jets. The use of ultrafast lasers allows for a high time resolution together with space resolved measurements. Electric field induced second harmonic light generation (E-FISH) for electric field measurements and femtosecond laser electronic excitation tagging (FLEET) for flow profile measurements are presented for plasma jets that can be used in plasma liquid interaction:
Plasma reaction kinetics is governed by the electron energy distribution function of the discharge, which can be controlled by the supplied electric field. We present 1D-electric field measurements, to study the electric field. To study the flow field of the plasma jet, we employ FLEET, which permits unseeded velocimetry in gas flows containing nitrogen and argon. A strongly focused femtosecond laser excites and ionizes nitrogen, which subsequently dissociates via electron ion recombination. Subsequent nitrogen recombination forms excited nitrogen species that can be tracked for flow field studies. Knowing electric field and gas flow development is of paramount importance for plasma tailoring. Single shot measurements allow to detect stochastic processes. The high resolution in space and time given by the described measurement techniques and the active probing by laser radiation advance insight into the reaction dynamics of plasma liquid systems.

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4:30 PM BM06.04.03
Emission Propagation in Low Energy Atmospheric Pressure Plasma Jet Hiromasa Yamada[1, 2], Tetsuji Shimizu[1, 3], Masanori Fujiwara[1], Susumu Kato[1], Jaeho Kim[1], Sanae Ikehara[4], Yuzuru Ikehara[1, 4, 3] and Hajime Sakakita[1, 3]; [1]Electronics and Photonics Research Institute, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan; [2]Nagano College, National Institute of Technology, Nagano, Japan; [3]Graduate School of Medicine, Chiba University, Chiba, Japan; [4]Biotechnology Research Institute for Drug Discovery, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan.

Abstract
Atmospheric pressure plasma jet (APPJ) is attracting attention in many research fields such as biology and medicine. Low energy atmospheric pressure plasma (LEAPP) equipment specially designed by Sakakita et al. [1] is one of the APPJ. Stop bleeding accompanied with blood coagulation without thermal damage using the LEAPP has been confirmed as an attractive surgical procedure necessary for minimally invasive surgery [2]. To control the interactions between the plasma and treated targets such as blood, it is necessary to understand the plasma's behavior. We have already reported several characteristics of the LEAPP, such as dynamic gas-flow behavior [3], gas temperature [4], spatial distribution of reactive species [5], electrical characterization [6], and emission propagation phenomena [7, 8]. In the emission propagation phenomena, a bullet-like emission [9] and a spatially continuous emission [10] were observed depending on the treated target conditions [8]. Moreover, striations have been also observed in the LEAPP [7, 8]. In this study, the characteristics of the emission propagation in the LEAPP under several experimental conditions were analyzed using a high-speed camera. All the observations were synchronized with the measurement of electrical characteristics such as the applied voltage, current and power consumption. As working gases, helium, argon, and neon gases were used. For the target, a copper plate was used and the emission propagation was measured with and without the target. In case with the target, the distance between the nozzle exit of the LEAPP equipment and target surface were changed. Furthermore, an optical emission spectroscopy (OES) was applied in order to identify the reactive species in the plasma. The experimental results are summarized and at the symposium, we discuss the mechanism of emission propagation associated with the characteristics of plasma such as electrical property and optical emission.

Acknowledgments
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4:45 PM BM06.04.04
Potential Formation on Insulator Film by Atmospheric Pressure Plasma Jet Tetsuji Shimizu[1], Kazuya Kikunaga[2] and Hajime Sakakita[1]; [1]Electronics and Photonics Research Institute, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan; [2]Advanced Manufacturing Research Institute, National Institute of Advanced Industrial Science and Technology, Tosu, Japan.

Biomedical applications using cold atmospheric plasmas (CAPs) have been studied extensively. At the beginning of this new field, sterilization and wound care were the main applications using CAPs because the plasma can supply reactive species similar to what the immune system produces. For sterilization, CAPs can inactivate bacteria including antibiotics-resistant strains, fungi, and viruses in tens of seconds to minutes. Within a few years, biomedical applications by CAPs expanded to include cancer therapy, gene transfection, activation of cell functions, blood coagulation, and regenerative medicine. From CAPs, there are several agents reacting with targets such as reactive oxygen and nitrogen species, charged particles, UV photons, heat, and electric field. So far, the biomedical effects driven by CAPs have been discussed mainly in terms of reactive species. However, the charged particles can also play an important role in the biomedical effects because they can also initiate chemical reactions. Moreover, the accumulation of charge on the target can influence the production of CAPs and the transport of reactive species. In addition, the developed electric field by the accumulated charge themselves can cause a biomedical effect. In order to understand the behavior of the charged particles, we aim to investigate a potential formation on an insulator film by the CAP treatment in this presentation.

The used CAP device was an atmospheric pressure plasma jet specially designed for blood coagulation. This device has a dielectric barrier discharge plasma source driven by an AC high voltage power supply with low energy consumption. The frequency of the applied voltage was ca. 62 kHz, and the peak-to-peak voltage was 3-6 kV with sinusoidal waveform. As the working gas, helium, argon or those admixtures was used in order to control the distribution of ion species. A plasma flare was ejected from a quartz tube of 1.4 mm in inner diameter and the insulator film was exposed to the plasma flare. The surface potential distribution on the insulator film was observed by using a static electricity scanner system which can measure an area of 30 x 30 mm^2 at a spatial resolution of 1 mm within 3 s by scanning an object surface along a vibrating linear array sensor. This method allowed us to measure the potential distribution in a non-contact manner. For the plasma treatment, the insulator films were treated by the CAP for a certain period of time and the potential distribution was measured immediately after the plasma exposure. In the presentation, we discuss the potential profile developed by the CAP treatment and the charging mechanism by the charged particles from the CAP.

SESSION BM06.05: Poster Session: Plasma Processing and Monitoring for Bioengineering and Biomedical Engineering
Session Chairs: David Graves, Emilio Martines, Deborah O'Connell and Tetsuji Shimizu
Monday Afternoon, November 26, 2018
8:00 PM - 10:00 PM
Hynes, Level 1, Hall B

BM06.05.01
Towards a Comprehensive Understanding of Plasma Activated Medium Treated Cells Masaru Hori, Hiromasa Tanaka, Masaaki Mizuno, Kenji Ishikawa, Shinya Toyokuni, Hiroaki Kajiyama and Fumitaka Kikkawa; Nagoya University, Nagoya, Japan.

We have previously developed plasma sources with high electron density, and applied for cancer treatments [1]. We found anti-tumor effects by plasma-treated medium, and this medium with anti-tumor effects was denoted "plasma activated medium" or PAM [2]. Anti-tumor effects by PAM have been

widely investigated in various cancer cell such as glioblastoma, ovarian, gastric, pancreatic, and lung cancers. We have also developed a novel plasma activated solution, plasma activated Ringer's lactate solution (PAL) for cancer treatments [3]. We have studied intensively mechanisms of anti-tumor effects by PAM based on plasma science and molecular biological science (plasma medical science) [4].
Plasma which consists of electrons, ions, radicals, and light interacts with oxygen, nitrogen, and water in humid air to produce molecules such as nitric oxide and hydroxyl radicals, and moves into the liquid phase to generate molecules such as hydrogen peroxide (H2O2), nitrites and nitrates. These bioactive molecules trigger signaling cascades that mediate plasma-induced effects in cells. PAM influence cell signaling networks, in turn affecting gene regulatory and metabolic networks.

We have focused on the effects on cells by PAM in gene regulatory networks and signaling networks. We treated glioblastoma cells with PAM or PAL, and prepared for cell lysates to investigate gene expression levels by real-time PCR methods and activation of specific proteins by western blotting methods.
Gene expression levels related in survival and proliferation signaling were affected by PAM and PAL, but the patterns were different with each other. Activation of proteins in the survival and proliferation signaling were also affected by PAM and PAL, and the activation/deactivation patterns were different with each other. Our results suggest that these approached are useful to understand PAM or PAL-treated cells comprehensively.

Obtaining a comprehensive understanding of gene regulatory networks, signaling networks, and metabolic networks will be important to accurately determine the effects of PAM on specific cellular processes.

Acknowledgements
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BM06.05.02
Possibility of Pore Formation in Supported Lipid Bilayer by Plasma-Induced Long-Lived Reactive Species in Liquid Phase Fumiya Oike[1], Ryugo Tero[2], Toru Harigai[1], Tsuyoshi Tanimoto[1], Hirofumi Takikawa[1] and Suda Yoshiyuki[1]; [1]Electrical and Electronic Information Engineering, Toyohashi University of Technology, Toyohashi-shi, Japan; [2]Environmental and Life Science, Toyohashi University of Technology, Toyohashi-shi, Japan.

Reactive oxygen and nitrogen species (ROSs and RNSs) produced by atmospheric pressure plasma would play crucial roles for those plasma-induced biological reactions. However, many aspects are still unclear how ROSs and RNSs affect and/or pass through cell membranes. Artificial lipid bilayers are useful cell membrane model systems for investigating the fundamental interaction between cell membranes and chemical and biological agents. Recently, we investigated the effects of atmospheric pressure plasma on the basis of dielectric barrier discharge (DBD) on a supported lipid bilayer (SLB), which is an artificial cell membrane system formed at a solid-liquid interface [1]. It was revealed that pores with nm ~ μm size is formed in DBD-irradiated SLB and the diffusion coefficient of the SLB is decreased prior to pore formation [2]. The main cause of pore formation is lipid oxidation by plasma-induced reactive oxygen species. In this study, we investigated the influence of major long-lived reactive species on pore formation. We focused on H_2O_2, O_3, NO_2^- and NO_3^- as long-lived reactive species as well as ClO_2^- and ClO^-. The reason for focusing on ClO_2^- and ClO^- is that KCl is contained in the buffer solution((100 mM KCl, 25 mM HEPES, pH7.4(NaOH)).
DOPC (dioleoylphosphatidylcholine) and Rb-DOPE (rhodamine B dioleoylphosphatidyl-ethanolamine) were used as a lipid and fluorescent dye-labeled lipid, respectively, and SLB consisted of them. SLB was prepared in a buffer solution (pH 7.4) by the vesicle fusion method, and introduced into a DBD-plasma irradiator [1], which was settled in a glove box and filled with He. We applied AC high voltage at 15 kHz for the DBD-plasma irradiation of DOPC-SLB with the electric energy in the range of 28 − 148 kJ/cm^2, which was standardized by electrode area. We observed the morphology of DOPC-SLB using an epi- fluorescence microscope (epi-FM) and atomic force microscope (AFM) [1].
Pack tests (RIKEN, Japan) were used for the measurement of H_2O_2, O_2^- , NO_3^-, ClO_2^- and ClO^- in the liquid phase. O3-3F (Kasahara Chemical Instruments Corp., Japan) was used for measurement of O_3 in liquid phase.
Measurement of the concentration of long-lived reactive species generated by the plasma showed that the main long-lived reactive species generated in this experimental system are H_2O_2, NO_2^- and NO_3^-. These reactive species concentrations increased with increasing the electric power. We predicted that when DOPC reacted with these reactive species, the concentration in the suspension was lower than that in the buffer solution. However, concentrations of H_2O_2, NO_2^- and NO_3^- were higher when DOPC vesicle was exist. Therefore, it is suggested that these reactive species may not react with DOPC. From the above, it is considered that lipid oxidation causing pore formation in SLB is induced by short-lived active species.

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BM06.05.03
Hemolysis and Coagulation by Plasma Treatment Kenji Miyamoto[1, 3, 2], Sanae Ikehara[2, 3], Hajime Sakakita[4, 2], Tetsuji Shimizu[4, 2], Takashi Yamaguchi[2], Nobuyoshi Takeuchi[2], Ken Wakai[2] and Yuzuru Ikehara[2, 3, 4]; [1]Graduate School of Engineering, Yokohama National University, Yokohama, Japan; [2]Graduate School of Medicine, Chiba University, Chiba, Japan; [3]Biotechnology Research Institute for Drug Discovery, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan; [4]Electronics and Photonics Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan.

We are integrating the plasma technology of low-temperature at atmospheric pressure into the medical sciences through the development of hemostatic equipment.
The first report on blood coagulation using LTP was from Drexel University, which exclusively highlighted the effects on the natural blood coagulation process but for hemolysis or serum protein aggregation. On the other hand, we have focused on the plasma effects on hemolysis to form coagulation from red blood cells and on aggregation of serum proteins that were not involved in natural blood coagulation system [1]. It was the volume of erythrocytes and concentration of albumin that are much higher than platelets and fibrinogen. In our previous studies, we succeeded to maximize the plasma effect that alters them to cellular and molecular "glue" in clot formation using the instrument to produce a plasma with a dielectric barrier discharge (66 kHz, sinusoidal peak-to-peak voltage of 6.0 kV applied to an electrode [1, 2].
In this study using the above setting, we monitored the clot formation, the input voltage onto the plasma generator, the gas flow rate, and the value of the

current flowing through the conductor A by measuring system connected to the toroidal coil (Rogowski coil). The formed aggregations in serum proteins solutions (albumin and Immune globulins) and RBC solutions were analyzed by either histological, ultrastructural or protein biochemical methods [3]. Albumin aggregation and hemolysis of erythrocytes correlated with current flowing, and there were thresholds for protein aggregation and hemolysis [4]. Moreover, the threshold for hemolysis was much higher than for aggregation of albumin and hemoglobin [4]. RBC clot formation didn't occur without exceeding a current limit for hemolysis [4]. Furthermore, the conductivity of the sample tray correlates with more active hemolysis. From the viewpoint of plasma physics and pathology, these findings that "electric current's contribution to hemolysis" accelerates blood coagulation help to develop more effective hemostatic equipment by applying these results [1].

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BM06.05.04

International Standardization of a Low Energy Ionized Gas Haemostasis Equipment Hajime Sakakita[1, 2] and Yuzuru Ikehara[1, 2]; [1]National Institute of Advanced Industrial Science and Technology, Ibaraki, Japan; [2]Chiba University, Chiba, Japan.

In the surgical procedure, surgeons conventionally control bleeding by cauterization, clipping, or ligation, depending on the types of blood vessel. Even in the bleeding case from capillaries and small vessels, cauterization has been used. Medical devices such as high-frequency electrical coagulator, ultrasonic wave equipment, laser, and high-temperature plasma induce cauterization, and produce smoke by the heat which is sometimes difficult to ensure the visual field during the operation. Moreover, cauterization causes prolonged postoperative disorder and scar tissue formation in the abdominal cavity. The scarring tissues are characterized as proliferation of fibroblast and blood vessels, so called as granulation tissue, which limits the performance of 2nd surgery. As a disorder caused by the digestive surgical operation, it is difficult to treat the subacute disorder by opioids [1]. Therefore, minimally invasive method to stop bleeding in capillaries is desired. It was reported that low temperature plasma treatment can reduce invasiveness under hemostasis, and risk of postoperative disorders [2,3]. Many of bleeding devices are already defined by international standards such as IEC 60601-2-2; Particular requirement for high frequency surgical equipment, but not for low temperature plasma. International standards on the basic performance and safety of medical plasma equipment for blood coagulation will accelerate and extend the usage of medical plasma equipment. To apply the plasma effectively, safely and reproducibly, it is necessary to clarify the correlation between the plasma components and biological effects. To ensure the safety of medical plasma equipment, specifications such as current must be measured and evaluated. Recently (2018/4/10), IEC 60601-2-76: 2018, Particular requirements for the basic safety and essential performance of low energy ionized gas haemostasis equipment was published. In the meeting, detail contents of this standard will be presented.

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BM06.05.05

A Model Analysis of the Optical Emission of Low-Energy Atmospheric-Pressure Neon Plasma Jets Susumu Kato[1], Tetsuji Shimizu[1], Masanori Fujiwara[1], Hiromasa Yamada[2, 1], Satoru Kiyama[1] and Hajime Sakakita[1]; [1]National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan; [2]National Institute of Technology, Nagano College, Nagano, Japan.

Atmospheric-pressure plasma jets (APPJs) have recently attracted much interest not only for biomedical applications [1] but also for plasma physics [2]. One of the most interesting phenomena in APPJs is bullet propagation [3], which has been observed in experiments and analyzed by computer simulations [2,4]. Another is a striation phenomenon which widely appears in discharges [5]. A stratified emission was observed and analysed in a positive column of a neon gas dc glow discharge [6]. The striations between a nozzle exit and a conductive target plate were observed in neon plasma ejected from low-energy atmospheric-pressure plasma device [7]. It is not clear, however, how the bounded plasma is sustained in APPJs. Especially, the role of the excited state (metastable) are not clear even though it is believed to be an important role [8]. In order to elucidate the sustaining mechanism of striations of APPJs, optical emission or laser absorption spectroscopy are used [9].
In this paper, we proposed a simple kinetic model to analyse the optical emission from the atmospheric-pressure neon. The kinetic model includes only noble gas atom, metastable, some excited states, ion, dimer, dimer ion and electron reactions. The most of the optical emission consists of the transition from excited Ne($2p^5 3p$) states to Ne($2p^5 3s$). The Ne($2p^5 3p$) is dominantly generated by both the electron collisional excitation of metastable and ground state and the dissociative recombination of ion dimer [10]. In the atmospheric pressure, the collisional deactivation of the Ne($2p^5 3p$) become significant and comparable to the radiative decay [11]. The spectrum strongly depend on the collisional deactivation.

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BM06.05.06
Evaluation of Amount of RONS Transport and Absorption of Seeds Kazunori Koga, Yosuke Wada, Ryoya Sato, Daisuke Yamashita, Kunihiro Kamataki, Naho Itagaki and Masaharu Shiratani; Kyushu Univ, Fukuoka, Japan.

Agricultural applications using nonthermal atmospheric pressure plasmas have attracted much attention not only from plasma researchers but also from biologists. So far, we have found that seeds irradiated by a scalable dielectric barrier discharge (DBD) plasma show growth enhancement of the plants [1]. Three minutes plasma irradiation to Arabidopsis thaliana seeds shows 11% harvest time reduction and 56% crop yield enhancement [2]. The growth enhancement of plasma-irradiated plants is probably caused by eustress response. However, the details of the molecular mechanism involved in plasma induced signal initiation and response regulation are unknown. Here we have evaluated the amount of reactive oxygen nitrogen species (RONSs) absorbed by seeds as a first step to understand the response regulation. First, we studied the relationship between growth of radish sprouts (Raphanus sativus L.) and a number density of seeds during the plasma irradiation using the scalable DBD device [2, 3]. We arranged the seeds within 20x20 mm2 at the center of the electrodes at 3 mm below the electrodes. After 180 s plasma irradiation, 30 seeds for each number density were cultivated under dark condition for 3 days. We obtained seed number dependence of the average length of radish. While the growth is suppressed for the seed number of 3 and 5, the growth is enhanced for the seed number of 10, 15 and 30. The plant growth for 30 seeds is slightly less than that for 15 seeds. The results suggest that the dose of RONSs depends on the number of seeds, leading to the plant growth suppression and enhancement. We also measured the electron spin resonance (ESR) spectra of seeds to detect the radicals in seeds [4]. The signal intensity of radicals correlated with the biochemical compounds in seeds is increased by the plasma irradiation. Comparative study of the effects of seeds density using the ESR measurements will reveal RONS transport and absorption mechanism in seeds.
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BM06.05.07
Porous Superstructural Raman Nanosensors for Ultrasensitive Biochemical Detection and Electrically Controlled Molecule Release Jianhe Guo[1], Jing Liu[1, 2] and Donglei (Emma) Fan[1, 3]; [1]Materials Science and Engineering, The University of Texas at Austin, Austin, Texas, United States; [2]Institute of Solid State Physics, Chinese Academy of Sciences, Hefei, China; [3]Department of Mechanical Engineering, The University of Texas at Austin, Austin, Texas, United States.

It is highly desirable, while extremely difficult to actively control the release dynamics of molecules from nanoparticle-carriers and to monitor the release process in real time. In this work, we report the design, fabrication, and manipulation of a superstructural Raman nanosensor, offering integrated dual functions in ultra-sensitive biodetection and dynamic control in molecule release. The device has a designed porous superstructure, consisting of gold (Au) nanorod cores and silica shells embedded with arrays of nanocavities arranged in concentric layers in three-dimensions (3D), where high-density plasmonic silver (Ag) nanoparticles are grown both in the nanocavities and on the outer surfaces. The Ag nanoparticles provide substantially enhanced Raman sensitivity for detection of molecules, owing to the large number of hotspots, as well as the near-field coupling of Ag nanoparticles due to their 3D concentric arrangement. Furthermore, by controlling the external electric field, the release of molecules can be facilely controlled at tunable rates owing to the induced electrokinetics at the junctions of Ag nanoparticles. Finally, the biosensing-release-unibody devices can be readily motorized, including transport and rotation, which opens new opportunities for single-cell bioresearch and precision medicine.

BM06.05.08
Preparation and Evaluation of Radiopaque and Biodegradable Microbeads Based on Lipiodol and Polycaprolactone for Transarterial Chemoembolization Yutaka Okamoto[1], Kenta Bito[1], Terumitsu Hasebe[1, 2], Shunto Maegawa[1], Kosuke Tomita[2], Tomohiro Matsumoto[2, 1] and Atsushi Hotta[1]; [1]Keio University, Yokohama-shi, Japan; [2]Tokai University, Hachioji, Japan.

Surface modification by the molecular grafting of a biocompatible polymer on biomaterials is an attractive technique in several fields such as tissue engineering and drug delivery. Especially, the air-plasma treatment can readily modify a polymer surface under atmospheric pressure at low cost without using inactive gas and vacuum chambers. Using the air-plasma treatment, we modified the surface of the gel composed of an oil and a polymer, from which new drug-eluting beads (DEB) were made for transarterial chemoembolization (TACE).
TACE is generally applied to liver cancer by embolizing arteries feeding tumors with DEB under fluoroscopy. During the embolization of the arteries, DEB are injected into the targeted tissue using a catheter. However, since the conventional DEB are not radiopaque in themselves, it is difficult to control an influx of the DEB in a blood vessel. An unintentional reflux into non-targeted blood vessels would end up in complicating diseases. Furthermore, since the conventional DEB are not biodegradable, problems would persist and become more serious.
Here, we focused on Lipiodol and polycaprolactone (PCL) to fabricate Lipiodol/PCL beads as new DEB. Lipiodol is an oil-based contrast agent, and PCL is one of the biomaterials actively studied owing to its excellent biocompatibility and biodegradability. However, Lipiodol/PCL beads agglutinate in water-soluble disperse media, which makes it hard to handle because of the hydrophobicity. Therefore, the air-plasma treatment was applied in order to graft gelatin molecules on the surface of the beads since gelatin is hydrophilic and biocompatible to prevent the beads' aggregation. The surface chemistry was characterized using the X-ray photoelectron spectroscopy (XPS), and the gelatin existence was confirmed by observing the N1s peak derived from gelatin molecules. In order to evaluate the biodegradability, the beads were immersed in phosphate-buffered physiological saline (PBS) or 1 mg/mL of lipase/PBS solution at 37°C before and after the surface modification with gelatin. Furthermore, as angiography was performed with the soluble contrast agent, the beads were injected into a hepatic artery of a healthy rabbit through a catheter, after which CT scanning was performed.
Our results indicate that the degradation of Lipiodol/PCL beads was promoted by lipase, and that the gelatin molecules grafted on the surface of the beads did not prevent the degradability. In addition, the surface-modified beads were successfully dispersed in soluble media. The embolization of the hepatic artery of the rabbit could be assessed by angiography, and the location of the beads in the rabbit could be recognized through the CT scan. Therefore, the Lipiodol/PCL beads were found to be a promising candidate for new DEB, and the success of the surface modification using air plasma on the beads indicated that the procedure was highly useful for the surface modification of the oil and polymer mixtures.

BM06.05.09
Enhanced Gas Sensing Performance by In_2O_3 Nanostructures Functionalized with Conducting Polymers Wang Wei Chien, Kuan-Wei Chen, Yu-Shan Hsu, Ying-Hao Pai and Chun-Hua Chen; National Chiao Tung University, Hsinchu, Taiwan.

A number of studies have demonstrated a strong correlation between exhaled breath components and specific diseases. Accurate detection of specific

volatile organic compounds (VOCs) can thus provide essential information for screening and diagnostic tests. For instance, the acetone concentration in exhaled breath of diabetes is much higher than that of healthy people (below than 1.1 ppm) and the CO concentration of people who smoke a pack of cigarettes per day is ~20 ppm where a nonsmoker is only less than 8 ppm. In this work, we have successfully synthesized a series of conducting polymer functionalized In_2O_3 nanostructures for sensing low-concentration VOCs. To achieve the required sensing performance, a series of conducting polymers functionalized In_2O_3 nanostructures with a high surface-to-volume ratio and porosity were successfully synthesized through hydrolysis of In_2Cl_3 in $NaBH_4$ aqueous solution at room temperature with the subsequent heat treatments. It was found that the novel organic-inorganic heterogeneous nanocomposites exhibit a high sensitivity and an excellent selectivity for specific VOCs, which evidently originated from the essential role of organic functional groups of the conducting polymer.

BM06.05.10
Accurate Point-of-Care Diagnosis of AIDS Based on Label-Free One-Step-Immunoassay Young-Eun Jang, Jounghyeok Kwon, Boram Lee, Ok Jeong Moon and Jeewon Lee; Chemical and Bioengineering, Korea University, Seoul, Korea (the Republic of).

We developed an accurate, rapid, simple, and label-free assay method for point-of-care diagnosis of AIDS, which quickly produces strong optical signals through one-step-immunoassay. The HIV proteins, gp41, p24, and/or gp120 were used as the probes to detect anti-HIV antibodies in AIDS patient sera. In particular, gp41 and p24 were genetically presented on the surface of engineered protein nanoparticles to prepare sensitive 3-dimensional (3D) probes. The 3D probes also present multi-copies of hexa-histidine peptide (H_6) on their surface to chemisorb gold ions (Au^{+3}), which is essential to producing strong optical signals. Point-of-care diagnostic performance of the developed one-step-immunoassay was compared with that of conventional lateral flow assay (LFA) using 30 AIDS patient sera. The sensitivity of LFA was only 63% when a single antigen (gp41) was used but enhanced to 90% when three different antigens (gp41, p24, and gp120) were used together as the assay probes. On the contrary, the one-step-immunoassay using gp41 only produced strong optical signals within 15 min without causing any false negative/positive signals, showing 100% sensitivity and 100% specificity. This technically advanced immunoassay method holds a promising potential as a clinical point-of-care diagnosis of AIDS.

BM06.05.12
Novel Nanoparticles as a Contrast Agent for *In Vivo* Computed Tomography Imaging for Vascular Inflammation Sun-Mi Lee and Kyung-Hwa Yoo; Yonsei Univ, Seoul, Korea (the Republic of).

Bismuth nanoparticle (Bi NPs) and gold nanoparticles (Au NPs) are a potential x-ray computed tomography (CT) contrast agent. We have designed multifunctional hybride nanoparticles of targeting vascular cell adhesion molecule 1 (VCAM-1), which is up-regulated in numerous inflammatory processes in atherosclerosis. Early diagnosis of high-risk plaques using nanoparticles as CT contrasts may be useful for preventing ischemic events. One major hurdle in detecting high-risk atherosclerotic plaques in coronary arteries is the lack of an imaging modality that allows for the identification of atherosclerotic plaque composition with high spatial and temporal resolutions. Here we show that VCAM-1 in atherosclerotic plaques of ApoE−/− mice can be detected with a clinical CT scanner after the intravenous injection of a contrast agent. These novel VCAM-1 targeting Bi-Au nanoparticles may become an important adjunct to the clinical evaluation of coronary arteries with CT.

BM06.05.13
Immobilization of Antibacterial Monomer onto Dentin Substrate by Non-Thermal Atmospheric Plasma Qi Liu[1, 2], Buling Wu[2] and Yong Wang[1]; [1]University of Missouri-Kansas City, Kansas City, Missouri, United States; [2]Southern Medical University, Guangzhou, China.

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-demineralized 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
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BM06.05.14
Low-Energy Ion Dose Thresholds of DNA Double Strand Breaks P. Thopan[1, 2] and L.D. Yu[2, 3]; [1]Rajamangala University of Technology Isan, Khonkaen, Thailand; [2]Chiang Mai University, Chiang Mai, Thailand; [3]Thailand Center of Excellence in Physics, Chiang Mai, Thailand.

DNA double strands break (DSB) is the critical damage of DNA to induce cell mutation/cancer or death. Energetic ion irradiation of biological living systems can possibly result in DNA DSBs via both direct physical and indirect physical and biochemical interactions with DNA. To investigate the interactions separately for understanding fundamentals, we focused our study in the direct interaction between ions and DNA. In this model study, we used ultra-low-energy ions generated and extracted from a plasma source and then decelerated for uniformly low ion energy which could be as low as a few eV/amu to bombard naked dry DNA so that physical and biochemical secondary effects could be avoided as much as possible. The plasma ion species used in the study included He, C, N and Ar, for He and Ar to investigate ion mass effect, for N to investigate ion activity effect when compared to He and Ar, and for C to investigate its effect in the medical application significance. The DNA used was plasmid pGFP which was a simple DNA model containing the green-fluorescence-protein character gene for easy detection of the DNA. The ion energy was ranged from 1000 eV down to 10 eV and the ion fluence was typically ranged in 1014 – 1016 ions/cm2, and the dose could be converted from both energy and fluence. The DNA damage was observed using the gel

electrophoresis which was operated after the ion bombardment of DNA. The electrophoresis could separate different DNA forms, including original supercoiled form, relaxed form which was normally caused by single strand breaks (SSBs) and linear form which was due to DSBs. We clearly observed that only under certain combinations of the ion energy and fluence which indicated a dose could DSBs start to occur. For He ions, the threshold was at the energy of 1,500 eV and the fluence of 2×1015 ions/cm2, for Ar ion the threshold was at the energy of 1,000 eV and the fluence of 1×1015 ions/cm2 or the energy of 750 eV and the fluence of 2×1015 ions/cm2, for N ion the threshold was not found even at the energy as low as 26 eV and the fluence of 2×1015 ions/cm2, indicating the threshold for the N ion case to be lower than this energy and fluence combination (which was already beyond the instrumental capability), and for C ion the threshold was at the energy of 50 eV and the fluence of 4×1015 ions/cm2 or the energy of 100 eV and the fluence of 1×1015 ions/cm2. The threshold was obviously dependent on the ion mass and activity, namely, the heavier the ion, the lower the threshold, and the more the ion activity, the lower the threshold. Why the DNA DSB has the threshold in such values is discussed in terms of the DNA double strand structure. There was a puzzle that the DSB dose threshold found here was considerably higher than the cell lethal dose. A discussion on the puzzle argued that our study implied cell death mostly caused by indirect interaction between radiation and cells.

BM06.05.15
Nanoscale Surface Engineering and Sterilization on Rice (*Oryza sativa* L.) Seed via Hybrid Nonthermal Discharge Plasma Siwapon Srisonphan; Department of Electrical Engineering, Kasetsart University, Bangkok, Thailand.

Well-controlled wettability and liquid spreading of seed surfaces can help achieve seedlings of better quality, especially in difficult-to-grow regions, including those affected by drought. Nonthermal (cold) atmospheric plasma (NAP) can provide the complex mixture of surface functionalities, leading to nanoscale surface modification and dramatically change in seed surface wettability. However, for coated biological objects, such as seeds, plasma interaction is not entirely understood. Herein, we employed atmospheric hybrid cold plasma (HCP) by combining the pulsed corona discharge plasma in conjunction with a dielectric barrier discharge (DBD) to elucidate how NAP fundamentally interacts with seed surfaces. Moreover, we applied HCP to inactivate microorganisms that commonly attach the rice (*Oryza sativa* L.) seed husk to elucidate seed surface modification and biological sterilization.

Overall results show that the HCP can provide NAP with low power consumption and high efficiency for surface activation. The cold plasma treatment modified the surface of the rice seeds, resulting in accelerated germination and enhanced water imbibition (WI). The HCP treatment completely inactivated pathogenic fungi and other microorganisms, enhancing the germination percentage and seedling quality without destroying the viable seed membrane. The SEM firmly indicate the nanoscale modification of the surface morphology and the decontamination of pathogen infestation. We also show that the modified surface was primarily attributed to the combined effects of physical ions-enhanced etching and chemical surface functionalization. The WI time of modified seeds is initially decreased as an exponential function of exposure time, and gradually reaching to the WI saturation time in which the absorption rate is constant. We explained such phenomena via electron-ions initiated impact ionization inducing the reactive species (RS) for surface functionalization.

Therefore, microcorona discharge on a single dielectric barrier provides a nonaggressive cold plasma that can be applied to organic materials without causing thermal and electrical damage, and open up new avenues for the NAP treatment for the surface sterilization and disinfection of organic and biological materials with large-scale compatibility.

SESSION BM06.06: Biomedical Imaging
Session Chairs: Jaeho Kim and Hiroki Kondo
Tuesday Morning, November 27, 2018
Sheraton, 2nd Floor, Liberty C

8:45 AM *BM06.06.01
Carbon Nanotubes for Biomedical Imaging Toshiya Okazaki; AIST, Ibaraki, Japan.

Optical imaging is one of the most important techniques in biomedical studies. Recently, usage of near-infrared light has been extensively tried because of the higher transparency in biomaterials than the visible light. Single-walled carbon nanotubes (SWCNTs) are nanocarbon materials that show optical absorption and fluorescence in the near infrared (NIR) wavelength region. Especially, due to the bright fluorescence in NIR, SWCNTs have been expected as imaging probes.

In this talk, we first review the optical properties of SWCNTs and then show our recent results about the bio-application of oxygen-doped SWCNTs.[1,2] Interestingly, covalent doping of the nanotube surface with a low concentration of oxygen atoms can create a new optically allowed defect state.[3] Consequently, nanotube fluorescence is red-shifted and can be over 10 times brighter. Fluorescence vascular angiography and observation of the intestinal contractile activity of mice are demonstrated by using the produced oxygen-doped SWCNTs as infrared fluorescent labels and imaging agents.[1] Further, the biodistribution analysis after the administration of the oxygen-doped SWCNTs in mice is also discussed by the resonance Raman spectroscopy and NIR fluorescence microscopy.[2]

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9:15 AM BM06.06.02
Ultra-Wideband Surface-Enhanced Raman Scattering in Hybrid Graphene/Fragmented-Gold Substrate *via* Cold-Drawing Tingting Wu, Yu Luo and Lei Wei; Nanyang Technological University, Singapore, Singapore.

Conventional surface-enhanced Raman scattering (SERS) substrate is well-known for its supreme electromagnetic enhancement and the corresponding ultra-high sensitivity in detecting molecules at low concentrations. However, the existing problems like limited stability and reproducibility, fluctuant SERS response, and severely subjected to the fluorescence interference should be overcome before practical application. Graphene makes up this deficiency by its homogeneous surface, chemical inertness, biological compatibility, and capability of quenching the molecules fluorescence, and therefore offers a more stable, more reproducible, and cleaner Raman spectrum. We report a hybrid graphene/fragmented-gold substrate to achieve ultra-wideband graphene-mediated SERS (GSERS) substrate. Cold-drawing, associated with mechanical-geometric instability, is a mechanical stretching process in which tensile stress reduces the dimension of a drawn film. Through a controllable fragmentation of a gold nanofilm by simple cold-drawing, a two-dimensional

fragmented gold substrate is achieved with periodically distributed gold nanoscale tips. The localized surface plasmon resonance created by the fragmented gold tips passes through the top integrated one-atom-thick graphene layer, leading to remarkable graphene Raman response enhancements. To be specific, we detect 44.5/59-fold graphene G/G' Raman band enhancements at 633 nm and 211.4/194.3-fold enhancement at 785 nm. Furthermore, the hybrid graphene/fragmented-gold substrate is beneficial for sensing the top bonded rhodamine 6G and methylene blue dye molecules, enabling a wideband tracing of analytes. The demonstrated hybrid GSERS substrate offers an atomically flat, thin, and chemically stable bonding interface with strongly enhanced Raman signals. These results provide a promising solution to realize wideband enhanced coupling of the electromagnetic field to graphene by localized plasmons and develop a new practical generation of compact molecular sensor. We anticipate our results will benefit the fabrication of a desirable practical GSERS platform.

9:30 AM BM06.06.04
Cancer Theragnosis with Superparamagnetic Gold Nanoparticle Synthesized on Protein Particle Scaffold Eunji Jo, Ok Jeong Moon, Boram Lee, Young-Eun Jang, Jounghyeok Kwon, Hyun Jin Kim and Jeewon Lee; Korea University, Seong-buk gu, Korea (the Republic of).

Cancer theragnosis with a single multimodality agent is a key of modern cancer diagnosis, therapy and management, but the clinically feasible agent with *in vivo* cancer therapeutic and targeting efficacy has not been developed. Here we report a new cancer theragnostic agent based on superparamagnetism of gold that is induced on a cancer-targeting protein nanoparticle carrier. We synthesized a superparamagnetic gold nanoparticle cluster (named SPAuNC) on a viral capsid nanoparticle engineered to present peptide ligands targeting a tumor cell-overexpressed receptor(TCR). We observed the potent multimodality of SPAuNC that can TCR-mediated targeting, T2-weighted magnetic resonance imaging, and magnetic hyperthermia therapy of liver and subcutaneous tumors in live mice under an alternating magnetic field. In particular, SPAuNC shows excellent biocompatibility without *in vivo* accumulation and holds a promising potential as a clinically effective agent for cancer theragnosis.

9:45 AM BM06.06.05
Measurement of Penetration Effect About High-Intensity Infrared Laser Pulses Through Body Tissues with Micro-Thermocouples Danhong Han, Jingjing Xu and Shengyong Xu; Key Lab for the Physics and Chemistry of NanoDevices, Department of Electronics, Peking University, Beijing, China.

Researchers have utilized infrared (IR) lasers as energy source for laser therapies for curing skin diseases and skin injuries with remarkable positive effects. Preliminary experiments also showed that high-intensity IR laser pulses could penetrate thick body tissues, resulting in remarkable effects for recovering from injuries in deep muscles and cartilage tissues. Yet for the deep-level IR laser therapies, it is not clear how much of the laser flux had penetrated the body tissues at certain depths, and which of the three major effects of laser irradiation, i.e., laser induced photo-chemical effect, photo-thermal effect and mechanical dragging effect, played the key role in the curing process. How to design a new applied device to measure the penetration effect about high-intensity infrared laser pulses through body tissues is our concern.
Here we developed sensitive micro-sized thin-film thermocouple (TFTC) arrays on freestanding Si_3N_4 thin-film windows as sensors for laser flux and local temperature. These devices showed excellent linear response in output voltage to a laser flux of wavelength 325 - 1064 nm, and meanwhile indicated the local temperature at the laser spot.
By using these devices we systematically measured the penetrating effect and thermal effect of near infrared, high-intensity Nd:YAG laser pulses passing through several kinds of thick porcine tissues. These laser pulses were practically used as laser therapy in clinic treatment for recovering functions of muscle and cartilage from athletic injury.
We obtained penetration depth for porcine tissues of fat, skin and muscle, and also obtained the increasing rate of local temperature at different tissue depth. Our results offered reliable references as the thresholds for maximum irradiation doses of IR laser in clinic treatments. The method also offered an alternative approach for measuring the flux and heat under laser irradiation. Therefore we think the novelty and technique presented in this work are valuable for the communities of medical applications of laser technology, health-care, sports biology and biophysics in tissues.

10:00 AM BREAK

SESSION BM06.07: Plasma Treatment toward Therapy and Pharmacology II
Session Chairs: Emilio Martines and Hiromasa Tanaka
Tuesday Morning, November 27, 2018
Sheraton, 2nd Floor, Liberty C

10:30 AM *BM06.07.01
Cold Atmospheric Pressure Plasma Treatment to Assist Bacterial Inactivation and Tooth Restoration in Endodontic Procedures Vittorio Colombo[1], Matteo Gherardi[1], Romolo Laurita[1], Emanuele Simoncelli[1] and Riccardo Tonini[2]; [1]Department of Industrial Engineering, Alma Mater Studiorum - Università di Bologna, Bologna, Italy; [2]School of Dentistry, University of Brescia, Brescia, Italy.

In recent years, cold atmospheric plasma (CAP) - an ionized gas where the electronic temperature is much higher than the macroscopic plasma temperature - have raised great interest for the treatment of living tissue. A potential application of CAP is in the field of dentistry, where preliminary studies have demonstrated its potential use to improve osteointegration, dental instrument cleaning, adhesive polymerization, tooth bleaching, root canal disinfection and other purposes. The present study aims to investigate the use of an innovative and handheld DBD-jet plasma source, properly designed to be translated to the clinical environment and in a realistic endodontic procedure for the disinfection and restoration of root canals.
Root canal disinfection experiments have been performed on tooth models and were designed to also address: i) the influence of the humidity of the root canal on the treatment efficacy ii) the possibility of employing plasma activated liquids with antibacterial properties as irrigants.
On the side of endodontic restoration in the coronal region, the adhesive-dentin interface has been well recognized as the weaker area for dental composite resin restoration; the improvement, through the development of new materials and techniques, of its characteristic is essential to extend the longevity of dental restorations. To evaluate the enhancement of adhesive properties induced by CAP treatment of dentin, a push-out analysis is carried out on extracted teeth, where the shape of the root-canal has been standardized, using EDTA and phytic acid as etching reagents.
Finally, the restoration of the apical region of root canal aims at avoiding a new bacterial colonization in the tooth apex. Filling materials such as guttapercha, are generally used to completely seal the root apex, but they are characterized by low adhesive performances; thus, endodontic cements, known as sealers, are generally applied despite their cytotoxicity to improve the adhesion with dentine. The present study investigates the enhancement of adhesion between these materials and apical dentine of ex-vivo teeth treated by a DBD-jet plasma source by means of pushout tests and confocal microscopy analysis.

Although investigations on long-term stability of adherent monoblock to dentine surface and clinical studies are required, the present study supports the exploitability of cold plasma devices in real-life endodontic clinics.

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11:00 AM *BM06.07.02

A Principle of Blood Coagulation Induced by Low-Temperature Plasma Treatment to Develop the Rational Medical Practices for Bleeding Control Yuzuru Ikehara[1, 2, 3]; [1]Chiba University, Chiba, Japan; [2]Biotechnology Research Institute for Drug Discovery, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan; [3]Electronics and Photonics Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan.

1. Introduction
The low-temperature plasma (LTP) technology at atmospheric pressure is being integrated into methodologies in the medical and biological sciences. Bleeding control using LTP was one of the applications to reduce the heat damages on hemostasis in surgery, based on the novel plasma effect to induce aggregation of soluble serum proteins. In my talk, I will introduce our concept of LTP use in hemostasis and illustrate our feature plan to integrate LTP technology into the biomedical manufacture.
2. Blood coagulation using LTP treatment
The first report on blood coagulation using LTP was from Drexel University, which highlighted the plasma effects on natural blood coagulation process, but for hemolysis or serum protein aggregation. On the other hand, we have focused on the plasma effects on hemolysis, and aggregation of albumin that was not involved in blood coagulation and the fibrogenesis system, because erythrocyte volume and albumin concentration are much higher than the amounts and levels of platelets and fibrinogen. To maximize the plasma effect that alters them to cellular and molecular "glue" in clot formation, we developed the instrument to produce plasma using a dielectric barrier discharge (66 kHz, sinusoidal peak-to-peak voltage of 6.0 kV applied to an electrode. In *in vivo* experiments, our plasma treatment succeeded to form clots solidly more than the naturally formed clot, and could generate aggregation on the solution that contained either albumin and immune globulins, resulting in the protein disc at 1-cm diameter with continuous contact with the plasma flare.
3. Significance in Medicine
A basic concept on the electronic surgical devices is to evaporate water components in tissue and to close bleeding points by shrinking the tissue, and the treatment using electronic surgical devices sometimes caused severe heat damage in parenchymal tissues. On the other hand, the LTP treatment is an innovative approach with minimal invasion because it can stop blood flow by sealing the bleeding point and creating a possibly favorable healing process. Moreover, the fundamental concept behind LTP hemostasis is to provide an essential method that can suppress excessive host inflammatory responses. In other words, plasma treatment is a tissue-processing technology, insulating local inflammation to start the scar formation. Consequently, I believe that LTP will become a conventional processing technology in tissue and biomaterial engineering with the progression of plasma science. I hope that our results will serve as a stepping-stone for the advancement of plasma science.

Acknowledgments
I thank Dr. Sakakita for our collaborative research. I thank Dr. Sakakita for our collaborative research. Grant Numbers 24108006 and 15K08413 in MEXT/JSPS KAKENHI Grants-in-Aid for Scientific Research on Innovative Areas supported this study.

11:30 AM BM06.07.03

Atmospheric Pressure Plasma Modification and Damage Quantification of Amino Acids Harold McQuaid, Mark Tweedie, Davide Mariotti and Paul Maguire; Ulster University, Belfast, United Kingdom.

Modification of biological material via non-thermal plasmas is continuing to gain much attention. While the general effects of plasma interactions with biological targets are becoming better understood, the mechanisms behind the damage enhancement and cell selectivity of plasmas are far from complete. As the number of mechanistic studies into plasma induced modification continues to grow, so does the diversity of plasma sources and biological targets used to determine this interaction. Cysteine, a key amino acid in proteins, has been previously used in plasma interaction studies due to its relatively simple analysis and suitability as a biological model[1]. Cysteine was also shown to be one of the most sensitive amino acids towards plasma reactive species[2]. Investigations into plasma interaction with cysteine are currently limited to DBD[1], COST-jet and kINPen[3], and less directly via a DC plasma jet[4]. Remote plasma sources offer certain advantages both for scientific study and plasma application. However the transport of reactive species well beyond the plasma region may impact on their efficacy.

In this study the plasma induced interactions with cysteine are investigated using a remote RF plasma source containing He-H_2O and isolated from atmospheric impurities in order to observe effects with a relatively simplified plasma chemistry. The liquid sample containing cysteine is located in the far effluent of the plasma source and the arrival species are predominantly H_2O_2 and OH. We also study the case of liquid exposure to the near effluent / afterglow region, where atmospheric gas impurities can be expected to influence the chemistry. This provides a reference to allow comparison with literature reports. We also present results using a droplet in plasma system[5] whereby cysteine is passed through the plasma for ∼100 µs and exposed to a high flux of electrons and OH as well as H_2O^+, H^+, H_2O_2 etc. High rates of electron solvation and reduction reactions, similar to that found with radiolysis, has previously been demonstrated with this system[6]. The corresponding modification of cysteine via each treatment method is analysed using FTIR and Raman spectroscopy. Differences in damage characteristics between individual methods and those previously published are detected and attributed to a change in the plasma induced chemistry. To aid the understanding of the plasma chemistry involved, buffer and radical scavenger solutions were also added. The droplet in plasma setup showed a significant increase in magnitude of cysteine modification in comparison to the other setups.

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11:45 AM BM06.07.04

Preparation of a Radiopaque and Biodegradable Microbead Surface-Modified Using Air-Plasma Treatment for Transarterial Chemoembolization Yutaka Okamoto[1], Kenta Bito[1], Terumitsu Hasebe[1, 2], Shunto Maegawa[1], Kosuke Tomita[2], Tomohiro Matsumoto[1, 2] and Atsushi Hotta[1]; [1]Keio University, Yokohama-shi, Japan; [2]Tokai University, Tokyo, Japan.

Drug-eluting beads (DEB), used as embolic agents for transarterial chemoembolization (TACE), are required to have radiopacity and biodegradability at the same time, to be visualized in a body under fluoroscopy and CT scanning, which should not lead to complicating disease.
In this study, we fabricated radiopaque and biodegradable beads composed of Lipiodol (ethiodized oil) and polycaprolactone (PCL), a biocompatible and biodegradable polymer. However, since the Lipiodol/PCL beads were hydrophobic, they aggregated in water-soluble disperse media, causing difficulty in handling. Therefore, the surface modification of the beads is necessary for the prevention of occlusion that is caused by the injected beads in catheters. The surface modification by a biocompatible polymer such as PCL through molecular grafting on biomaterials is an attractive technique in tissue engineering. For the surface modification and the molecular activation for grafting, plasma treatments are widely used. It is expected that air-plasma treatment could easily modify polymer surface under atmosphere pressure at low cost without using reactive gases and vacuum chambers. Thus in this study, air-plasma treatment was studied to graft gelatin molecules on the bead surface, as gelatin is hydrophilic, biocompatible, and low cost, which may prevent the bead aggregation. Such Lipiodol/PCL beads may be applied to several parts in the drug-delivery system and the tissue engineering.
Lipiodol/PCL beads were first fabricated with a home-made microfluidic device. Then the beads were treated with air plasma to introduce -COOH groups on the surface, followed by the covalent grafting of gelatin molecules using carbodiimide as a coupling agent. The surface chemistry was characterized using the X-ray photoelectron spectroscopy (XPS), and the existence of gelatin was confirmed by the presence of the N1s peak coming from the gelatin molecules. The surface-modified beads were injected with water-soluble disperse media into a hepatic artery of a rabbit, and the CT image of the liver confirmed that the beads were visible *in vivo*. Furthermore, the beads before and after the surface modification with gelatin molecules were immersed in phosphate-buffered physiological saline (PBS) or in 1 mg/mL of lipase/PBS solution at 37°C to analyze degradability by measuring the weight loss. It was found that the degradation of the beads was significantly promoted by lipase, and that the gelatin molecules grafted on the bead surface did not prevent the degradability. It was, therefore, concluded that the Lipiodol/PCL bead obtained in this study was a promising candidate for new DEB, and that the surface modification of the beads by air plasma was useful to induce the mixture of oil and polymer.

SESSION BM06.08: Plasma Agriculture
Session Chairs: Eric Robert and Hiromasa Yamada
Tuesday Afternoon, November 27, 2018
Sheraton, 2nd Floor, Liberty C

1:30 PM *BM06.08.01
Air Discharges and Plasma Activated Water for Applications in Bio-Decontamination, Agriculture and Food Processing Zdenko Machala; Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia.

Non-thermal plasmas generated by electrical discharges in atmospheric pressure air are rich sources of various reactive species. Plasmas enable the transfer of reactive oxygen and nitrogen species (RONS) into the water or aqueous solutions when generated in contact with water and so generate the *plasma activated water* (PAW). PAW is typically a strong antibacterial agent and besides multiple uses in medicine for disinfection it has the potential for food processing or agriculture applications.
We prepare PAW by a self-pulsing streamer corona (SC) and transient spark (TS) discharges operated in air with water electrospray or water electrode. The production of active species (e.g. O_3, NO, NO_2 and OH) in the gas and consequently the PAW properties can be controlled by the discharge regime and gas-flow and liquid-flow parameters. Low power air corona discharge are dominated by O_3 production, which enhanced the biocidal effects. In the higher power TS, dominant gaseous products are NO_x that lead to significant NO_2^- and NO_3^- in the PAW and practically no O_3. Both discharges produce H2O2. The antibacterial action is then mainly due to the synergy of H_2O_2, NO_2^- and acidic milieu (via ONOOH formation) and typically decays in time within several hours post plasma activation, depending on temperature and pH [1,2]. We use UV-VIS colorimetric and fluorescence methods for the analysis of RONS in the PAW.
PAW produced by TS air discharge has been tested for agriculture applications, such as enhanced plant growth (lettuce, radish, tomato, wheat) or seed germination rate. Testing various medical applications of PAW or plasma activated media in dentistry (periodontal biofilms, endodontics), wound disinfection, urinary tract infections, or cancer cells are under way.
In a similar concept, TS air discharge was successfully demonstrated to induce antimicrobial effects in fresh fruit juices to extend their shelf lifetime without thermal pasteurization and without reducing their composition and nutrition/vitamin qualities. Potential effects of cold plasma on chemical (changes of pH, degradation of organic acids, polyphenols, sugars) and sensory juice properties (color, taste) were carefully tested and it was shown that the juice quality was not significantly affected.
This work was supported by Slovak Grant Agency VEGA 1/0419/18 and Slovak Research and Development Agency APVV-0134-12 and APVV-17-0382.
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2:00 PM *BM06.08.02
Innovative Agricultural Productivity Improvement Using Atmospheric Pressure Plasmas Kazunori Koga and Masaharu Shiratani; Kyushu Univ, Fukuoka, Japan.

Seeking for low energy consumption and high productivity processes for biomass and harvest is an important topic on agriculture. The conventional improvement methods of the agricultural productivity are irrigation, fertilization, and crop protection. Atmospheric pressure nonthermal plasmas can contribute to such methods by various ways such as sterilization, fertilization, water treatment and purification, soil treatment, seed treatment, storage improvement, insecticide, pre-harvest treatment, and post-harvest treatment. Nonthermal atmospheric plasmas are attractive for agricultural applications because they provide a large amount of reactive oxygen species (ROSs) and reactive nitrogen species (RNSs) with a little thermal damage to plants, crops, and fruits [1-8]. So far, we developed a scalable dielectric barrier discharge (DBD) device [5]. Using the device, we found that plasma-irradiated seeds of Arabidopsis thaliana show 11% reduction in a first harvest period from sowing and 56% increase in seed yield [1]. Plasma irradiated seeds of Sorghum, which is a strong candidate of biomass plants to produce ethanol [9], show 74 % increase in the volume of the plant. The energy consumption of 4.7MJ/ha for plasma irradiation corresponds to only 0.14% of 3.3 GJ/ha for cultivation and harvesting. The energy gain by plasma irradiation is 43.2 GJ/ha, in other words, the energy leverage is a quite high value of 1x104. Thus, atmospheric pressure nonthermal plasma offers an innovative agricultural productivity enhancement method with a high impact on our society.
This work was partly supported by JSPS KAKENHI JP16H03895 and JAXA.
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2:30 PM BM06.08.03
Optical Diagnostics of Atmospheric-Pressure Microwave-Excited Plasma Jets Jaeho Kim and Hajime Sakakita; National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan.

Non-equilibrium air plasmas at atmospheric pressure have been attracting special attentions for practical applications in various industrial fields including sterilization, decontamination, pollution control, surface materials processing, aerodynamics, high-speed combustion, microwave propagating, and lighting discharge control. Recently, their medical applications such as blood coagulation, bleeding control, and wound healing have been also reported [1-6]. Natural air is a mixture of gases including N_2, O_2, Ar, CO_2, H_2O, and so on. Air plasms can provide molecular radicals such as OH, NO, CN, atomic radicals such as H, O, N, and other active species.
In this work, we have developed a minimalized microwave-exited plasma source using a 2.45 GHz magnetron source. The plasma source produces stable air plasma jets even at atmospheric-pressure. We have considered their materials processing applications as well as biological and medical applications. Optical diagnostics were carried to better understand the fundamental properties of the plasma jets for the applications using an optical emission spectroscopy. The kinds of air plasma-induced radicals were identified with a variety of operation conditions. Temperature properties of a plasma jet were also investigated by measuring rotational temperatures and vibrational temperatures of nitrogen molecules. In the conference, we will present these experimental results.

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[6] I. H. Won et al., IEEE T. Plasma Sci. 42,2788-2789 (2014).

2:45 PM BREAK

SESSION BM06.09: Panel Discussion–Plasma Processing and Monitoring for Bioengineering and Biomedical Engineering
Session Chairs: David Graves and Yuzuru Ikehara
Tuesday Afternoon, November 27, 2018
Sheraton, 2nd Floor, Liberty C

3:15 PM PANEL DISCUSSION