Plasma Processing for Biomedical Applications Focus Topic
Room 101A - Session PB+BI+PS-TuM

Plasma Processing of Biological/Biomimetic Surfaces

Moderators: Uroš Cvelbar, Jozef Stefan Institute, Slovenia, Satoshi Hamaguchi, Osaka University, Japan

8:00am **PB+BI+PS-TuM1 Investigation of Discharge Propagation on Cell and Plasmid Suspension in Plasma Gene Transfection**, ***Yugo Kido***, Pearl Kogyo Co., Ltd., Japan; *H. Motomura, Y. Ikeda,* Ehime University, Japan; *S. Satoh,* Y's Corp., Japan; *M. Jinno,* Ehime University, Japan

The authors have been developing a plasma gene transfection technique and averaged transfection efficiency up to 20% and cell survivability up to 90% are achieved for more than 20 kinds of cells. A typical procedure of this method is as follows. Target cells are cultured on a 96 well plate and gene suspension is added. The plate is placed between a high voltage electrode made of copper capillary with the diameter of 70 µm and a copper plate grounded electrode. By exposing the suspension to a microplasma generated at the tip of the capillary electrode, the cells are transfected by the genes. In this method, both chemically reactive species (chemical factors) and discharge current (electrical factors) are indispensable to the transfection process and their synergistic effect has been experimentally verified. Moreover, the transfection occurs on the whole area of each well although only the central area is exposed to the microplasma. In this study, to clarify how the discharge current contributes the transfection process with the synergistic effect with the chemical factor, discharge propagation phenomenon on the cell and plasmid suspension is investigated.

As a target, COS-7 cells are cultured on a 35 mm dish and 24 µg of plasmid pCX-EGFP suspended in 120 µL of TE/PBS buffer is added. The applied voltage between the electrodes is 20 kHz sinusoidal waveform and the amplitude is set at 10 kV peak to peak. The gap length between the capillary electrode and the suspension is set at 1 to 5 mm. The discharge propagation is observed with an ICCD camera equipped with a UV lens.

When only TE/PBS buffer solution exists in the dish, the discharges reach the buffer solution surface and then they propagate radially up to 15-20 mm of diameter. On the other hand, when the cells are cultured on the dish, remarkable radial propagation of the discharge is not observed and the discharge irradiation area is limited within the diameter of about 1 mm, which is narrow compared with the area in which the transfection occurs. Therefore, as a contribution of the electrical factors, not only the direct effect of the discharge current, charge on the plasmids, conduction current in the solution etc. should be analyzed. As a first step of the chemical factor investigation, similar observation is performed by the ICCD camera with an interference filter to observe the emission of OH radicals. The results of the discharge propagation paths study by means of equivalent circuit simulation and comparative analysis between the discharge propagation and the transfection will be shown at the symposium.

8:20am **PB+BI+PS-TuM2 Spectroscopic Study of Permeability of Stratum Corneum by Plasma Treatment for Transdermal Drug Delivery**, ***Jaroslav Kristof***, *N. Tran, M. Blajan, K. Shimizu,* Shizuoka University, Japan

Application of drugs by needles presents risk of infections and causes pain. On the other side, oral application of drugs can be toxic for human body because drug has to be transported through alimentary tract and higher amount of active agent is required. Transdermal delivery could be ideal painless and effective way but barrier function of skin has to be reduced for improving permeability of drugs. Research of last years proves that plasma can interact with skin and cause decreasing barrier function of skin [1-3].

We used plasma jet and microplasma discharge for investigation of barrier function of stratum corneum – horny layer of of Yucatan micropig skin. Helium or argon was used as working gas. These rare gases were later enriched by liquids like water or ethanol through the bubbling system to achieve higher amount of active particles like OH.

Physical changes of the pig skin were observed by microscope. As the human body is not non-conductive, we can expect different results when conductivity of layer under skin is changed. We compared effect of plasma on conductive and non-conductive material. Placement of skin on conductive material caused burned spots on skin by plasma jet [3]. While it was isolated, no damage was observed with plasma jet irradiation. In case of treatment of skin by microplasma, physical damage was hardly observed.

Changes in stratum corneum layer were observed by Attenuated Total Reflectance – Fourier Transform InfraRed (ATR-FTIR) spectroscopy. ATR-FTIR spectrum offer information about water, lipid bilayer and proteins in stratum corneum. Permeability of skin for drugs correlates with shift of asymmetric stretch of CH_2 band to higher wavenumbers. This information describes behavior of lipid bilayer. Information about reaction of proteins on plasma treatment content Amide I and Amide II bands. Reaction of stratum corneum layer of pig skin depended on used discharge type and gas. Effectivity of plasma sources and used gases or gas mixtures for transdermal drug delivery was analysed.

References

[1] Lademann J., A Patzelt A., Richter H., Lademann O., Baier G., Breucker L., Landfester K., *Laser Phys. Lett.***10**, 083001 (2013).

[2] Wu A. S. et. al., *Journal of surgical research***179**(1), E1-E12 (2013).

[3] Shimizu K., Hayashida K. and Blajan M, *Biointerphases***10**(2), 029517 (2015).

8:40am **PB+BI+PS-TuM3 Cell Attachment to Microwave Plasma-oxidized Titanium and Titanium Alloy Substrates**, ***Denis Dowling***, University College Dublin, Ireland; *M. Naciri,* University Mohamed V of Rabat, Morocco; *M. Al-Rubeai, A. Breen,* University College Dublin, Ireland **INVITED**

Titanium and its alloys have been widely investigated for use in orthopedic and dental implant devices, particularly for osteointegration and bio-compatibility. This paper evaluates the influence of titanium surface oxidation using a microwave plasma treatment technique on cell attachment. Commercially pure titanium (CpTi) and titanium alloy (Ti6Al4V) discs were treated in an oxygen atmosphere for 5 min-utes at 850 °C using a microwave (2.45 GHz) plasma system, operating at 2 kPa. After the 5-minute treatment, the thickness of the oxidized layer was 2.3 µm on the CpTi discs and 4.7 µm on the Ti6Al4V discs, with growth rates of 0.5 and 1 $\mu m.min^{-1}$ respectively. Reduced plasma oxidation rates were observed on a high surface area beaded surface (Porocoat). In contrast to the plasma treatments, the use of air furnace oxidation only achieved an oxide layer thickness for the CpTi of 1 µm, when treated at the same temperature. Optical profilometry measurements were performed to determine the surface roughness; XRD, EDX, and SEM examinations were also car-ried out to determine the properties of the oxide layers and their morphologies. Cell attachment to the treated discs was also assessed after exposure times of 25 and 100 minutes. A 40% increase in MG63 osteoblast cell attachment on the Ti6Al4V discs was observed, when compared with that on the CpTi discs. Alkaline phosphatase (ALP) specific activity of MG63 cells grown on control and plasma oxidised surfaces were compared after 21 days. A statistically significant difference between Ti6Al4V and CpTi oxidised surfaces ($P<0.05$), when compared to that obtained for the control surface that had not been plasma treated. The acicular morphology of the oxidised Ti6Al4V surface was found to have the most significant influence on enhancing cell attachment, combined with higher oxide layer roughness and thickness.

9:20am **PB+BI+PS-TuM5 The Role of Electrical and Chemical Factors in the Molecular/Gene Transfection by Micro-Plasma Irradiation**, ***Masafumi Jinno***, *Y. Ikeda, H. Motomura,* Ehime University, Japan; *Y. Kido,* Pearl Kogyo Co. Ltd., Japan; *S. Satoh,* Y's Crop., Japan **INVITED**

The plasma gene transfection is expected as a safe and useful method of gene transfection. However, this method had a problem of a difficulty in keeping both high transfection efficiency and less cell damage simultaneously. The authors have evaluated four different plasma sources, such as arc discharge, plasma jet, DBD (dielectric barrier discharge) and microplasma, in terms of the transfection efficiency and the cell viability. High transfection efficiency is achieved by the styles of arc discharge and microplasma in which the electric current flows via the cells. Our experimental results suggests that an electric current may play an important role in plasma gene transfection, and that total volume of the gas flow must be small or zero and the area in which the cells are directly irradiated by plasma must be small in order to achieve higher cell viability. Among the various types of plasmas, which the authors have tried, the microplasma satisfies these conditions and brings both the high transfection efficiency and the high cell viability simultaneously.

We evaluated the contribution weight of three groups of the effects and processes inducing gene transfection, i.e. electrical, chemical and biochemical ones through three experiments. The laser produced plasma (LPP) was employed to estimate the contribution of the chemical factors. The liposomes were fabricated and employed to evaluate the effects of plasma irradiation on membrane under the condition without biochemical

reaction. The clathrin-dependent endocytosis, one of the biochemical processes was suppressed. It also turned out the clathrin-dependent endocytosis is the process of the transfection against the 60% in all the transfected cells. The endocytosis and electrical poration are dominant in plasma gene transfection, and neither permeation through ion channels nor chemical poration is dominant processes.

By scavenging the H_2O_2 generated by plasma irradiation using catalase, the transfection efficiency decreased to 40% of that of without catalase. On the other hand, when the H_2O_2 solution is dropped in the cell suspension without plasma irradiation, the transfection is not observed. These results suggest that the synergistic effect of H_2O_2 with electrical factors or with other reactive species generated by plasma irradiation is important. Consequently it becomes clear that chemical factors, radicals such as H_2O_2 and reactive oxygen/nitrogen species, do not work by itself alone, and that the electrical factors (electrical current, charge and field) are essential to plasma gene transfection.

11:00am **PB+BI+PS-TuM10 Control of Plant Growth by RONS Produced Using Nonthermal Atmospheric Air Plasma**, ***Kazunori Koga,*** Kyushu University, Japan*; T. Sarinont,* Kyushu University*; M. Shiratani,* Kyushu University, Japan

Nonthermal atmospheric plasmas have been widely used for biomedical applications because of their non-equilibrium feature and synergy effects [1-3]. The non-equilibrium feature allows us to introduce reactive oxygen and nitrogen species (RONS) to biomaterials with a significantly wide dose range compared with the conventional irradiation methods such as X-ray and γ-ray [4]. For an agricultural application, we succeeded in reducing harvest period and enhancing crop yield by plasma irradiation to plant seeds [5]. We found irradiation of RONS with an appropriate dose to seeds brings about growth enhancement in all growth stages of plants. To understand the growth enhancement mechanism, here we have studied dependence of irradiation dose of RONS produced by plasma to seeds on growth of *Arabidopsis thaliana L.* Experiments were carried out using a scalable DBD device [2, 3, 5]. The device consisted of 20 electrodes of a stainless rod of 1 mm in outer diameter and 60 mm in length covered with a ceramic tube of 2 mm in outer diameter. The discharge voltage and current were 9.2 kV and 0.2 A. 20 seeds of Arabidopsis thaliana L. were set 3 mm below the electrodes. The RONS dose was controlled by the irradiation time. After plasma irradiation, they were grown on soil tab in incubators. To evaluate plant growth, the stem length was measured as a function of cultivation days. The stem length was normalized by the stem length of the plants without plasma irradiation. To evaluate statics of the measured values, we used a two-tailed ANOVA statistically significance different at $\alpha = 0.05$ ($p < 0.05$). The normalized stem length increases to 1.3 for 3 min irradiation, then decreases to zero for 10 min irradiation. The results indicate the plant growth is activated by plasma irradiation less than 3 min and inactivated by plasma irradiation of 5-10 min. Above 10 min irradiation, no seeds were germinated. We have succeeded in growth control of plants from death to activation with irradiation dose of RONS produced by plasma. The mechanism will be discussed in the presentation.

[1] J. Raiser and M. Zenker, J. Phys. D, 39, 3520 (2006).

[2] T. Sarinont, et al., JPS Conf. Proc. 1, 015078 (2014).

[3] S. Kitazaki, et al., Curr. Appl. Phys., 14, S149 (2014).

[4] A. Pankaj, et al., Scientific Reports, 5, 17781 (2015).

[5] K. Koga et al., Appl. Phys. Express, 9, 16201 (2016).

11:20am **PB+BI+PS-TuM11 Generation of Reactive Species in Medium Irradiated Laser-Induced-Plasmas**, ***Yukihiro Kurokawa,*** *N. Kurake, K. Takeda, K. Ishikawa, H. Hashizume, H. Tanaka, H. Kondo, M. Sekine, M. Hori,* Nagoya University, Japan

The non-equilibrium atmospheric pressure plasma (NEAPP) was irradiated to the cell culture medium as liquid. The antitumor effect, showing the selective killing effect for cancer cells without killing normal human cells, was reported [1,2]. This effect are considered to be caused by large amounts of reactive nitrogen and oxygen species (RONS) generated by the plasma. However, chemical reactions during transport of plasma in ambient to the liquid surface is complicated; therefore we have applied the laser-induced plasma.

Previously, we reported that the high ratio of NO_2^-/ H_2O_2, even in low H_2O_2 contained in the plasma activated medium [3]. However, relations of reactive species concentrations with antitumor effects have not been fully elucidated. Here, we focus on the concentrations of reactive species generated in culture media by the laser-induced plasma.

A Nd:YAG laser and harmonic generators (Quanta Ray Pro 230, Spectra Physics) provided the pulsed-laser light with a wavelength of 266 nm, a frequency of 30 Hz, a power at sample surface of 25 mW. The light was focused on the gas-liquid interface of ultrapure water or Dulbecco's Modified eagle Medium (DMEM; cat. no. 5796; Sigma) by using plano-convex lens, made of synthetic quartz. 2 mL of the liquid was typically irradiated for 5 min. This is called as LPAM. Just after irradiation, H_2O_2 and NO_2^- concentrations were measured by using absorption that was measured by ultraviolet-visible near infrared spectrometer (V-650, JASCO). Moreover, HeLa cells were incubated in the LPAM and cell survival was measured after 24 h incubation. For analysis of killing mechanism, activated caspase3/7 as apoptosis marker (CellEvent Caspase-3/7) was measured after fluorescent staining by a fluorescent microscope.

The LPAM generated effectively H_2O_2 causing by photo-dissociation of water, hydroxyl radicals (・OH) works a precursor of H_2O_2 with the reaction of ・OH + ・OH →H_2O_2. Survival of HeLa cells in the LPAM was dependent on dilution of the LPAM and standard DMEM. We prepared the diluted LPAM for a half of killing of HeLa cells. After the cultivation for 24 h in the diluted LPAM, the caspase-3/7 activity of dead cells as apoptosis death was observed clearly. Notably, the cell-death was almost inhibited by catalase.

We will discuss on the generation mechanism of active species and the mechanism of antitumor effect of the LPAM with comparison of the PAM.

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[1] H. Tanaka *et al*., Plasma Medicine, 3, 265 (2013); [2] F. Utsumi *et al*., PLoS ONE, 4, e81576 (2013); [3] N. Kurake *et al*., Arch. Biochem. Biophys. (2016) doi:10.1016/j.abb.2016.01.011

11:40am **PB+BI+PS-TuM12 Electric Fields in kHz-driven Plasma Jets**, *ET. Slikboer, Y.N. Nguyen,* Eindhoven University of Technology, The Netherlands*; O.Y.N. Guaitella,* Ecole Polytechnique, Palaiseau, France*; G. Sretenović,* University of Belgrade*; A. Obrusnik,* Masaryk University, Brno*;* ***Ana Sobota,*** Eindhoven University of Technology, The Netherlands **INVITED**

Non-thermal atmospheric pressure plasma jets have been developed for use on thermosensitive targets at atmospheric pressure, for example polymers or for biomedical applications. Diagnostics on these plasma sources is challenging because of their transient nature, often associated jitter and very small volume. Electric fields, fundamental property essential for the understanding of the discharge, are not well known. In this talk two methods of electric field measurements will be shown applied to a He kHz-driven jet, one based on spectroscopy and one on polarimetry and the obtained results will be discussed.

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