## Coatings for Biomedical and Healthcare Applications
## Room Royal Palm 1-3 - Session D4

### Biointerfaces: Improving the Cell Adhesion and Avoiding Bacteria Adhesion. What Kinds of Coatings Should be Used?

**Moderators:** Marcela Bilek, The University of Sydney, Margaret Stack, University of Strathclyde, Vincent Fridrici, Ecole Centrale de Lyon - LTDS

2:10pm **D4-3 Titanium Oxide Coatings to Improve Cell Adhesion and Differentiation**, *V Garcia-Perez, A Almaguer-Flores,* Universidad Nacional Autónoma de México, Mexico*; R Olivares-Navarrete,* Virginia Commonwealth University, USA*; A Fonseca-Garcia,* ***Sandra Rodil,*** Universidad Nacional Autónoma de México, Mexico **INVITED**

Amorphous titanium oxide ($aTiO_2$) coatings were produced by magnetron sputtering using a Ti target and a reactive $Ar/O_2$ atmosphere. The coatings were deposited on commercially pure titanium ($aTiO_2$/cpTi) and stainless steel ($aTiO_2$/SS) substrates with a thickness of about 60-70 nm. For the SS substrates, a Ti buffer layer was used to improve the film-substrate adhesion. The results from different cell-surface interactions clearly show that a thin but dense and stoichiometric $TiO_2$ oxide film present a better biological response than the cpTi, even when deposited on the SS substrate. A significantly larger initial attachment (2 hours) of human osteoblasts cells was observed on the TiO2 films in comparison to cpTi even at protein-depleted conditions, i. e, using serum-free culture media. The attachment was comparable to that obtained on collagen-coated plastic dishes (100%), while on cpTi only a 40% of attachment was obtained. The cell adhesion at longer period of times (24h and 7 days) was also demonstrated for human mesenchymal stem cells (MSCs). Similarly, a larger differentiation into osteoblasts of the MSCs was observed on the $aTiO_2$/cpTi coatings in comparison to the native oxide layers (cpTi) for two different surface roughnesses: smooth (0.3 μm) and micro-rough (2.6 μm). As a final test, the cell adhesion, differentiation and inflammatory response of MSCs on $aTiO_2$/SS surfaces was compared to the cpTi and the SS metallic surfaces. The results clearly show that the amorphous TiO2 surfaces presented the highest expression of integrins and production of osteogenic proteins in comparison to the uncoated SS surfaces, reaching a very similar response to that presented by the typically used titanium surfaces. Moreover, the pro-inflammatory factors were inhibited while anti-inflammatory factors were up-regulated, demonstrating the advantage of using thin $TiO_2$ films for the development of orthopedic and dental implants with improve bone regeneration and osseointegration.

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2:50pm **D4-5 Antibacterial Thin Films with Controlled Antibiotics Release Based on Plasma Polymer**, ***Vitezslav Stranak,*** *J Kratochvil, D Kahoun, J Sterba, H Langansova, J Lieskovska,* University of South Bohemia, Czech Republic*; J Hanus, J Kousal, A Kuzminova, O Kylian,* Charles University in Prague, Czech Republic

Bacterial infections developed after implant surgery can cause serious medical complications for the patients. Current approach for infection suppression is to use systemic treatment of the patient by antibiotics. An alternative and very promising approach, that gains increasing attention, is to cover the surface of the implant with bioactive thin film, which prevents creation of the biofilm. Main benefit of this method are local treatment and possible supporting effect to the conventional systemic treatment.

Two different methods, which are able to gradually release antibacterial agents, will be presented. First method is based on immobilization of Ampicillin (i.e. common antibiotic) into magnetron sputtered Nylon 6,6 thin films. It was proven that Ampicillin is immobilized equally in the volume of Nylon 6,6 film, so it is possible to easily tune the amount of antibiotics in the coatings simply by changing their thickness. Controlled release kinetics can be achieved by deposition of diffusion barrier. Nanocomposite consisting of Cu nanoparticles, embedded into plasma polymerized PTFE represent the second method. Advantage of nanoparticles is their huge area against their volume, which reduces side effects of antibacterial metals in human body to minimum. Both methods are applicable to any substrate including smooth metals or polymers.

Acknowledgement: This work is supported by GACR 16-14024S.

3:10pm **D4-6 Development of a Microfluidic Based Multianalyte Biosensor Device for Medical Diagnostics**, ***Emma MacHugh,*** Dublin Institute of Technology, Centre for Research in Engineering Surface Technology (CREST), Ireland*; B Duffy, M Oubaha,* Centre for Research in Engineering Surface Technology (CREST), Ireland

Over the past decade, the biosensor research community has intensively investigated the development of innovative point-of-care (POC) devices often targeting the improvement of the platforms sensitivities for single analyte detection. However, in certain situations the detection of several in parallel is desired for economical and practical reasons, making the development of multianalyte platforms one of the most promising methodologies in the medical diagnostic industry.

Most biosensors also require an integrated microfluidic system for the flow of analyte liquids (blood, saliva and urine) onto the sensor areas of the POC. In order to enable a rapid and efficient delivery of these analytes, of the most important parameters, the surface properties of the microchannels have to exhibit as high a surface energy as possible. Unfortunately, to date, most materials employed in the fabrication of microfluidics are based on hydrophobic materials, the most popular of those being PDMS, and utilises low resolution fabrication processes, such as injection moulding and often require external pumps to activate the circulation of the liquids.

In this study, we propose a new fabrication concept of optical multianalyte biosensor platforms based on the integration of multiple sensor spots onto a microfluidic platform. The originality of the study resides in the development of high surface energy hybrid sol-gel materials that can be simultaneously photoreactive for microstructuration of high resolution microchannels by standard photolithography processes and irreversible immobilization of biological species. The preparation and characterisation of these innovative materials as well as the development of multianalyte biosensors will be presented. Correlation between the structure and surface properties of these materials along with the correlation of these properties against the fluidic performances of the biosensors platforms will be discussed. Finally, demonstration of concept of the multianalyte capability of the biosensor platform via optical fluorescence and a sandwich ELISA will be presented.

3:30pm **D4-7 Bactericidal Activity and Cytotoxicity of a Zinc Doped PEO Titanium Coating**, ***Luciane Santos,*** Pontificia Universidade Católica do Paraná, Brazil*; K Popat,* Colorado State University, USA*; P Soares,* Pontificia Universidade Católica do Paraná, Brazil

Metallic implants are susceptible to bacterial colonization even years after the implantation impairing the osseointegration process. The treatment of a colonized implant is highly demanding, and in most cases implant replacement is the only effective solution. To avoid the bacterial attachment and proliferation, bactericidal coatings are proposed as a long-term prevention tool. Those coatings must assure a bactericidal activity for a long period and cannot induce cytotoxic responses in eukaryotic cells. Among all the bactericidal agents Zinc is one of the most investigated due to its broad bactericidal activity spectrum and its stimulatory effect on bone formation. The aim of this study is to obtain a titanium oxide coating containing Zinc and evaluate its bactericidal activity, cytotoxicity and ion release profile. The coating was obtained by Plasma Electrolytic Oxidation (PEO) on commercially pure titanium grade 4 at 350 V for 60 s. Samples were divided in two groups, the reference group was obtained in a base electrolyte containing calcium acetate and calcium glycerophosphate (called CaP group). The experimental group has added Zinc acetate as a Zinc source to the base electrolyte (called Zn-CaP group). The surface was characterized by Scanning Electron Microscopy (SEM) and X-ray Photoelectron Spectroscopy (XPS), while the ion dissolution was evaluated by Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES). The bactericidal activity was determined against *Staphylococcus aureus* by fluorescence microscopy using a live/dead viability kit. The cytotoxicity against eukaryotic cells was evaluated using adipose derived stem cells (ADSC) using the lactate dehydrogenase (LDH) assay. Zinc, Calcium and Phosphorous were incorporated to the titanium oxide coating and no changes on the coating structure and morphology were observed by the addition of Zn to the electrolyte. ICP-AES results show the coatings released Ca, P and Z ions after 28 days of immersion in DI water. The ICP-AES profile suggests the ion release reach an equilibrium state after 7 days of immersion. The Zn-CaP coating presented bactericidal activity against *S. aureus*, showing a higher number of dead bacteria after 6 h of incubation and a lower number of living bacteria after 24 h compared to CaP group. No cytotoxic effect was observed against ADSC by the presence of Zn on

the coating, indicating the Zn-CaP coating has a potential to prevent bacterial colonization in metallic implants.

3:50pm **D4-8 Antibacterial Effects of Titanium Embedded with Silver Nanoparticles Based on Electron-Transfer-Induced Reactive Oxygen Species***, **Guomin Wang**, W Jin, A Qasim, A Gao, X Peng, W Li, H Feng, P Chu,* City University of Hong Kong, Hong Kong

Although titanium embedded with silver nanoparticles ( Ag-NPs@Ti) are suitable for biomedical implants because of the good cytocompatibility and antibacterial characteristics, the exact antibacterial mechanism is not well understood. In the present work, the antibacterial mechanisms of Ag-NPs@Ti prepared by plasma immersion ion implantation (PIII) are explored in details. The antibacterial effects of the Ag-NPs depend on the conductivity of the substrate revealing the importance of electron transfer in the antibacterial process. In addition, electron transfer between the Ag-NPs and titanium substrate produces bursts of reactive oxygen species (ROS) in both the bacteria cells and culture medium. ROS leads to bacteria death by inducing intracellular oxidation, membrane potential variation, and cellular contents release and the antibacterial ability of Ag-NPs@Ti is inhibited appreciably after adding ROS scavengers. The whole process can be found in Fig. 1. Even though ROS signals are detected from osteoblasts cultured on Ag-NPs@Ti, the cell compatibility is not impaired. This electron-transfer-based antibacterial process which produces ROS provides insights into the design of biomaterials with both antibacterial properties and cytocompatibility.

4:10pm **D4-9 Tribocorrosion and Cytotoxicity of FeB-$Fe_2B$ Layers on AISI 316 L Steel***, I Campos-Silva,* Instituto Politecnico Nacional, Surface Engineering Group, Mexico*; M Palomar-Pardavé,* Universidad Autonoma Metropolitana-A, Mexico*; R Perez Pasten-Borja,* Instituto Politecnico Nacional, ENCB Zacatenco, Mexico*; O Kahvecioglu,* Argonne National Laboratory, USA*; D Bravo-Bárcenas,* Universidad Autonoma Metropolitana-A, Mexico*; C López-García, **RodolfoYael Reyes-Helguera**,* Instituto Politecnico Nacional, Surface Engineering Group, Mexico

All metallic biomaterials are required to satisfy various criteria, such as adequate strength, high resistance to corrosion, biocompatibility, and high wear resistance. However, the various biomaterials that have been developed thus far do not satisfy all of the above requirements. Wear and corrosion have been reported to be the primary reasons for the failure of implant elements.

One alternative to reduce corrosion and wear is the boriding process. Boride layers have excellent resistance to crevice and pitting corrosion, high temperature performance as well as outstanding mechanical properties in corrosive environments. Based on that, new results about the tribocorrosion resistance and cytotoxicity of borided AISI 316 L steel are presented in this work. The powder-pack boriding process was conducted at 1273 K with 4 h of exposure, whereas a FeB-$Fe_2B$ layer, with 50 microns of thickness, was obtained at the material surface. The tribocorrosion tests were performed in Hank's solution, using a ball-on-flat tribometer, which was connected with a three electro-chemical cell. The system comprised $Al_2O_3$ ball as the counterpart, the borided AISI 316 L steel as the working electrode, platinum rod as the counter electrode, and Ag/AgCl as the reference electrode. All sliding test, in the presence or absence of corrosion, was performed under 20 N normal force, considering a total sliding distance of 100 m.

The *in vitro* cytocompatibility of borided AISI 316 L steel was evaluated and compared with a conventional AISI 316 L steel. The immortalized human fibroblast CHON-002 and Vero established cell line from ATCC collection were used. Cells were exposed to conditioned leachates produced by immersion of the materials in culture medium (DMEM). Polyurethane film containing 0.1% zinc diethyldithiocarbamate (ZDEC) and polyurethane film containing 0.25% zinc dibuthyldithiocarbamate (ZDBC), as well as high density polyethylene films were used as reference materials. Cells cultured in fresh medium was used as negative control. Cell viability was established with the cellular metabolic activity assay by means of MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5diphenyl- tetrazolium bromide). The data were analyzed statistically by ANOVA, considering a significance of 5%.

The results showed that the presence of FeB-$Fe_2B$ layer increases the tribocorrosion performance in comparison with the AISI 316 L steel. In addition, the AISI 316 L steel samples modified by boron denoted satisfactory properties in terms of effects on survival and proliferative activity of human fibroblasts; results that reveal that the boride layers are excellent candidates for the use as biomedical layers.

4:30pm **D4-10 Optical Spectroscopic study for Atmospheric Pressure Plasma by Radio Frequency Power***, **Chuan Li**,* National Yang Ming University, Taiwan*; J Hsieh,* Ming Chi University of Technology, Taiwan*; C Yu,* National Yang Ming University, Taiwan

Atmospheric plasma techniques developed rapidly in the past decade. High-performance atmospheric plasma harnesses the power of plasma for surface treatment such as cleaning and coating. Due to the nature of atmospheric environment, the atmospheric plasma functions more like an ion carrier rather than reaction producer. This particular indicates that much less destructive processes such as ionization and excitation occur in the plasma zone. The less destruction implies more intact molecules or atoms survive their journeys through the plasma zone, which is crucial if one would like to maintain certain levels of the integrity of molecules delivered by the plasma to the surface of a substrate. Such a condition is particularly necessary for depositing macromolecules such as proteins, DNA/RNA in biomedical applications. It is also found useful in the task of surfaces activation and modification where the atmospheric plasma can be straightforwardly utilized for large scale productions without complicate vacuum facilities. The roll-to-roll process for coating and etching metallic or polymeric surfaces is a typical example in aviation, marine, automotive and civil applications. In this study, we investigate the effects of radio frequency of power and gas flow rates on the chemical compositions and morphology of atmospheric He plasma. A customized plasma system was setup and equipped with a radio frequency power supply, an optical emission/absorption spectrometer, deuterium halogen light source, x-y-z automated table, intensified charged coupled device camera, various flow controller and pressure gauges. The study focuses on the analysis of optical emission and absorption of spectra, temperature and power of He plasma by varying the radio frequency and flow rates. The chemical compositions of plasma are further analyzed using the optical spectra to identified possible ions and radicals. Along with the assistance of digital camera, additional information on the density of plasma is acquired for visualization. As a final touch, films of lactic acid are deposited on glass substrates via the He atmospheric plasma to demonstrate its capability as a carrier and reaction center.

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