Coatings for Biomedical and Healthcare Applications
Room Pacific Salon 3 - Session D3-TuM

Surfaces and Coatings to Promote Tailored Biological Responses

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8:40am **D3-TuM-3 *In Vitro* Evaluation of Macrophage Response to Ionic Liquid-Coated Titanium:***, **Sutton Wheelis**, L Guida, D C. Rodrigues,* University of Texas at Dallas, USA

Introduction: Dicationic Imidazolum-based ionic liquids with amino acid anions (IonL) have been proposed as a multifunctional coating approach for dental implants. IonL are able to form a temporary, stable coating on the surface of titanium (cpTi), providing lubrication and antimicrobial properties to address the multiple causes of early dental implant failures while maintaining bone and soft tissue cell compatibility *in vitro.* However, there is limited information regarding the response of immune cells to this coating, which is significant as they are often determining factors in implant healing. The aim of this study is to evaluate the proliferation and cytokine gene expression of human macrophages in response IonL-coated cpTi *in vitro* to infer the possible effect of the coating on the onset of the acute immune response *in vivo*.

Methods: IonLs with the best combination of antimicrobial and host cell compatibility properties (IonL-Phe, IonL-Met) were chosen as the coatings for investigation. CpTi disks (5 X 2 mm) were drop coated in 1 umol of each IonL on the disk surface. Uncoated cpTi disks and 1 umol of pure IonL were used as controls. Samples and controls were plated individually in 6 well plates in experimental duplicates (n = 36), seeded with primary human macrophages (M0, Cellprogen), then incubated for 1 or 3 days with a 135,000 or 50,000 cells per well. One set of experiments underwent a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay to determine cell proliferation in each well. The other experimental replicate were evaluated with a quantitative polymerase chain reaction (qPCR) to determine changes in IL6, TNFα, TGFβ1, iNOS, ARG1 and FIZZ1 gene expression to determine M0 polarization. Statistical analysis was performed using ANOVA ($\alpha = 0.05$)

Results: Results from 1 day MTT assays showed a significant difference in viability in IonL-Met coated samples and uncoated cpTi control. Results from 3 days showed a significant difference in viability of the control versus both IonL coatings, as well as between IonL-Met and IonL-Phe coated samples. However, in both 1 and 3 day experiments, viability in all samples did not drop below 70% when scaled to the uncoated cpTi.

Conclusions: MTT assays show that although there is a significant drop in viability after 3 days of IonL exposure, the coating is still not considered cytotoxic. Similar viability results were seen when the same assay was performed on bone and soft tissue cells. Therefore, the IonLs retain their cell compatibility and qPCR results will likely corroborate viability results, showing a lack of significant stimulation towards pro- or anti-inflammatory phenotypes.

9:00am **D3-TuM-4 Materials To Control Biological Function***, **Karine Anselme**,* CNRS, France **INVITED**

Surface modifications of materials are used to monitor and evaluate cellular function for several decades. An example of this is the ability to control tissue integration of medical prostheses by changing their composition, surface topography, chemistry, energy, or their mechanical properties. Similarly, control of the surface of materials is also a key element in the effectiveness of diagnostic tools, devices for cell culture, bio-sensors or drug delivery systems (1).

The cells have the ability to discriminate and specifically react to surface characteristics of the materials considered at the micrometer scale as well as the nanometer scale. In this talk, I will present our experience on the response of cells to functionalization of implants with mixed coatings associating inorganic and organic compounds such as hydroxyapatite and biologically active proteins (fibronectin, bone morphogenetic proteins) (2). Also, our last experience on response of living cells to topography at their own scale will be detailed and in particular our recent discovery of a new cellular ability which we term "curvotaxis" (3).

References:

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9:40am **D3-TuM-6 Comparison of Elution of Antibiotic and Biofilm Inhibitor from Manually Applied and Spray Deposited Phosphatidylcholine Coatings***, **Zoe Harrison**, R Awais, R Gopalakrishnan, J Jennings,* University of Memphis, USA

When implanting medical devices, the resultant wounds are often at high risk of infection. This infection is often caused by the formation of a biofilm, which occurs when microorganisms attach to the surface of the implanted device. Previous work in our lab has shown the effectiveness of cis-2-decenoic acid (C2DA) to disperse and inhibit biofilm, especially when combined with antibiotics. Previous studies have shown that phosphatidylcholine (PtC) can be loaded with C2DA and antibiotics to create a crayon-like solid, which can be used to "draw" a thick layer of coating onto an implant and thus prevent infection. While this application method showed promising results for drug elution and prevention of bacterial growth, this method of application has drawbacks of slow application process and uneven coating, especially for devices with complex shapes. To overcome these clinical issues, a system has been developed to spray phosphatidylcholine directly onto the device via an aerosol sprayer. This study seeks to determine if this aerosol spraying method provides similar drug elution capabilities as the previously manual coating method. PtC crayons loaded with 15% ciprofloxacin and 15% C2DA were dispersed in water and sprayed onto stainless steel coupons. Manually applied coatings were used as controls. Elution over 7 days in PBS was analyzed using high performance liquid chromatography. Results indicate that though mass of coating applied was lower using the spray setup, coating uniformity is improved with this method. Elution study results indicate that while the thicker manual coating showed higher elution of ciprofloxacin per day, the spray coating only eluted concentrations above inhibitory levels of ciprofloxacin through day 3. Current studies are being conducted to test the efficacy of sprays dispersed in polyethylene glycol 400 (PEG 400) and phosal 53 MCT (a 50/50 blend of PtC and medium chain triglycerides), which may increase elution and spray deposition. Furthermore, modifications are being made to the aerosol sprayer to improve the portability of the system, the speed of coating, and the thickness of the sprayed coating.

10:00am **D3-TuM-7 *In vitro* Osseointegration Aanalysis of Bio-functionalized Titanium Samples in a Protein-rich Medium***, S Rao, S Hashemiastaneh, J Villanueva,* University of Illinois at Chicago, USA*; F Silva,* University of Minho, Portugal*; C Takoudis,* University of Illinois at Chicago, USA*; D Bijukumar,* University of Illinois College of Medicine, USA*; J Souza,* University of Illinois at Chicago, USA*; **Mathew T. Mathew**,* University of Illinois College of Medicine, USA

Titanium (Ti) or Ti-based alloys are commonly used for the biomedical implant applications. The long-term survivability of the implants is strongly influenced by the osteointegration aspects of the metal-bone interface. Several techniques on Ti surface modification have been reported in the literature to improve osteointegration of implants to bone. Here, biological materials such as protein are used to functionalize titanium surfaces to enhance the ability of implants to interact with human tissues for accelerated osseointegration. The main aim of this study is to functionalize titanium surfaces in a medium enriched with fibrinogen.

Commercially pure titanium grade IV discs (8 mm Dia x 3 mm thick) are etched using two different acidic substances (HF/HNO_3 and H_2SO_4/HCl). Fibrinogen is processed and precipitated by a standard procedure to obtain high molecular weight fibrinogen under repeated centrifugation. Fibrin gel is prepared by adding thrombin to fibrinogen. Surfaces coated with and without fibrin are analyzed by white light microscope, SEM, and WCA to check for the surface properties. Osteoblasts are cultured on the surfaces to assess cell proliferation, adhesion as well as the mineralization process. Osteoblastic proliferation on these surfaces is quantified by Alamar blue assay and visualized under confocal microscope and SEM; qPCR is performed to investigate the expression of genes specific to osteogenic cells and mineralization assay is done to check calcium and phosphate deposits.

This study demonstrates a method of increasing roughness and hydrophilicity of the implant surface by etching followed by protein functionalization. Such a method can reduce the time for osseointegration that can decrease risks in early failures of implants. Also, the adsorption of fibrinogen on titanium surfaces is influenced by the method of acid etching.

10:20am **D3-TuM-8 Microstructural and Electrochemical Properties of TiAlN- (Ag,Cu) Nanocomposite Coatings Deposited by DC Magnetron Sputtering for Medical Applications***, H Mejía,* ***Aida Echavarría****, G Bejarano,*
Universidad de Antioquia, Colombia

AISI 420 stainless steel is currently used in the manufacture of surgical and dental instrumentation due to its hardenability, acceptable biocompatibility and resistance to corrosion. However its resistance to wear is relatively low, and therefore multiple strategies of surface modification are offered such as plasma nitriding, hard coatings and self-lubricating ceramic coatings deposited by the physical vapor deposition techniques PVD, among others, to reduce the wear rate and to confer this steel other variety of specific properties. In the case of coatings that include metallic solid lubricants, the anticorrosive properties can be affected by the formation of galvanic pairs and the high chemical reactivity of the metallic phase. Therefore, it is absolutely necessary to evaluate the electrochemical behavior and the corrosion resistance of these composite coatings. In this work TiAlN coatings doped with four different contents of Ag and Cu nanoparticles (11 at.% to 20 at.%) were deposited onto 420 steel by means of DC magnetron sputtering equipment using two composited targets of Ti/Al and Ag/Cu (both 50/50 at.% and 99,95 purity), which were facing each other at 180 degrees. The microstructure, chemical and phase composition were analyzed by scanning and transmission electron microscopy (SEM/TEM), energy dispersive X-ray spectroscopy (EDX) and X-ray diffraction, while the roughness were determined using atomic force microscopy (AFM), respectively. The corrosion rates were obtained from the polarization curves and the electrochemical behavior was evaluated by electrochemical impedance spectroscopy (EIS) in artificial saliva. The reduction in the corrosion rates of the steel was evident once it was superficially modified with the TiAlN (Ag,Cu) nanocompound, forming a substrate protection barrier when exposed to the selected body fluids. The TiAlN (Ag-Cu) coatings presented an electrochemical activity superior to the TiAlN matrix, and consequently exhibited higher corrosion current densities. This behavior becomes greater with the increase of the Ag-Cu content in the compound and is correlated mainly with the continuous and increasing dissolution of silver and copper in the surrounding medium and to the greater chemical activity of the Ag- particles as compared to the ceramic matrix. However, all coated samples showed an enhanced corrosion resistance compared to the steel substrate, obtaining the best electrochemical behavior by the sample coated with TiAlN (Ag-Cu) with 17at.% Ag-Cu. The development of this nanostructured coating system might be considered for potential application in in surgical and dental instrumentation.

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