Coatings for Biomedical and Healthcare Applications
Room Pacific D - Session D3-TuA

Biointerfaces: Coatings to Promote Cell Adhesion while Inhibiting Microbial Growth

Moderators: Dr. Valentim A.R. Barão, University of Campinas (UNICAMP), Brazil, **Dr. Sandra E. Rodil**, Universidad Nacional Autónoma de México

1:40pm **D3-TuA-1 Chemical Vapor Deposition of Tantalum for Enhanced Cell Adhesion***, **Jessica DeBerardinis**,* Ultramet, USA **INVITED**

Tantalum is a refractory metal with demonstrated strength, ductility, resistance to corrosion and oxidation, and biocompatibility. It has long been used as a biomaterial for medical devices such as pacemaker electrodes, contrast media and markers, and cell growth matrices, and for decades Ultramet's open-cell tantalum foam has been used in orthopedic implants. The use of chemical vapor deposition (CVD) has enabled these biomedical applications of tantalum. Biocompatibility research has demonstrated the following:

1. CVD tantalum coatings can enhance the binding of collagen and proteoglycans because of their surface roughness and tension [1,2].
2. CVD tantalum has antioxidant properties, which limit the negative effects of reactive oxygen species on osteogenic differentiation [1,3].
3. CVD tantalum can up-regulate integrins, which are transmembrane adhesive proteins that enhance cell adhesion and reduce apoptosis [1,4].
4. In the presence of CVD tantalum, cells demonstrate enhanced proliferation and autophagy, promoting cell survival in the presence of an implant [1,5].
5. Porous tantalum increases cell proliferation and enhances osseointegration in comparison with porous titanium substrates [6].

CVD allows small amounts of metallic material, such as tantalum, to be evenly applied over three-dimensional structures. CVD is not line-of-sight limited, so it can be used to uniformly coat and infiltrate extremely complex three-dimensional structures, such as Ultramet's reticulated (porous) carbon foam (Figure 1). A thin film of CVD tantalum can be applied over less expensive and/or less biocompatible materials to create a biointerface that promotes cell adhesion. Furthermore, Ultramet can modify its CVD processing to deposit tantalum such that it forms a diffusion bond, which occurs at the atomic level, so the tantalum cannot peel or delaminate from the substrate (Figure 2). Ultramet's CVD processing can also be modified to grow a textured tantalum surface (Figure 3), which has been demonstrated to affect the adhesion of human bone marrow stromal osteoprogenitor cells. Examination of the microscale topography of textured tantalum showed increased cell spreading with significant growth of actin fibers, and metabolic activity was also notably higher than with the bare substrate and untextured tantalum.

Ongoing research of textured CVD tantalum coatings at Ultramet involves modifying the texture according to tissue type and incorporating other bioactive layers. Process development continues to improve the application of CVD tantalum coatings to various medical grade substrates to create more economical orthopedic and dental implants.

2:20pm **D3-TuA-3 The Functionalization of N95 Masks Using Atomic Layer Deposited Silver Nano-Islands to Induce Antimicrobial Activity***, **Harshdeep Bhatia**, C. Takoudis,* University of Illinois, Chicago, USA

Due to the recent COVID-19 pandemic, the demand and use for antimicrobial textiles has increased. This demand saw a similar surge in these textiles during the SARS-COV-1 outbreak, resulting in many patents, after which the use of Silver as a potential material to give a material some antibacterial/antiviral properties was popularized. Additionally, the use of disposable N95 masks has become popularized as the safest and easily available barrier against these viral outbreaks. In this study, a novel strategy to deposit nano-islands of Silver on N95 masks using ALD is developed to give it some antibacterial properties. X-ray Photoelectron Spectroscopy (XPS) and X-ray adsorption fine structure (XAFS) were used to characterize the as-deposited silver nano-islands. The size and roughness of the nano-islands was calculating using atomic force microscopy (AFM). Furthermore, inductively coupled plasma mass spectrometry (ICP-MS) was used to study the leaching of these Silver nano-islands in standard 1x phosphate buffer over predetermined times to simulate the effect of biological fluids such as saliva or mucus. A microbiological assay was also conducted to study the effect Ag- coated N95 had on *Staphylococcus Aureus*. The films grown at the two temperatures, 90 °C and 120 °C, were stable in ambient conditions. The deposited silver nano-islands were stable on the N95 filter media against washing. A comparison of the characteristics of the films grown at different temperatures has been made.The functionalization of materials using ALD of silver provides a repeatable method to impregnate textiles and induce antimicrobial activity which releases silver at a slow rate.

2:40pm **D3-TuA-4 Cold Atmospheric Plasma Jets Generated from Flexible Sources***, C. Corbella, **Sabine Portal**, H. Solomon, M. McCraw, M. Keidar, S. Solares,* George Washington University, USA **INVITED**

Atmospheric pressure plasma jets (APPJs) are excellent resources for a myriad of applications in energy and healthcare industries, being cancer therapy a major breakthrough. Their working principle is based on the generation of a rich plasma chemistry and photon emission in open air, which can gently modify surfaces of different materials at room temperature. APPJs originated from a flexible source are able to adapt to complex topologies and to treat delicate samples, like soft matter and organic tissues. Stable and reproducible operation of a flexible multi-jet array has been proven in sources set with planar (concave and convex modes) and radial (cylindrical source) configurations. Also, plasma plumes with different shapes have been achieved by conveniently modifying the nozzle geometry, thereby demonstrating the wide control over plasma performance. The participation of reactive oxygen and nitrogen species (RONS) in combination with energetic UV photons emitted by flexible APPJs as a function of the nozzle-sample distance are crucial aspects that need thorough characterization. Indeed, the action of these plasma species must be considered for the sake of APPJ safety in applications that require proximity of sensitive samples, especially in medical practices. Here, we demonstrate methods to estimate macroscopic parameters of relevance at the plasma-surface interaction region, namely local temperature, relative humidity (RH), pH variations, electric field (jet potential), and UV dose irradiation including shortest vacuum-UV wavelengths (VUV<100 nm). Portable platforms of flexible APPJ arrays exhibiting specific configurations and nozzle geometries will constitute the next generation of plasma devices aimed at biomedical applications.

4:00pm **D3-TuA-8 Multifunctional Coating Approach Integrating Visible-Light Driven Photodynamic Therapy and Photocatalytic Activity for Controlling Biofilm Accumulation and Reinforcing Wear Protection***, **Bruna Nagay**[1], C. Dini, R. Costa, A. Santos,* University of Campinas (UNICAMP), Brazil*; J. Cordeiro,* Centro Universitário das Faculdades Associadas de Ensino, Brazil*; B. Gomes,* University of Campinas (UNICAMP), Brazil*; E. Rangel, N. Cruz,* Sao Paulo State University, Brazil*; J. van den Beucken,* Radboud University Medical Center, Netherlands*; V. Barão,* University of Campinas (UNICAMP), Brazil

The accumulation of biofilm and further establishment of infections on implant devices is one of the major concerns in the biomedical industry and clinical management. Among current therapies, although antimicrobial photodynamic therapy (aPDT) has been considered effective, inconsistencies regarding its outstanding performance in battling the burden of growing peri-implantitis prevalence have raised concerns towards the development of novel strategies. To overcome these drawbacks, we proposed an integrated coating strategy combining visible-light-driven photocatalytic activity and aPDT to simultaneously face titanium implant infections and optimize the implant biointerface for biomedical and dental implant applications. A multifunctional bismuth (Bi)-doped TiO_2 coating was synthesized upon titanium (Ti) substrate using plasma electrolytic oxidation (PEO). Polished Ti and pure TiO_2 coating were used as controls. PEO produced a crystalline, rough coating on Ti surface with superhydrophilicity features. The incorporation of Bi into the TiO_2 matrix was confirmed by X-ray photoelectron spectroscopy. UV-vis diffuse reflectance spectroscopy revealed that Bi effectively narrowed the band gap of TiO_2, making Bi-TiO_2 promising to exhibit photocatalytic activity under visible-light irradiation, which was confirmed by the methylene blue (MB) degradation assay. In addition, because the visible light used herein has a wavelength compatible with the MB used in aPDT, we hypothesized that the combination of reactive oxygen species generated by the photocatalysis mechanism along with MB-mediated aPDT could potentiate

[1] **Graduate Student Award Finalist**

the eradication of microorganisms. As such, in vitro experiments using human saliva as inoculum revealed that Bi-TiO_2 potentiated the polymicrobial biofilm reduction mediated by aPDT, and that 1 min of light exposure had similar antimicrobial effects when compared to 5 min. Furthermore, the Bi-TiO_2 coating was not cytotoxic to human bone mesenchymal stem cells and human gingival fibroblasts, even under light exposure. Finally, PEO coatings presented higher wear resistance, hardness, and albumin adsorption than control groups, indicating their outstanding properties to optimize the implant biointerface. The proposed proof-of-concept research holds great promise to face peri-implant infections by using a visible-light-driven photocatalytic coating and aPDT in a smart and safe manner to reduce biofilm while maintaining the propertiesthat affect the longevity of biomedical devices (e.g., wear resistance, cell-material interactions). This opens a new perspective for the development of effective antimicrobial surfaces by the implant industry.

4:20pm **D3-TuA-9 ZnO_X Nanolayers as Antimicrobial Surfaces***, L. Reyes-Carmona,* Universidad Nacional Autónoma de México*; O. Sepulveda-Robles,* Instituto Mexicano del Seguro Social, Mexico*; A. Almaguer-Flores, C. Ramos-Vilchis,* ***Sandra E. Rodil****,* Universidad Nacional Autonoma de Mexico

The regular use of disinfectants is not an ecologically friendly solution to control the transmission of viruses and bacteria, and it could promote antibacterial resistance. Antimicrobial surfaces are a plausible solution applicable to rigid and flexible surfaces, as well as to protective equipment for healthcare personnel.

In this work, we investigated the antiviral and antimicrobial properties of sputtered ZnO_x nanolayers deposited on polypropylene (PP) fabrics for their use in respiratory protection equipment. Because of the fast traveling of respiratory drops containing viruses and bacteria, our study emphasizes the effect of contact time on the antimicrobial response. Since the substrate cannot be heated, the films were substoichiometric and amorphous.

Two methods were developed to test the antimicrobial response as a function of contact time. The shortest contact time was simulated using a bactericidal/virucidal filtration system where the material was exposed to an aerosol loaded with microorganisms (bacteria and surrogate viruses). Larger contact times between 0.5 and 24 hr. for the viruses and 24 hr. for the bacteria were tested by placing a drop containing the microorganisms on the surface for the specified contacting time. After such time, the virus was recovered, and the infectivity was evaluated by counting the plaque-forming units. Colony-forming unit determination was used for the bactericidal tests to evaluate the survival of aerobic and anaerobic bacteria.

Virus viability assays were used to study the survival of PaMx54, PaMx60, PaMx61 (ssRNA, Leviviridae), and PhiX174 (ssDNA, Microviridae) as surrogates for non-enveloped viruses. An approximate 40% PFUs reduction was obtained after 12 hr. for the RNA viruses, but only 12% was achieved for the DNA virus compared to the uncoated PP. For larger contacting times, the RNA viruses were completely reduced after 12 hr. in the ZnO-coated fabric, but no reduction was observed for the DNA virus or the uncoated PP.

For the aerosols containing the anaerobic bacteria, which are typically found in the oral environment, inhibition ratios between 53-96% were obtained, depending on the strain. Similarly, for the aerobic bacteria aerosols, the inhibition was between 26 and 90%, being slightly more resistant. However, after 24 hr of direct contact between the bacteria and the ZnO-coated surface, most strains were inhibited (80-90%).

These results suggest that ZnOx nanolayers deposited by magnetron sputtering reduce the infectivity of non-enveloped RNA respiratory viruses and inhibit the growth of anaerobic and aerobic pathogen bacteria.

4:40pm **D3-TuA-10 Cytocompatibility of Chitosan-Silver Coated Titanium Coupons***, E. Coleman Montgomery,* ***J. Amber Jennings****, M. Atwill, J. Bumgardner,* University of Memphis, USA

Introduction

Titanium is commonly used in orthopedics due to its strength, resistance to corrosion, and bone-like mechanical properties. Silver ions affect microbials by blocking transport in and out of the cell, inhibiting the production of energy, and interacting with DNA to prevent replication. These characteristics lead to broad spectrum antimicrobial properties against bacteria and fungi and therefore support the advantage of silver ions as an implant coating using chitosan biopolymer as a complexing agent and coating to localize silver.

Methods

Treated Coupons: Titanium coupons were polished with 400, 600, 800, and 1200 grit sandpaper before being sonicated in soapy water, acetone, and ethanol to remove oil and residue for 10 minutes each. The coupons were then soaked in 5M NaOH for 24h to allow accumulation of hydroxide reactive groups on the titanium surface and rinsed with deionized (DI) water twice. The coupons were treated with a linking agent and dried for 10 minutes in a 110°C oven. Chitosan-silver solution (Chitozan Health) was added and left to dry overnight. The coated coupons were immersed in phosphate buffer for 1 hour, rinsed with DI water, and dried fully.

Untreated Coupons: Titanium coupons were polished with 400, 600, 800, and 1200 grit sandpaper before being sonicated in soapy water, acetone, and ethanol to remove oil and residue for 10 minutes each. The uncoated coupons were rinsed with DI water and dried fully.

Cytocompatibility: Coupons were UV-sterilized for 20 minutes and washed in cell medium. Soas-2 cells were seeded at 90,000 cells/well in a 12-well plate before exposure to 3 test groups: treated coupons, untreated coupons, and tissue culture plastic (TCP) control. After 24 hours, cell viability was determined using CellTiter-Glo Viability Assay (n=3), and cell morphology was determined using Live/Dead staining (n=1).

Results

The Saos-2 cell viability for treated coupons was not statistically different than untreated coupons and was about 70 percent of the TCP control. Live/Dead staining also produced similar results, with mostly living cells in all groups.

5:00pm **D3-TuA-11 Nonsurgical Decontamination Protocols for 3D-Printed Implant Surfaces***,* ***Valentim Barão****, R. Costa, T. Takeda, C. Dini,* University of Campinas (UNICAMP), Brazil*; M. Bertolini,* University of Pittsburgh, USA*; M. Feres, J. Shibli, J. Souza,* Guarulhos University, Brazil

There is still a lack of a predictable nonsurgical protocol for effective dental implant decontamination, including for 3D-printed surfaces. Therefore, this study aimed to scrutinize the deleterious effects of different mechanical and chemical decontamination treatments on titanium (Ti) surface, electrochemical properties, biofilm cleaning potential, and cell behavior. 3D-printed Ti discs obtained by direct metal laser sintering were used. These samples were coated with polymicrobial biofilm from human saliva *in vitro*. Biofilm-covered surfaces were decontaminated with mechanical [Ti curette, plastic curette, Ti brush, water-air jet device, and Er:YAG laser] and chemical [amoxicillin; minocycline; tetracycline; H_2O_2 3%; chlorhexidine 0.2%; NaOCl 0.95%; and hydro-carbon-oxo-borate-based formula antiseptic] protocols isolated. Negative control using 0.9% NaCl was adopted and PVPI 0.2% as a biofilm matrix-degrading agent applied before all chemical protocols. Surface deterioration and corrosion were analyzed before and after mechanical instrumentation as well as fibroblast adhesion on these degraded surfaces. The best *in vitro*mechanical/chemical protocol was tested in combination using *in situ* biofilm model. Er:YAG laser treatment displayed optimum surface cleaning by biofilm removal with minimal deleterious changes on the surface, smaller Ti release, anti-corrosion performance, and improved cell spreading. NaOCl 0.95% was the most effective chemical agent to reduce *in vitro* and *in situ*biofilms when applied isolated and more prominent results when associated with PVPI as pre-treatment to disrupt biofilm matrix. The combination of mechanical and chemical treatments promoted an optimum mechanical cleaning ability with biofilm matrix disruption and killing remnants *in situ* biofilms (~99% biofilm eradication). We conclude that Er:YAG laser + PVPI 0.2% + NaOCl 0.95% is considered an optimized decontamination protocol by demonstrating a potential to eliminate *in vitro* and *in situ*biofilms with minimum deleterious effects on 3D-printed Ti surfaces, opening new perspectives to improve implant-related infection therapies.

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