Blood Interaction and Protein Adsorption on Engineered Titania Nanotubular Arrays

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Statement of Purpose: As nanomaterials continue to be studied for their promising role in tissue engineering, there is a growing need to identify their effects on biological systems. Hemocompatibilty and inflammation continues to remain a concern for the long term success of nanomaterials [1]. Materials which mimic the natural physiological design of the body provide one possible solution to the problem of biomaterial rejection. In this study we have developed titania nanotube arrays for implantable devices. Titania nanotube arrays have potential applications in dental and orthopedic materials, stents and wound healing bandages. Driven by a need for reduced material rejection, and thus obtaining an enhanced biocompatibility between the implant/body interactions, a study focusing on an improved understanding of the physiological response to nanomaterials, specifically hemocompatibility, is considered here. Previous research on titania nanotube arrays has focused on the cellular response [2]. In this study, we have examined one of the earliest stages in the physiological immune response by considering the *in vitro* adsorption of blood proteins, the adhesion and activation of platelets, and the clotting kinetics of whole blood. The goal of this work is to facilitate a better understanding of the natural blood response and overall hemocompatibility of titania nanotube arrays for biomaterial applications.

Methods: Titania nanotubular surfaces were fabricated by the Grimes laboratory at The Pennsylvania State University using an anodization process described elsewhere [3] (**Fig. 1**).

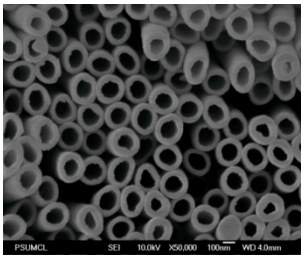

Fig. 1 *SEM image (50,000X) of titania nanotube arrays with 70-80 nm pore size*

Smooth titanium (medical grade) surfaces were used as controls. Whole blood from healthy individuals was spun down to produce platelet rich plasma (PRP) with a final concentration of 4 x 10^8 platelets/sample. The substrates were incubated in 500 µl of PRP for 30 minutes. The substrates were tested for the platelet adhesion and activation using both Methylthiazol Tetrazolium (MTT) assay and calcein AM live stain fluorescence microscope imaging. The substrates were also imaged using scanning electron microscopy (SEM) to visualize platelet activation. Clotting time measurements were performed at 10 min intervals for up to 60 minutes using whole blood from healthy individuals. Further, blood protein adsorption was studied on both test and control substrates. The substrates were incubated in 100 µg/mL human fibrinogen, albumin and immunoglobin G for two hours. Sodium dodecyl sulfate (SDS) was used to detach the adsorbed protein and its concentration was measured using a microBCA assay. Surface characterization was accomplished through the use of X-ray photoelectron spectroscopy (XPS), and data was converted to atomic percentage.

Results and Discussion: Increased adhesion and activation of platelets (**Figs. 2 and 3**) and adsorption of proteins was identified along with accelerated clotting times on titania nanotube arrays. The figures below indicate amplified platelet response in titania nanotubular arrays when compared to smooth titanium.

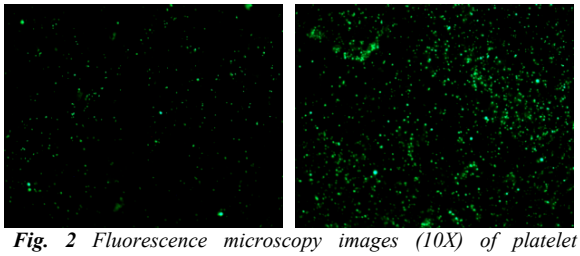

Fig. 2 *Fluorescence microscopy images (10X) of platelet adhesion on biomedical grade titanium (left) and titania nanotube arrays (right)*

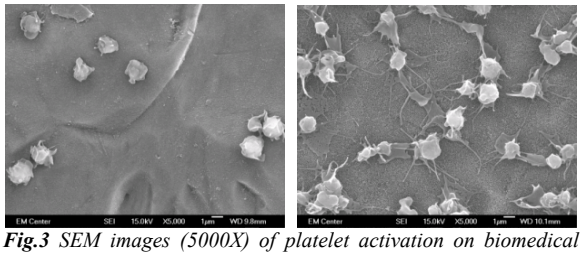

Fig.3 *SEM images (5000X) of platelet activation on biomedical grade titanium (left) and titania nanotube arrays (right)*

Conclusions: Our results show increased platelet adhesion and activation, increased clotting times, and increased protein adsorption on titania nanotube arrays. This work provides a proof of concept that opens the way for the use of titania nanotubular arrays as an optimal candidate for fast clotting bandages as well as coatings for implantable devices. Further studies are directed towards development of nanotube arrays of different sizes to control their hemocompatibility and protein adsorption.

References:

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2. Popat, K.C., et al., *Influence of engineered titania nanotubular surfaces on bone cells.* Biomaterials, 2007. **28**(21): p. 3188-97.
3. Roy, S.C., et al., *The effect of TiO2 nanotubes in the enhancement of blood clotting for the control of hemorrhage.* Biomaterials, 2007. **28**(31): p. 4667-72.