Smooth Muscle Cells Interaction with Titania Nanotube Arrays

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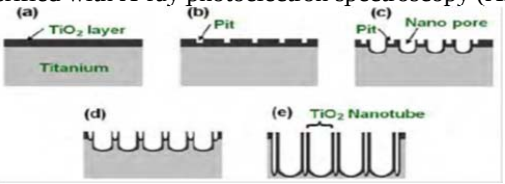
Statement of Purpose:

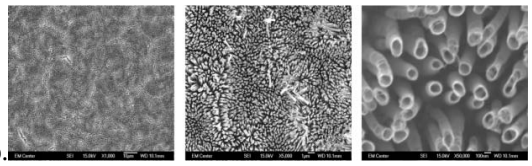
With the enormous escalation in the world's populace, there are vast requirements each year for diverse biomedical transplants to restore ailing or missing tissues. It is plainly imperative to generate new generation alternatives to restore tissue at defect locations that will endure for the lifetime of the patient. Titanium and titanium alloys are widely used in biomedical devices, particularly as solid tissue replacements as well as in cardiovascular and cardiac treatments, because of their advantageous properties, such as fairly low modulus, suitable fatigue strength, machinability, formability, corrosion resistance, and biocompatibility. Since these transplant surfaces interact closely with nanostructured extra-cellular matrices (ECM), the characteristics of nanomaterials are crucial in promoting cell growth, migration, differentiation and proliferation as well as influencing tissue restoration [1]. Smooth muscle tissue is one of the major cell types that are in contact with vascular stents; therefore the interaction between the cells and the nanotube titania surface is of the utmost importance. This study investigates key smooth muscle cell (SMC) proteins, in vitro adhesion, proliferation and viability on titania nanotubes as compared to flat CP titanium in vitro. Previous studies have shown increased cell adhesion and growth on osteoblasts, mesenchymal stem cells, fibroblasts, and chondrocytes on titania nanotubes, yet few have explored smooth muscle interaction.

Methods:

Fabrication of the titania nanotubes arrays was accomplished through an electro-chemical process known as anodization. A two-electrode reactor was utilized for the anodization process by connecting the platinum (99.9% Alfa Aesar) to the cathode terminal and Commercially Pure titanium (Ti) to the anode of the power supply. The anodization electrolyte solution contained 95% diethylene glycol with 2% hydrofluoric acid and 3% de-ionized water with all reactions run at room temperature at 60 Volts for 24 hours. Post-anodization, the nanotube samples were cleaned, dried and annealed in air at 530^{o}C for 3 hours allowing crystallized substrates to be acquired. Surface topography of the nanotubes was done utilizing a Scanning Electron Microscope (SEM). The elemental composition was verified with X-ray photoelectron spectroscopy (XPS).



a. [2]



b.

Figure 1*: a). Schematic of Titania nanotube fornation b). SEM images at 5000x, 5000x and 50000x.*

Human aortic smooth muscle cells were cultured, multiplied, and seeded in 48 well plates at a density of 20,000 cells/cm^2 in culture media. The cultures were sustained at 5% CO_2 at 37^o C. MTT assay was utilized to determine cell viability at days 1, 4 and 7 on both Ti and TiO_2 nanotubes. SMCs on each substrate were viewed under SEM and also with fluoresce microscope at days 1, 4, 7, 10 and 14 using CMFDA, DAPI and rhodamine phalloidin that stained the cytoplasm, nucleus, and actin respectively. Automated DAPI nucleus cell counting was determined after staining employing ImageJ software.

Results:

XPS confirm toxic fluorine left from the anodization process was removed through annealing. Cell counts and viability increased relatively for each tested day of the study. More uniform, proliferated, 'contractile' SMCs were observed on nanotube substrates than flat Ti under SEM and Fluoresce microscopy.

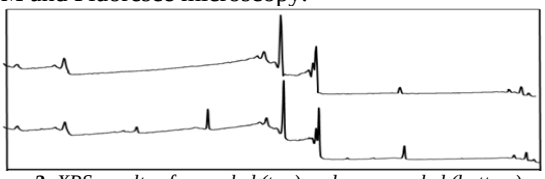


Figure 2: *XPS results of annealed (top) and un-annealed (bottom).*

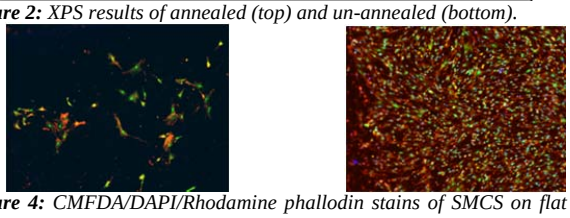


Figure 4: *CMFDA/DAPI/Rhodamine phallodin stains of SMCS on flat Ti (left) and TiO2 nanotubes (right) on day 7 at 5x.*

Conclusions:

The fundamental processes of the in vivo connections between nanofeatures and cells at the molecular level will considerably expand the progress of this discipline [1]. Titania nanotubes show an almost infinitely greater surface area, hydrophilic and are produced to mimic the membrane proteins and ECM nanofeatures in vivo. With further application of coatings of growth factors and antibiotics even greater cell proliferation and adhertion might be achieved to increase longevity of titanium medical devices such as vascular stents.

References:

[1] Zhang, Lijie, and Thomas J. Webster. Nano Today 4.1 (2009): 66-80.

[2] Brammer, K. S., Oh, S., Frandsen, C. J., & Jin, S. Progress in Polymer Science 35(10): 1217-1256.