Neural Stem Cell Interaction with Titania Nanotube Surfaces

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Statement of Purpose: Neural implants have become increasingly popular in the treatment of neurological disorders such as Parkinsons Disease as well as for recording brain activity. The longevity and utility of these devices may be hindered by the cellular response to the implant. Glial scar tissue has been shown to encapsulate the implant preventing neuronal attachment and growth, and limit electrically conductivity[1]. Nanostructure surfaces of these implants may improve their neurological integration and biocompatability, increasing their useful life. Here the in-vitro cellular response of C17.2 maurine neural stem cells to titania nanotube arrays is analyzed.

Methods: Densely packed, highly ordered titania nanotube arrays were prepared using an anodization technique with a 1% hydrofluoric acid in deionized water electrolyte. Commercially pure (c.p.) titanium samples were cut to size and cleaned, before being attached to an anodization fixture with a platinum catalyst. The anodization was voltage limited at 20 V and was run for three hours. The samples were left attached to the fixture until they had been fully rinsed of the electrolyte solution. The samples were subsequently detached, further rinsed and dried. Nanotube arrays were annealed at 530C for three hours before being cut to size. The nanotubes arrays were characterized using a JEOL JSM-6500F field emission scanning electron microscope. After samples had been sized, they were sterilized in a biosafety cabinet. Sterilized samples were then used for cell and protein adsorption studies.

Samples of c.p. titanium and prepared titania nanotube arrays were placed into well plates and seeded with 1000, 1500, and 2000 cells/well of C17.2 Maurine neural stem cells. Media was changed every two days. The samples were analyzed on day 1, 4, and 7 by staining for the nucleus, cytoplasm, and cytoskeleton. A protein adsorption study was carried out on the substrates using a micro-BCA assay (Pierce Biotechnology). Square 6mm samples were placed in 48 well plates, incubated in 100 ug/mL of human albumin (Sigma), bovine serum albumin (Pierce Biotechnology), and human laminin (Sigma). Samples were placed on a shaker table at 100 rpm for two hours. The remaining solution was aspirated, and the samples were rinsed with PBS. The samples were incubated with 1% SDS in PBS for one hour on the shaker plate at 100 rpm. The rinses were pooled and analyzed colorimetrically using the micro-BCA assay kit and plate reader (BMG Labtech).

Results: The titania nanotube arrays are densely packed and highly ordered. Diameters range from 80 to 120 nm with the lengths near 500nm.

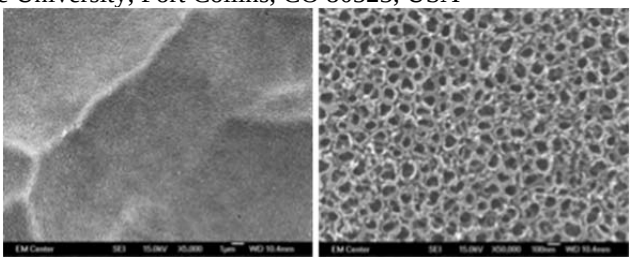

Figure 1: Prepared **titania** nanotube arrays **Left**: 5,000x magnification. **Right**: 50,000x magnification.

Examining the cell counts of the different substrates with varying initial cell seeding concentrations reveals larger initial seeding concentration cause a more rapid loss in cell count as the study over longer durations.

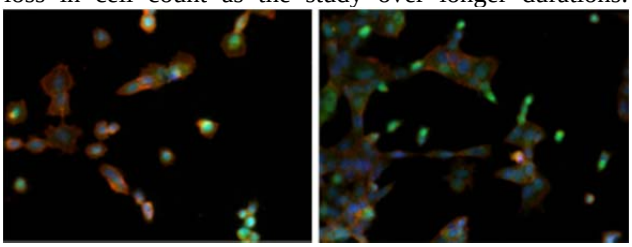

Figure 2: Fluorescence images of C17.2 cells stained for cytoplasm (green), cytoskeleton (red), and nucleus (blue). **Left**: c.p. titanium surface 10x magnification. **Right**: TiO2 Nanotube surface 10x magnification

Titania nanotube substrates show a less significant loss in cell count than c.p. titanium.

The protein adsorption assay reveals that commercially pure titanium has a slightly lower adsorption of all proteins than that of the nanotube arrays. Laminin is adsorbed on both substrates more than the albumin.

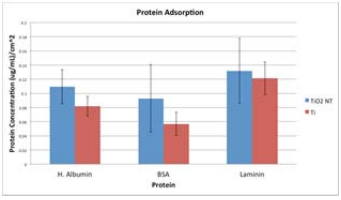

Figure 3: Protein Adsorption on TiO2 NT and C.P. Ti

Conclusions: The C17.2 cells proliferate slower, but adhere better to the titania nanotube substrates. The adsoprtion of proteins that may both promote astrocyte and glial cell formation, as well as neuron attachment is similar for both comercially pure titanium, as well as the titania nanotube arrays. Further testing on cell morphology as well as differentiation will be performed to understand the cell interaction with the surfaces and whether that nano architecture promotes differentiation. Surface resistivity testing will to be performed to validate the efficacy of the surface for use in implants.

References: [1]Polikov VS. J Neuro Sci Meth. 2005;148:1-18