Skin Cell Functionality on Titania Nanotube Arrays

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Statement of Purpose: Skin contacting biomaterials are prevalent in numerous clinical applications including prosthetics and dental implants. These applications and many others may be treated through the use of transcutaneous implants, which penetrate across the depth of the skin. Favorable interactions between the material surface and the respective tissue layers are critical for the long-term success of such devices. Wound infection resulting from loose material/skin interactions is one of the major causes for failure in prosthetics [1]. Recent studies have shown that materials which mimic the natural physiological design of the body provide one possible solution to the problems of infection and biomaterial rejection [2-3]. In this study, we have developed titania nanotube arrays to investigate the nano-biomaterial/skin interaction. This study evaluates human dermal fibroblasts (HDF) responsible for the underlying dermal structure, and human epidermal keratinocytes (HEK) responsible for the production and the retention of the epidermis. Skin compatibility is a key consideration for the long-term use of transcutaneous implantable devices; hence, there is a critical need to understand the physiological response elicited from skin/nano-biomaterial interactions,

Methods: Titania nanotubular surfaces were fabricated by the Grimes laboratory using an anodization process described elsewhere (Figure 1) [4-5].

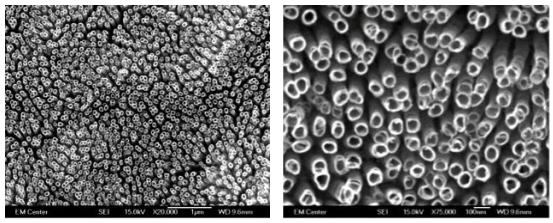
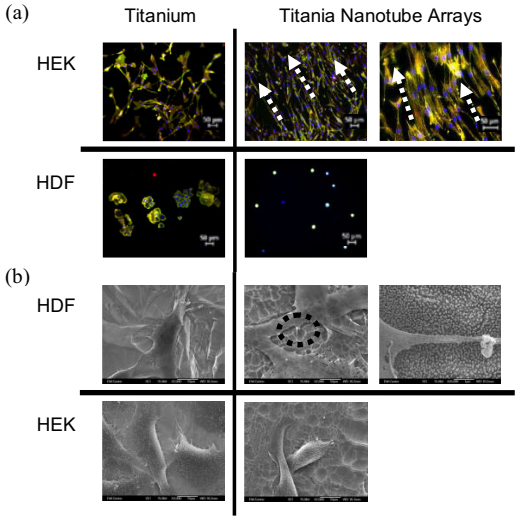


Figure 1 SEM images (20,000X and 75,000X) of titania nanotube arrays with 70-90 nm pore size

Biomedical grade titanium was used as the control. Cryopreserved HDF and HEK cell suspensions were thawed and cultured prior to seeding. Second passage cells were used for this study. HEK's were seeded at a cell density of $3x10^4$ /ml and HDF's were seeded at $1x10^4$ /ml. The substrates were incubated for 1, 2 and 4 days, and tested for cellular adhesion, proliferation, orientation, morphology, cytoskeletal orientation and protein expression. Cellular adhesion, proliferation, and orientation were evaluated using calcein-AM live stain and DAPI nucleus stain fluorescence microscope imaging, and the Methylthiazol Tetrazolium (MTT) assay. The cell morphology was investigated using scanning electron microscope (SEM) imaging. Cytoskeletal reorganization was evaluated by FITC-conjugated anti-vinculin and rhodamine-conjugated phalloidin fluorescence microscope imaging. Protein expression was identified using specific marker proteins for HDF cells (collagen-Iα1 and fibrillin-1) and HEK cells (cytokeratin-19 and laminin-β3) by fluorescence microscope imaging. Further, cell coverage and cell counts were obtained using the Image J Software.

Results and Discussion: The results of this study show adhesion, proliferation, orientation, cytoskeletal reorganization and protein expression to be significantly increased in HDF cells and decreased in HEK cells on titania nanotube arrays as compared to the control substrate (Figure 2).

Figure 2 (a) Fluorescence microscopy images of HDF stained with DAPI, FITC-conjugated anti-vinculin, and rhodamine-conjugated



phalloidin on control (10X, left) and titania nanotube arrays (10X and 20X, right.) (b) SEM images (2000x and 20000x) of HDF (4 days) and HEK (1 day) cells on control (left) and titania nanotube arrays (right).

Conclusions: In this study, we have demonstrated that the titania nanotube array architecture provides a favorable template for the growth and maintenance of HDF cells while preventing the adhesion and proliferation of HEK cells. This may translate into an implant interface that allows for increased cellular adhesion and enhanced matrix deposition by dermal fibroblasts; while preventing competitive adhesion of epidermal keratinocytes, thus restricting them from traveling downward along the implanted biomaterial. The skin cell response may be optimized for specific *in vivo* applications by precisely tuning the nanotube dimensions by altering the anodization parameters [5].

References:

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