Interfacial Peptide Coatings Facilitate Biological Control on Material Surfaces

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Statement of Purpose: Devices implanted into the body will generally be isolated in a fibrous capsule due to the actions of the foreign body response.[1] One method of overcoming this inherent limitation is by creating a surface coating of proteins that serves to properly direct cellular interaction with the material. A prototypical method of accomplishing this goal is to present the well-studied arginine-glycine-aspartate (RGD) tri-peptide motif at the biological-material interface. This trimer binds to integrins expressed by cells, which facilitates attachment, but more importantly has been linked to control of the cellular processes of differentiation, apoptosis, and growth cycling.[2] Herein, we investigate unique short peptides, terminated with RGD, that non-covalently bind to polystyrene (PS) or titanium (Ti) surfaces and control the differentiation and apoptosis of bound cells.

Methods: A custom phage display library was used to select peptides with high affinity for PS and Ti. This procedure was previously used by our group to select peptides that bind strongly to PS to create a cytophobic coating.[3] Briefly, peptides were expressed on the surface of M13 phage. The phage were exposed to the target Ti or PS substrate, non-specific interactions were removed by washing, and the phage with affinity for the substrate were amplified. The process was repeated three more times with the previous round serving as the input to the next. Apoptosis studies were accomplished by treating wells of a PS plate with either PBS or peptide (FFSFFFPASAWGS-SSG-RGD) dissolved in PBS (0.1 mg/mL) for 2 hours. Next, human umbilical vein endothelial cells (HUVECs) were seeded and left for 1 day before an apoptotic drug cocktail of tumor necrosis factor alpha (TNFα) and actinomycin D (AMD) was added at various concentrations for an additional day. Fluorescent images of the wells were then taken using fluorescein diacetate as a vital stain. Differentiation studies were accomplished by coating a Ti disk with either PBS or peptide (SCSDCLKSVDFIPSSLASS-SSG-RGD) dissolved in PBS (0.1 mg/mL). Adipose derived adult stem cells (Zen-Bio; Research Triangle Park, NC) were seeded onto the coupons and were given medium containing appropriate differentiation inducers. After 3 weeks, progress was monitored using scanning electron microscopy (SEM) and X-ray Photoelectron Spectroscopy (XPS) to judge the extent of surface calcification.

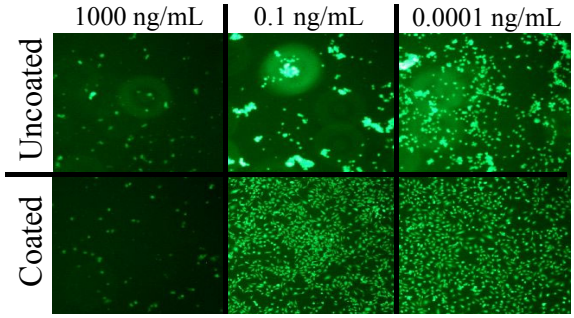

Figure 1: Vital staining of HUVECs exposed to various concentrations of TNFα. Cells on the uncoated surface are quite stressed in all conditions, while there is a survival shift at 1 ng/mL TNFα for the coated wells.

Results/Discussion: Integrin attachment to extracellular matrix (ECM) proteins has been shown to promote survival when challenged by apoptotic agents.(Cheresh DA. Nat Med. 2002;8:193-194) The interfacial peptide coating replicates the function of the ECM by providing the cytoskeletal interactions absent on many biohostile materials. We have tested this hypothesis by challenging HUVECs on coated and uncoated surfaces with varying levels of TNFα/AMD (Figure 1). As can be seen the cells on the coated surface are capable of surviving drug cocktail levels of at least 3 orders-of-magnitude greater than those on the natural surface. An additional assay confirmed the presence of apoptotic caspases. Similar results can be seen when examining differentiation of attached stem cells (Figure 2). Under identical medium conditions calcium-phosphate deposits are only witnessed with the coated Ti surface upon viewing under SEM. The granule composition was verified through XPS and the Ca and P spikes can be clearly observed (Figure 2 inset).

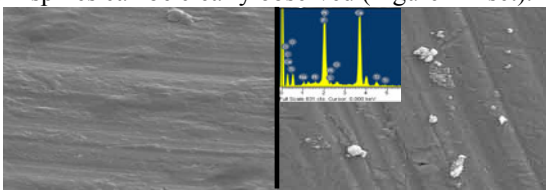

Figure 2: (Left) Uncoated Ti surface with preadipocyte coating after 21 days incubation. (Right) Coated Ti surface after the same incubation period. White granules are Ca-P deposits, verified with XPS (insert).

Conclusions: Controlling cell biology appropriately on implants remains one of the major challenges in biomaterials. Indeed, most implants fail due to improper integration with the host tissue. We have shown that a small, adsorbed peptide coating has the capability of controlling such key cellular processes as apoptosis and stem cell differentiation. Further refinements to the system will substitute the ubiquitous RGD motif for more selective interactions with surrounding cells, thereby adding additional control over the biological processes. The authors wish to thank the NIH for their support.

References:

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