The Use of Interfacial Peptide Coatings for Vascular Device Applications

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Statement of Purpose: Vascular implants are increasingly being utilized to treat a myriad of medical conditions. One such example, drug eluting stents (DES), seek to selectively prevent the proliferation of smooth muscle cells and allowing endothelial cells to reline the artery. However, the DES prolongs the natural healing process and serves as a nucleus for the development of fatal late-stent thrombosis which occurs in an estimated 3500 patients per year.[1] Other vascular stent coatings under investigation also seek to combat the natural response of the body by hiding from or poisoning the surrounding cells. We believe that an increase in device integration with the surrounding tissue could be produced by creating a biomimetic surface. To accomplish this goal we have created a novel short peptide sequence that binds non-covalently to unmodified titanium (Ti) and promotes cell adhesion and spreading under both static and dynamic conditions.

Methods: Peptides with high Ti affinity were selected using a custom phage display library. We have previously used this procedure to select peptides that bind to polystyrene.[2] Briefly, genes coding for 19-mer peptides were expressed on the coat of a bacteriophage. The phage were exposed to Ti beads, washed to remove non-specific binding, and the phage that bound Ti were amplified. The encoded sequences were translated and the peptide with the strongest interaction was synthesized using solid-phase Fmoc chemistry. The peptide was terminated with a well-studied integrin binding arginine-glycine-aspartate motif (SCSDCLKSVDFIPSSLASS-SSG-RGD).[3] The resultant peptide is soluble in aqueous buffer and readily absorbs to Ti surfaces. A thin film of Ti metal (20 nm) was evaporated onto glass coverslips for all imaging studies. The coverslips were treated with either the peptide dissolved in PBS (0.1 mg/mL), or plain PBS for 2 hours, followed by blocking with 1% BSA. Washes were performed between each step and human umbilical vein endothelial cells (HUVECs) were introduced for 1 hour during which time images were taken of the cell spreading. Average cell surface areas were quantified using ImageJ (NIH; Bethesda, MD). After the hour the slides were washed and the remaining cells were imaged and immunostained with a phalloidin-rhodamine conjugate (Sigma; St. Louis, MO). Additional experiments were accomplished using a peristaltic pump to produce shear flows over a bulk Ti surface. The Ti disks were treated using the same procedure outlined above. The HUVEC were then added on top of the disks in medium and after 15 minutes flow was started (~ 5 dynes/cm^2) and continued for 1 hour. The cells remaining were quantified using an MTS proliferation assay (Promega; Madison, WI).

Results/Discussion: We began by examining HUVEC spreading and attachment to coated and uncoated Ti surfaces. Cell surface areas were calculated at 30 and 60 minutes after being placed onto the surfaces. The cells that were placed onto the blocked uncoated Ti had very little change is surface area increasing from 487 ± 188 μm^2 at 30 minutes to only 594 ± 256 μm^2 after 60. The cells on the coated surface rapidly adhered to and spread on the biomimetic coating increasing from 791 ± 379 μm^2 to 1539 ± 877 μm^2 over the same time course. These slides were then washed and retained cells were imaged and stained (Figure 1; Left). To determine the stability of coating cellular retention under dynamic flow, Ti surfaces were either coated or left uncoated and placed into a custom chamber. Cells were placed on the Ti for 15 minutes and then shear flow was applied for 1 hour. The results show that with both a high and low serum concentration the biomimetic surface retains cells 3-fold better than the uncoated control (Figure 1; Right).

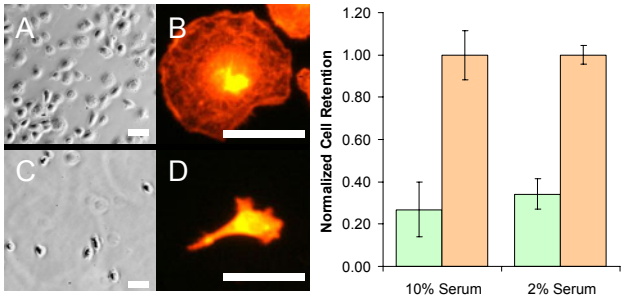

Figure 1: (Left) Attachment results for peptide coated (A, B) and uncoated (C, D) Ti films. Slides were washed with PBS 1 hour after cells were placed on the surface. Images A and C were taken with phase contrast while B and D were from fluorescent microscopy of the actin microstructure. Scale bars represent 50 μm in all photos. (Right) Normalized cell count of cells remaining attached to the coated (orange) and uncoated (green) Ti disks after 1 hour of shear flow in two different serum conditions. Error bars are SD (n=4, $p<0.0005$).

Conclusions: A biomimetic coating for vascular implants is being designed to promote a pro-healing response, thereby reducing the likelihood for thrombosis and rejection. In general, it represents a fundamentally different approach to vascular implant design and illustrates the validity of this approach in promoting the adhesion and interaction of ECs with devices under static and dynamic conditions. The authors wish to acknowledge the support of the NIH.

References:

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