A Novel competitive Co-culture system for Investigating Vascular Cell Responses to Underlying Stent Topography

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Statement of Purpose: The main limitation of post coronary and peripheral stent implantation lies in restenosis [1]. Although the mechanism of in-stent restenosis is not fully understood yet, it has been clear that proliferation of smooth muscle cells (SMC) and the slow recovery rate of the endothelium play important roles in initiating intimal hyperplasia which causes in-stent restenosis [2]. In order to inhibit in-stent resteosis for designing a more effective vascular stent, efforts should focus on developing stents that not only inhibit hyperproliferation and inflammatory responses, but also approaches that facilitate vascular endothelial cell attachment, migration and growth. For this purpose, nanotechnology may provide a promising approach to replace current drug-eluting or bare metal stents through the implementation of nano-structured vascular stent surface features. An *in vitro* competitive co-culture system was designed in this study to investigate the effects of vascular stent nanoscale surface roughness and associated surface energy on the functions of vascular cells.

Materials and Methods: Three different titanium roughness values: flat (F-Ti), nano (N-Ti) and sub-micron (S-Ti) were created by depositing a layer of titanium on glass coverslips using E-beam evaporation. A co-culture system was used to investigate rat aorta endothelial cell (RAEC) function in the presence of rat aorta smooth muscle cells (RASMC) for up to 5 days. The synthesis of intracellular collagen and elastin by RAEC and RASMC were also measured for up to two week to test long term functions of the two cells with underlying titanium substrates with different roughness.

Results: Result of RAEC and RASMC proliferation showed that after 1, 3 and 5 days, while more RAEC adhered on S-Ti and N-Ti than F-Ti, the RAEC proliferation rate on both substrates was greater than on F-Ti, resulting in a faster formation of a monolayer of endothelial cells. Unlike RAEC, RASMC showed no correlation with the various Ti roughness for any of the substrates tested here after 5 days of culture.

Results of the RAEC and RASMC co-culture proliferation experiments (Figure 1) confirmed previous results in which cells were cultured separately, and demonstrated for the first time more RAEC than RASMC on S-Ti than any other substrate tested here. In contrast, there were no significant differences between the growth of RAEC and RASMC on F-Ti.

Results of collagen synthesis showed that RAEC synthesized the largest amount of collagen (Figure 2) and elastin on S-Ti compared to all other substrates after 7 and 14 days of culture. Collagen and elastin synthesis was also greater on N-Ti by RAEC than F-Ti after the same periods. For RASMC for collagen synthesis, there was no significant difference with respect to any of the substrates after 14 days. But for elastin synthesis, RASMC synthesized more elastin on S-Ti compared to any other substrates after 7 and 14 days.

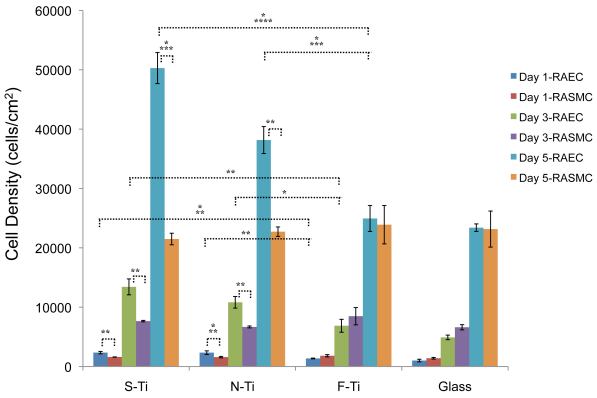

Figure 1. Competitive RAEC and RASMC co-culture proliferation on submicron (S-Ti), nanometer (N-Ti), flat (F-Ti) titanium surface features and borosilicate glass (Glass) after 1, 3 and 5 days of culture. The cell seeding densities were 2,000 cells/cm^2. All error bars are mean ± SEM; n=9; *$p<0.1$, **$p<0.05$, ***$p<0.01$, ****$p<0.005$ and *****$p<0.001$.

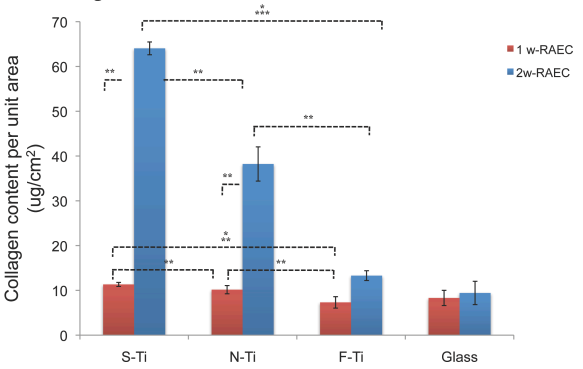

Figure 2. Intracellular total collagen synthesis from RAEC cultured on submicron (S-Ti), nanometer (N-Ti), flat (F-Ti) titanium surface features and borosilicate glass (Glass) after 7 and 14 days of culture. RASMC seeding density was 10,000 cells/cm^2. All error bars are mean ± SEM; n=9; **$p<0.05$, ***$p<0.01$ and ****$p<0.005$.

Conclusions: The results of the competitive co-culture and intracellular protein synthesis study indicated the great potential sub-micron/nanometer surface roughness on titanium has to promote vascular cell interactions for improving current vascular stent applications.

References:

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[2]. Cejna M. J Vasc Interv Radiol. 2002;13(8):823-3