Smooth Muscle Cell Response to Hydroxyapatite Coated Endovascular Stent Materials

Brad Winn [1], Vipul Taneja [1], Eugene M. Langan III [1,2], Martine LaBerge [1].
[1] Department of Bioengineering, Clemson University; [2] Department of Surgery, Greenville Hospital SystemUniversity Medical Center

Statement of Purpose: Hydroxyapatite (HA), present in various tissues of the body, has been extensively used as implant materials for orthopedic and dental applications. HA coating of stents is a relatively new idea, but may be beneficial for several reasons. HA forms a porous coating on the stent surface that can be utilized as a unique drug delivery platform for drugs. Additionally, HA coating might provide a beneficial surface for stented artery re-endothelialization as demonstrated by Okada et al. [1,2]. Additionally, reduction in the release of metallic ions including nickel from coated implants including nickel-titanium alloys and stainless steel as compared to a non-coated control has been reported for HA coating in in-vitro studies [3,4].

Although the effect of HA coatings on endothelial cells (ECs) has been well evaluated, its effects on SMCs have not. With more SMCs than ECs remaining at the site of stenting [6], evaluating the response of SMCs to the stent/coating material becomes very critical in establishing the prospects of using HA coating on stents. The objective of this study, therefore, is from a biomaterials perspective to evaluate the response of SMCs in terms of cell morphology, proliferation, and phenotype, to HA coating as compared to the current industry standard for bare metal stents – electropolished 316L stainless steel (SS).

Methods: Surface preparation: Annealed 316L SS specimens (Brown metal company, Rancho Cucamonga, CA) were divided into two groups – electropolished and HA coated (Enbio, Ireland). Prior to experimentation, disks were ultrasonically cleaned and EtO sterilized.

Cell culture: Vascular SMCs, isolated from aortas of 6-10 week old female Sprague-Dawley rats, were maintained in DMEM (10% FBS, 1% antibiotic/antimycotic) under standard cell culture conditions. 15000 SMCs were seeded per disc at passage 4-7

Cell Morphology: At 48 hrs, SMCs were stained using rhodamine phalloidin and DAPI then imaged (Nikon LV-UDM, Nikon Instruments Inc., Melville, NY).

Cell Proliferation: At days 1, 2, 3, and 4, Cell Titer 96 AQ_{ueous} ONE Solution Cell Proliferation Assay (MTS) (Promega Corporation, G-3580, Madison, WI) was used to evaluate cell proliferation with absorbance measured at 490 nm (Beckman Instruments, Inc., Model# DU® 640B, Fullerton, CA).

α-actin expression: α-actin expression was analyzed using a modified colorimetric cell-based ELISA assay [6]. Absorbance was read at 405 nm with a microplate reader (Beckman Instruments, Inc., Model# DU® 640B, Fullerton, CA). Cells were then DAPI stained and counted to normalize to absorbance to cell count for each disk.

In all cases, data were evaluated using an ANOVA statistical analysis paired with Tukey analysis using SAS statistical analysis software (SAS Institute Inc., Cary, NC) with $p<0.05$ indicating a significant difference.

Results:

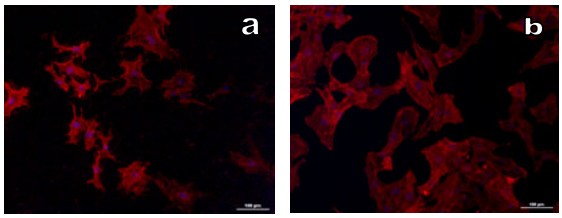

Figure 1: Representative micrographs of F-actin (Red) and cell nuclei (Blue) stained SMCs cultured on HA coated (a) or electropolished (b) SS disks Magnification: 100X; Bar represents 100μm

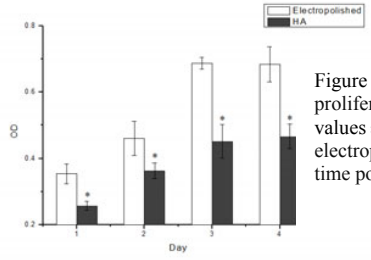

Figure 2: AQueous ONE Cell proliferation assay. Data represents mean values ±SD; n=3; *$p<0.05$ compared to electropolished metal surface at the same time point.

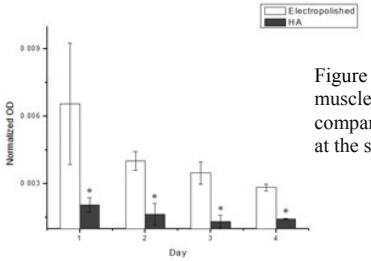

Figure 3: Effect of HA coating on smooth muscle α-actin expression; n=3; *$p<0.05$ compared to electropolished metal surface at the same time point.

Conclusions: SMCs on the HA coating were more spread with pronounced cellular processes and showed significantly reduced proliferation over the electropolished bare metal surface. Additionally SMCs lost a substantial amount of α-actin when cultured on the HA coating compared to the bare metal surface. HA may provide a favorable surface to intrinsically reduce SMC proliferation while providing a possible drug delivery platform. Additional testing is needed to investigate the possible osteogenic potential of the HA coating.

Acknowledgements: The authors thank Fionan Keady and EnBio for HA coating; this work was supported by the Page Morton Hunter Fellowship.

References:

[1] Okada, M. J Biomed Mat Res. 2008. (Vol 86A, p. 539-596).
[2] Furuzono, T. ASAIO Journal. 2006. (Vol 52, p. 315-320).
[3] Jiang, H. Surface & Coatings Technology. 2006. (Vol 201, p. 1017-1021).
[4] Sousa, S. J Mater Sci - Mater Med. 1995. (Vol 6, p. 818-823).
[5] Roach, P. J Mater Sci - Mater Med. 2007. (Vol 18, p. 1263-1277).
[6] Zhang, H. J Immunol. 1997. (Vol 158, p. 1392-1399).