Influence of Topography of Endovascular Stent Material on Smooth Muscle Cell Response

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Statement of Purpose: Following endovascular stenting, neointimal hyperplasia, characterized by a phenotypic shift of smooth muscle cells (SMCs) from contractile to synthetic phenotype, has been deemed to be the predominant cause of restenosis [1, 2-5]. A number of stent surface parameters, including topography, have been attributed to play important roles in stent performance. Although endothelial cell and thrombogenic response to surface roughness have been well evaluated, the effect of topography of the outer stent struts on SMCs has not yet been reported. Therefore, the purpose of this study was to examine the in vitro effects of topography of a commonly used endovascular stent material (316L stainless steel) on vascular smooth muscle cell phenotype as an indicator of potential neointimal hyperplasia and restenosis in vivo.

Methods:

Surface Preparation: Annealed 316L stainless steel specimens (Brown metal company, Rancho Cucamonga, CA) were divided into two groups – electropolished and micro-grooved. Micro-grooved surfaces were produced using a surface grinder (Exakt D-2000, Exakt Technologies Inc., Oklahoma, OK) with 60 grit silicon carbide paper. The metal discs were ultrasonically cleaned, air dried, and EtO sterilized . Non-contact profilometry (NT-2000, Veeco, WYKO Corp., Tuscon, AZ) was conducted on each sample at 25X with cut-off area of 736x480µm. These measurements allowed for calculations including roughness average (R_a), and root mean square roughness (R_q).

Cell culture: Vascular smooth muscle cells, 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, with medium replaced every second day.

Quantification of cell elongation: Cellular aspect ratio measurements (axis major/axis minor) were performed on images of rhodamine phalloidin stained SMCs using NIS Elements 3.1 (Nikon Instruments Inc., Melville, NY).

Cell proliferation: At day 1, 2, 3, and 4, SMCs were DAPI stained and imaged (Nikon AZ-100, Nikon Instruments Inc., Melville, NY). Cell number was counted for each disc and reported as cell count per field of view.

Smooth muscle α-actin expression**:** α-actin expression in SMCs grown on stainless steel discs of varying topography were analyzed using a modified cell-based colorimetric ELISA assay [6]. Absorbance was read at 405 nm with a microplate reader (Beckman Instruments, Inc., Model# DU® 640B, Fullerton, CA). Absorbance due to non-specific binding was subtracted from that of the experimental groups, with the net absorbance normalized against cell count on each disc giving a number directly related to the α-actin content per cell.

All data were evaluated via ANOVA statistical analysis paired with LSD/Tukey analysis using SAS statistical analysis software (SAS Institute Inc., Cary, NC) with $p<0.05$ indicating a significant difference.

Results:

Table1: Roughness values of three surface types

	R_a (in nm)	R_q (in nm)
Electropolished	89.2±8.1	110.8±9.7
Micro-grooved	2009.8±361.4	2474.1±4

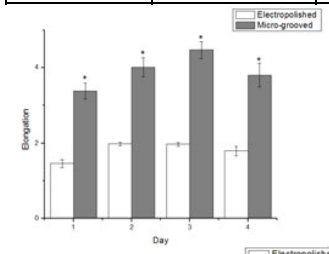

Figure 1: Effects of surface topography on cell elongation. Data represents mean values ±SD; n=3, *p<0.0001 compared to electropolished surface at the same time point.

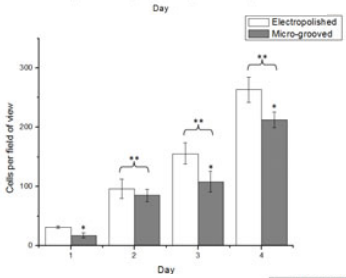

Figure 2: DAPI cell count per field of view. Data represents mean values ±SD; n=3; *p<0.05 compared to electropolished surface at the same time point; **p<0.05 compared to respective surface type on day 1.

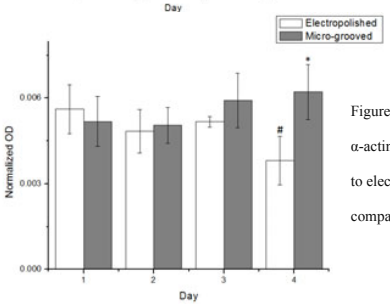

Figure 3: Effect of surface topography on smooth muscle α-actin expression – ELISA assay; n=3; *p<0.05 compared to electropolished surface on the same time point; #p<0.05 compared to same surface type on day 1.

Conclusions: Results show that micro-grooves approximately 13µm deep on 316L stainless steel are able to preserve the contractile phenotype of SMCs while limiting their proliferation when compared to the current industry standard for bare metal stents – electropolishing. This research, therefore, demonstrated the potential of micro-grooves on outer stent surface as an intrinsic tool to prevent neointimal hyperplasia and thus in-stent restenosis.

References:

[1] Inoue, T. Circ J. 2009. (Vol 73, p. 615-621).
[2] Kipshidze, N. J Am Coll Cardiol. 2004. (Vol 44, p 733-739).
[3] Acampora, K. Ann Vasc Surg. 2010. (Vol 24, p. 116-126).
[4] Shanahan, C. Arterioscler Thromb Vasc Biol. 1998. (Vol 18, p. 333-338).
[5] Welt, FG. Arterioscler Thromb Vasc Biol. 2002. (Vol 22, p. 1769-1776).
[6] Zhang, H. J Immunol. 1997. (Vol 158, p. 1392-1399).