Interaction of Endothelial and Smooth Muscle Cells with Paclitaxel-Immobilized Self Assembled Monolayers

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Introduction: Polymer-based drug delivery carriers in stents cause adverse reactions in patients [1]. Hence, the use of self-assembled monolayers (SAMs) as a polymer-free drug delivery platform for stents has been demonstrated [2]. An anti-proliferative drug, paclitaxel (PAT), has been immobilized on a stent material cobalt-chromium (Co-Cr) alloy surfaces using SAMs and released it for a period of time [3]. In this study, the interaction of endothelial cells (ECs) and smooth muscle cells (SMCs) with PAT immobilized SAMs on Co-Cr alloy was studied. The hypothesis of the study is that the SAMs platform do not elicit an adverse response from ECs while the PAT released from SAMs will inhibit the growth of SMCs.

Methods: Co-Cr alloy plates (1cm x 1cm) used in this study were mechanically polished using 600, 800, and 1200 grit SiC papers and chemically cleaned by sonication in ethanol, acetone, and methanol for 10 minutes each followed by N_2 gas drying. A carboxylic acid-terminated phosphonic acid SAM was coated on the Co-Cr alloy surfaces [3]. A dose of 100 µg of PAT was loaded on the SAMs coated surfaces by microdrop deposition as described previously [3]. The control, SAMs coated, and PAT deposited Co-Cr alloy surfaces were characterized using Fourier transform infrared spectroscopy (FTIR), 3D optical surface profilometry (OSP), scanning electron microscopy (SEM), and contact angle goniometry (CAG). A density of 15×10^3 human aortic endothelial cells (HAECs) and human aortic smooth muscle cells (HASMCs) were seeded separately (not co-cultured) on control, SAMs coated, and PAT deposited alloy surfaces. The viability and proliferation of cells were investigated at 1, 3, and 5 days using a Resazurin fluorometric assay. The morphology of cells was investigated using fluorescence microscopy (FM) after staining the cells with fluorescein diacetate. A one-way analysis of variance (ANOVA) was used to determine the statistical significance at $p < 0.05$.

Results: The IR peaks for the symmetric and asymmetric stretches of $–CH_2$ groups of SAMs were observed at 2849 and 2917 cm^{-1}, respectively (Fig 1A). For PAT coating, the IR peaks for the C=O stretches of ester and amide bonds were observed at 1733 and 1647 cm^{-1}, respectively. Also, the peaks for the finger print region of PAT were observed at 716, 1073, and 1248 cm^{-1} (Fig 1B). Thus, the FTIR spectra confirmed the successful coating of SAMs and PAT on Co-Cr alloy surfaces. SEM (Fig 2A) and OSP (Fig 2B) images showed the spherical and oval shaped morphology of PAT crystals on alloy surfaces. The average surface roughness values of control, SAMs, and PAT coated surfaces determined by OSP were 0.004 ± 0.000, 0.005 ± 0.001, and 0.111 ± 0.017 µm, respectively. The contact angles of control, SAMs, and PAT coated surfaces were 68.3 ± 4.8, 72.6 ± 3.9, and 69.8 ± 1.5, respectively. The EC adhesion increased in the following order: PAT < control = SAMs (Fig 3A, day-1). The ECs continued to proliferate on all the three surfaces with maximum number of cells observed on SAMs coated surfaces on days – 3 and 5 (Fig 3A). The spreading of ECs on all the surfaces with typical polygonal shape indicated that these surfaces are conducive to endothelialization (Fig 3B). The number of SMCs on PAT coated surfaces was significantly lesser when compared to that of other surfaces (Fig 4A). The fluorescent microscopy images also showed only very few irregular shaped (not the characteristic spindle shaped) SMCs on PAT coated surfaces (Fig 4B). These results show that the PAT released from SAMs inhibited the growth of SMCs.

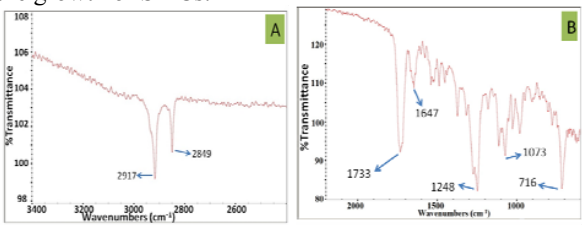

Fig 1: FTIR spectra of SAMs (A) and PAT (B).

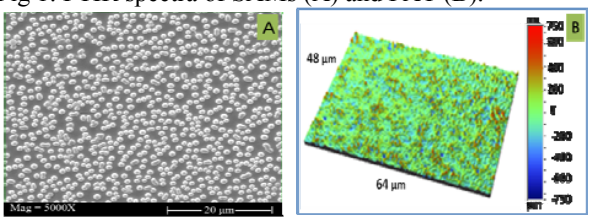

Fig 2: SEM (A) and OSP (B) images of PAT-Co-Cr.

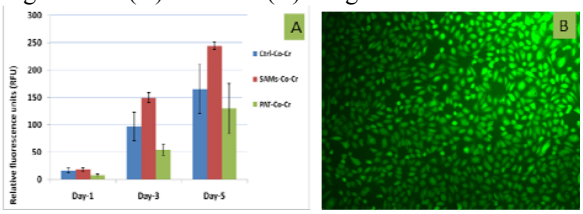

Fig 3: Resazurin data (A) and Fluorescent microscopy image (SAMs-CoCr, after day-5) (B) of HAECs.

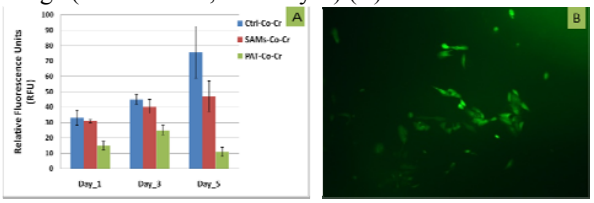

Fig 4: Resazurin data (A) and Fluorescent microscopy image (PAT-Co-Cr, after day-5) (B) of HASMCs.

Conclusions: SAMs did not elicit an adverse response from ECs. Also, the PAT released from SAMs inhibited the growth of SMCs. Thus, this study demonstrated the use of SAMs platform to control cell behavior on stents.

References: (1) Virmani R. Circulation 2004; 109: 701-705; (2) Mani G. Biomaterials 2008; 29: 4561-4573; (3) Mani G. Biointerphases 2011, 6, 33-42.

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