Native Endothelium Mimicking Self-Assembled Nanomatrix for Drug Eluting Stent Applications

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Statement of Purpose: Deployment of stents has been a major therapeutic method for cardiovascular diseases (CVD). However, intimal hyperplasia and poor endothelialization of bare metal stents (BMSs) lead to restenosis of the artery. Drug eluting stents (DESs) are designed to deliver therapeutic drugs to tackle the shortcomings faced by BMSs. However, recently, DESs have been associated with higher risk of late thrombosis which may be related to lack of endothelialization. Therefore, the main goal of this project was to develop an innovative strategy for next generation DES using a native endothelium mimicking self-assembled nanomatrix which will inhibit restenosis and thrombosis while enhancing endothelialization. Two different peptide amphiphiles (PAs) were developed for this study: PA-KKKKK containing nitric oxide (NO) donors and PA-YIGSR containing endothelial cell adhesive ligand YIGSR[1], both along with enzyme-mediated degradable sites[2].

Methods: PA-YIGSR and PA-KKKKK were separately synthesized using F-moc Chemistry. PA-YK (9:1 molar ratio mixture of PA-YIGSR and PA-KKKKK) was used for further studies. NO producing PA-YK-NO was developed by reacting pure NO gas with PA-YK solution. PAs were self-assembled into nanofibers on glass cover slips by solvent evaporation and verified for self-assembly with TEM. Effect of PA-YK-NO on behaviors of Human Umbilical Vein Endothelial Cells (HUVECs) and Human Aortic Smooth Muscle Cells (AoSMCs) was evaluated by Proliferating Cell Nuclear Antigen (PCNA) staining. Platelet attachment on the collagen, PA-YK, and PA-YK-NO was investigated with human blood. Preliminary animal studies were conducted by implanting PA-YK-NO coated stents in a rabbit iliac artery. ANOVA analysis was performed to evaluate statistical significance.

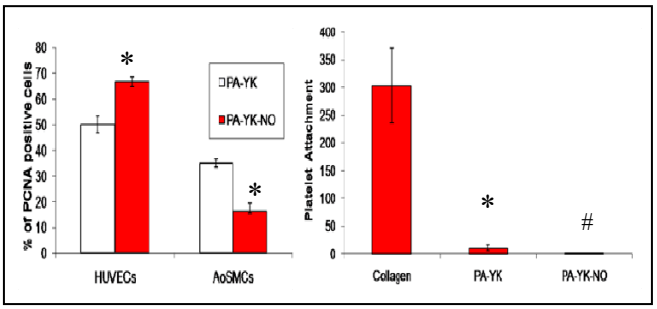

Figure 1. (A) Proliferation of HUVECs and AoSMCs on PA-YK and PA-YK-NO nanomatrices after 48 hours, quantitatively assessed by PCNA staining (B) Platelet attachment on Collagen, PA-YK, and PA-YK-NO nanomatrices after incubation with human blood for 15 minutes. (*, # $p<0.05$)

Results: Successful self-assembly of all PAs into nanofibers (6 nm - 10nm) was confirmed by TEM. NO release from the PA-YK-NO nanomatrix was studied, and an initial burst release, followed by sustained release was observed. PA-YK-NO showed significantly higher proliferation of HUVECs and significantly lower proliferation of AoSMCs when compared to PA-YK *(Figure 1 A)*. This indicates that PA-YK-NO may have potential to enhance endothelialization but reduce restenosis. In addition, PA-YK was found to limit platelet attachment compared to the positive control, collagen I. PA-YK-NO further prevented platelet attachment (*Figure 1 B)*. This result indicates that PA-YK-NO may have potential to prevent thrombosis. Preliminary rabbit studies showed that stents were successfully deflated, stent coatings are stable, minimal inflammation was observed, and no thrombosis was found as shown in *Figure 2*.

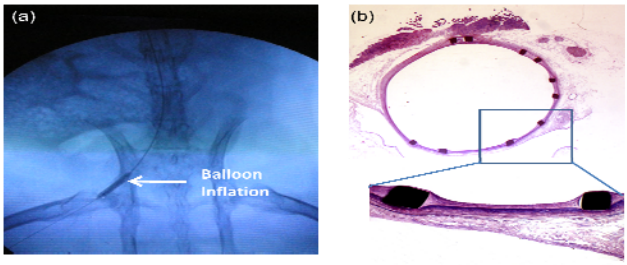

Figure 2. (a) Balloon inflation to deploy stent in rabbit iliac artery. (b) Histology section of PA-YK-NO coated stents after 4 weeks of implant.

Conclusions: We have successfully developed a nitric oxide releasing native endothelium mimicking nanomatrix that limits platelet adhesion and smooth muscle cell proliferation which are the key events in thrombosis and restenosis. Also, the nanomatrix was found to enhance endothelial cell proliferation which is critical for re-endothelialization. From preliminary rabbit studies, the nanomatrix coating showed stability under blood flow. Endothelialization was evident, and neointimal proliferation and thrombosis were limited. Therefore this nanomatrix could have a great potential to improve clinical patency of DESs as a coating by enhancing endothelialization while reducing restenosis and thrombosis.

References:

1. Hubbell JA *et al.* Bio/Technology 1991 ; 9 : 568–572
2. Jun HW *et al*. Adv. Materials 2005; 17(21):2612-17

Acknowledgments

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