Biomimetic Hybrid Nanomatrix for Cardiovascular Applications

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Statement of Purpose: Current cardiovascular implants are limited by poor endothelialization, restenosis, and thrombosis. This study proposes to develop a hybrid nanomatrix that mimics a native endothelium by combining unique properties of electrospun poly (ε-caprolactone) (ePCL) nanofibers with self-assembled peptide amphiphiles (PAs). Electrospinning produces uniform nanofibers with an interconnected porous structure and high surface area to volume ratio. However, these nanofibers are limited by the lack of surface bioactivity to control cellular behaviors. It is hypothesized that PAs can be self-assembled onto the surface of the ePCL nanofibers and endow them with the characteristic properties of a native endothelium. PAs consist of hydrophobic alkyl tails attached to functional hydrophilic peptide sequences, which comprise enzyme-mediated degradable sites[1], coupled to either endothelial cell adhesive ligands (PA-YIGSR)[2] or nitric oxide (NO) donating residues (PA-KKKKK).

Methods: ePCL nanofibers were synthesized by electrospinning a solution of PCL in 1:1 (v/v) methanol/chloroform, at 21 kV. 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 synthesized by reacting pure NO gas with PA-YK solution. PA-YK-NO was self-assembled into nanofibers on ePCL nanofibers by solvent evaporation to form a hybrid nanomatrix (ePCL-PA-YK-NO), and this was verified with transmission electron microscopy (TEM). NO release from ePCL-PA-YK-NO was studied using Greiss assay. Effect of ePCL-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. ePCL coated with PA-YK (ePCL-PA-YK) was used as a control. ANOVA analysis was performed to evaluate statistical significance.

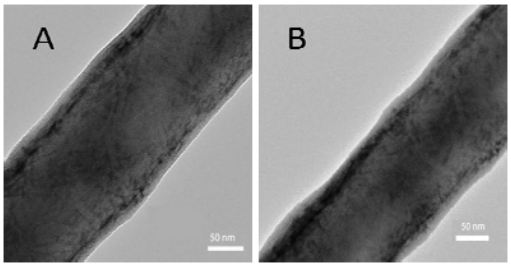

Figure1. (A) TEM image of a hybrid nanomatrix (ePCL+PA-YK-NO) at 67000x. (B) TEM image of ePCL+PA-YK-NO tilted to 21°, to confirm uniform self assembly. 67000x.

Results: Uniform ePCL nanofibers of diameter approximately 300-500 nm were produced and confirmed by scanning electron microscopy. PAs were successfully self-assembled onto the surfaces of ePCL nanofibers, and this was confirmed by TEM (*Figure 1)*. NO release from ePCL-PA-YK-NO was studied, and an initial burst release, followed by sustained release over 30 days was observed. ePCL-PA-YK-NO promoted the spreading and adhesion of endothelial cells. ePCL-PA-YK-NO also displayed a higher percentage of proliferating HUVECs and a significantly lower percentage of proliferating AoSMCs when compared to control ePCL-PA-YK, as shown in *Figure 2*. This indicates that ePCL-PA-YK-NO enhances the proliferation of HUVECs and also reduces the proliferation of AoSMCs.

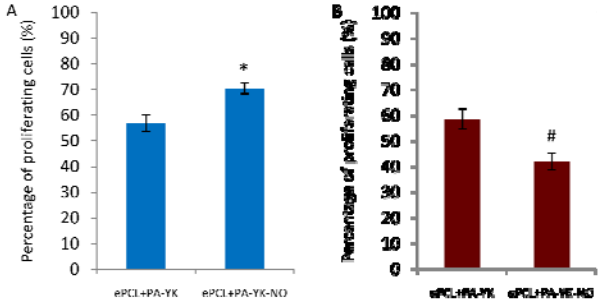

Figure 2. Percentage of proliferating cells at 48 hours. (A) HUVEC proliferation. (*) ePCL-PA-YK-NO shows significantly greater proliferation when compared to ePCL-PA-YK (control). (B) AoSMC proliferation. (#) ePCL-PA-YK-NO shows significantly reduced proliferation when compared to ePCL-PA-YK.

Conclusions: We have successfully developed a hybrid nitric oxide releasing biomimetic hybrid nanomatrix (ePCL-PA-YK-NO) that limits smooth muscle cell proliferation, which is essential for preventing restenosis. Also, this hybrid nanomatrix was found to enhance endothelial cell adhesion and proliferation which is critical for re-endothelialization. The effect of this hybrid nanomatrix on adhesion and activation of platelets is currently being investigated. This hybrid biomimetic nanomatrix could improve clinical patency of cardiovascular implants by enhancing endothelialization and reducing restenosis, and therefore, has great potential for applications in vascular grafts and artificial heart valves.

References:

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2. Hubbell JA *et al.* Bio/Technology 1991; 9: 568–572

Acknowledgments

Funding for this project was provided by Wallace H. Coulter Foundation and Caroline P. Ireland Research Scholarship.