Hybrid Biomimetic Nanomatrix for Cardiovascular Applications

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Statement of Purpose: This study proposes to develop a native endothelium mimicking hybrid nanomatrix by combining the unique properties of electrospun polycaprolactone (ePCL) with peptide amphiphiles (PAs) to overcome the limitations such as lack of endothelialization, restenosis and thrombosis faced by current cardiovascular implants. Electrospinning has been gaining attention recently due to its ability to produce nanofibers similar in dimensions to extracellular matrix (ECM) proteins. However, they lack surface bioactivity to control cell behaviors. It is hypothesized that PAs self-assemble onto the surface and endow these ePCL nanofibers with native endothelium mimicking characteristics. PAs[1] consist of hydrophobic alkyl tails attached to hydrophilic peptide chains, which in turn comprise enzyme mediated degradable sites, endothelial cell adhesive ligands (PA-YIGSR)[2] or nitric oxide donors (PA-KKKKK)

Methods: PCL solution in 1:1 (v/v) methanol/chloroform, was electrospun at 21 kV. Fmoc chemistry was used for synthesis of PA-YIGSR and PA-KKKKK separately. PA-YK (9:1 molar ratio mixture of PA-YIGSR and PA-KKKKK) was used for further studies. PA-YK was reacted with pure NO to produce NO releasing PA-YK-NO. 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 and PicoGreen DNA assay. ePCL coated with PA-YK (ePCL-PA-YK) was used as a control. Platelet adhesion was studied by incubating mepacrine labeled whole human blood on the hybrid nanomatrix. ePCL-PA-YK, uncoated ePCL and collagen films were used as controls. ANOVA analysis was performed to evaluate statistical significance.

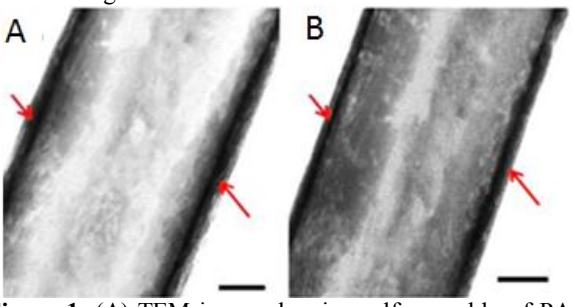

Figure 1. (A) TEM image showing self-assembly of PA on ePCL in the hybrid nanomatrix (ePCL+PA-YK-NO) at 67000x. (B) TEM image of ePCL+PA-YK-NO tilted to 21°, to confirm uniform self assembly. 67000x. Scale bar = 50 nm

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 promoted the proliferation of HUVECs but limited the proliferation of AoSMCs compared to control ePCL-PA-YK. This was confirmed by PCNA (*Figure 1*) and PicoGreen assays. ePCL-PA-YK-NO also limited the adhesion of platelets when compared to control ePCL-PA-YK, ePCL and collagen films (*Figure 2*).

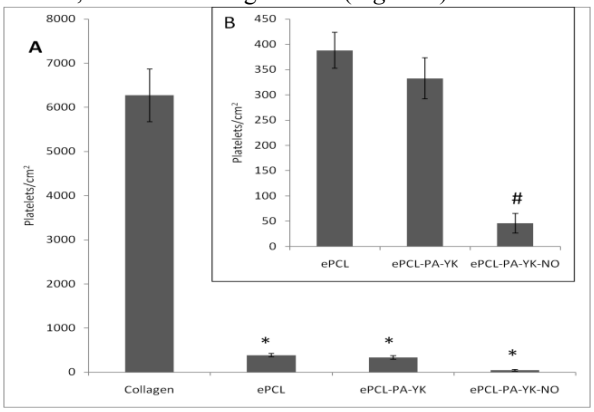

Figure 2. Platelet adhesion on the hybrid nanomatrix. (A) Platelets showed significantly reduced adhesion on ePCL, ePCL-PA-YK and ePCL-PA-YK-NO when compared to collagen control (*). (B) ePCL-PA-YK-NO (#) showed significantly reduced platelet adhesion when compared to ePCL and ePCL-PA-YK.

Conclusions: A nitric oxide releasing hybrid endothelium mimicking nanomatrix (ePCL-PA-YK-NO) was developed by combining ePCL with NO releasing PAs. This hybrid nanomatrix limits smooth muscle cell proliferation and platelet adhesion, which is essential for preventing restenosis and thrombosis respectively. It was also found to promote endothelial cell adhesion and proliferation which is critical for re-endothelialization. This hybrid biomimetic nanomatrix could improve clinical patency of cardiovascular implants and therefore, has great potential for applications in vascular grafts and artificial heart valves.

References:

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2. Andukuri *et al*. Nanomedicine 2010; 6:289-297

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