

Novel Nanomatrix Reduces Inflammation in Dynamic Conditions *in Vitro* and Dilates Arteries *ex Vivo*

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Statement of Purpose

Inflammatory responses play a critical role in tissue-implant interactions, often limiting current implant utility. This is particularly true for cardiovascular devices. Existing stent technology does little to avoid or mitigate inflammation or to influence the vasomotion of the artery after implantation. We have developed a novel endothelium-mimicking nanomatrix composed of peptide amphiphiles that enhances endothelialization while decreasing both smooth muscle cell proliferation and platelet adhesion. Here, we evaluated whether the nanomatrix could prevent inflammatory responses under static and physiological flow conditions. The goal was to demonstrate the clinical potential of this nanomatrix by both preventing inflammatory responses and promoting vasodilation, critical improvements in stent and cardiovascular device technology. Our overall hypothesis is that the prohealing multifunctional endothelium nanomatrix will both prevent inflammatory induction and attenuate pre-existing inflammatory conditions.

Materials and Methods

Peptide amphiphiles (PAs) with either a cell adhesive ligand (YIGSR) or polylysine nitric oxide donors (KKKKK) were synthesized and mixed in a 9:1 molar ratio to form PA-YK. Nitric oxide-releasing PA-YK-NO was developed by reacting PA-YK with nitric oxide. PA-YK-NO self-assembled into nanofibers through water evaporation without use of organic solvents and was verified by TEM, SEM, and FTIR. Nitric oxide release profiles were studied with Chemiluminescence and Griess assays. Adhesion of U937 monocytes, both with and without TNF- α stimulation, was evaluated on human umbilical vein endothelial cells. Monocyte inflammatory gene expression of TNF- α , MCP-1, IL-1 β , and IL-6, again both with and without TNF- α stimulation, was assessed by RT-PCR. *Ex vivo* vasodilation experiments were performed in rat mesenteric arterioles.

Results

SEM, TEM, and FTIR confirmed that PA-YK successfully assembled in nanofibers of uniform diameter of 7-8 nm. The nitric oxide release profile from PA-YK-NO displayed an initial burst release in the first 48 hours of measurement followed by slow, sustained release over 60 days. Adhesion of U937 monocytes on human umbilical vein endothelial cells with and without TNF- α stimulation was significantly decreased on PA-YK-NO compared to PA-YK and TCP controls. qRT-PCR results, both with and without TNF- α stimulation, demonstrated significantly reduced expression of the inflammatory

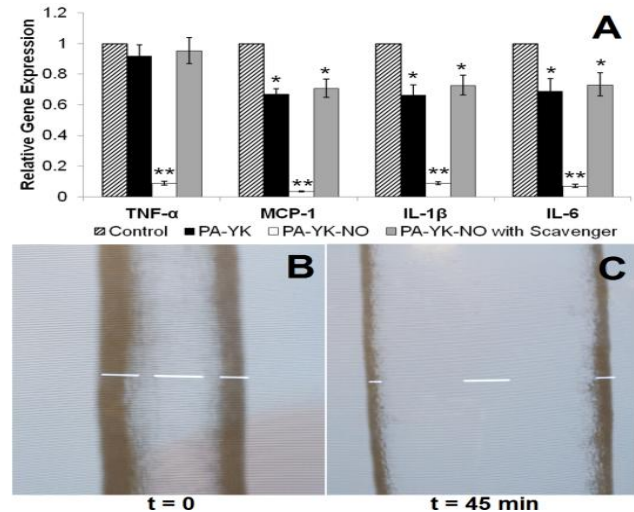


Figure 1: (A) qRT-PCR of monocyte gene expression with TNF- α stimulation at 24 hours. (B, C) Vessel dilation from PA-YK-NO. * $p < 0.01$, ** $p < 0.001$.

genes TNF- α , MCP-1, IL-1 β , and IL-6 in monocytes on the PA-YK-NO nanomatrix compared to PA-YK and TCP controls (figure 1). This affect was abolished by addition of a nitric oxide scavenger. *Ex vivo* vasodilation studies demonstrated NO's bioactivity, bioavailability, and desired release kinetics (figure 1). Statistical analyses were done with one-way ANOVA.

Conclusion

We have successfully developed a novel prohealing multifunctional endothelium nanomatrix that can enhance endothelialization and inhibit smooth muscle cell proliferation and platelet adhesion. Experiments conducted under static and dynamic conditions demonstrate that this self-assembled nanomatrix does not induce inflammation, and may reduce a pre-existing inflammatory environment; *ex vivo* studies demonstrate its bioactivity, bioavailability, and desired release kinetics in promoting vasodilation. Therefore, this nanomatrix has vast potential to enhance stent efficacy by promoting endothelial healing and reducing neointimal formation, inflammation, and thrombosis. Future studies include further evaluation of the nanomatrix within a physiological flow-modeling bioreactor prior to evaluation *in vivo* within rabbit iliac arteries.

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