# An Endothelium Simulating Multifunctional Nanomatrix for Drug Eluting Stents

Adinarayana Andukuri[1], Young-Doug Sohn[2], Young-Sup Yoon[2], Brigitta C. Brott[3], Ho-Wook Jun[1]
[1]Dept of Biomedical Engineering, University of Alabama at Birmingham, [2]School of Cardiology, Emory University,
[3]School of Medicine, University of Alabama at Birmingham.

**Statement of Purpose:** The overall goal of this study is to develop an endothelium simulating multifunctional nanomatrix comprising peptide amphiphiles (PA) for coating drug eluting stents. Deployment of stents is a major therapeutic technique for treatment of cardiovascular diseases. The requirement for new generation stents is caused by characteristic limitations of conventional stents, including neointimal hyperplasia, poor endothelialization, restenosis, and thrombosis. This study demonstrates the synergistic effects of multiple bioactive functions combined with nitric oxide (NO) on cellular behaviors of vascular cells, endothelial progenitor cells (EPCs), blood cells, and inflammatory cells *in vitro* and *in vivo*.

**Methods:** PA-YIGSR containing an endothelial cell adhesive YIGSR and PA-KKKKK containing nitric oxide (NO) donors were separately synthesized and mixed in a 9:1 molar ratio to produce PA-YK[1]. NO producing PA-YK-NO was developed by reacting pure NO gas. PAs were self-assembled into nanofibers by solvent evaporation and verified for self-assembly with TEM. NO release from PA-YK-NO was studied using Greiss assay. Human Umbilical Vein Endothelial Cells (HUVECs) and Human Aortic Smooth Muscle Cells (AoSMCs) proliferation on PA-YK-NO was evaluated by Proliferating Cell Nuclear Antigen (PCNA) staining. Platelet attachment on the collagen, PA-YK, and PA-YK-NO was investigated with mepacrine labeled human blood. Endothelial progenitor cell (EPC) behavior on PA-YK-NO was also studied. Peripheral blood mononuclear cells (PBMNCs) were isolated, and their adhesion was studied by DAPI staining. PBMNC differentiation was studied using flow cytometric analysis for endothelial markers vWF, VEGFR2, CD31, and CD34. Preliminary animal studies were conducted by implanting PA-YK-NO coated stents in a rabbit iliac artery. Currently, the effect of the nanomatrix on inflammatory cells is being investigated by incubating U937 monocytes on PA-YK-NO and evaluating the expression of TNF-α, IL6, and MCP.

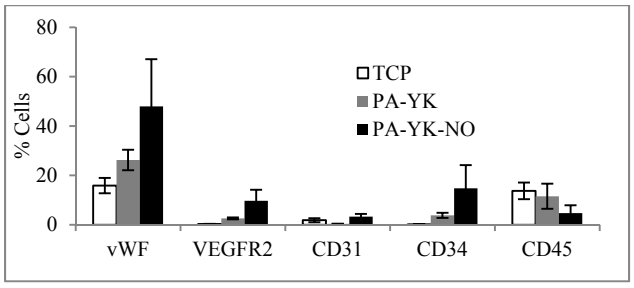


**Figure 1.**Flow cytometric analysis of EPCs at 14 days on PA-YK-NO, PA-YK, and TCP[2].

**Results:** Successful self-assembly of all PAs into nanofibers (8 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 over a 2 month period. PA-YK-NO showed significantly higher proliferation of HUVECs and significantly lower proliferation of AoSMCs when compared to PA-YK. PA-YK was also found to limit platelet attachment compared to the positive control, collagen I. PA-YK-NO further prevented platelet attachment. EPC adhesion was significantly higher on PA-YK-NO and PA-YK when compared to the control. The expression of endothelial markers on EPCs was significantly increased on PA-YK-NO when compared to controls (*Figure 1*). These results indicate that PA-YK-NO may have potential to enhance endothelialization, prevent intimal hyperplasia, restenosis and prevent thrombosis. Inflammation studies showed reduced expression of inflammatory genes on PA-YK and PA-YK-NO when compared to controls. 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*.

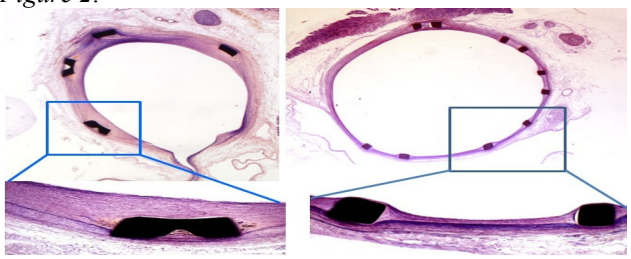

**Figure 2.** Histology section of PA-YK-NO coated stents after 4 weeks of implant. Control uncoated stent (left). PA-YK-NO coated stent (right).

**Conclusions:** We have successfully developed an endothelium simulating nanomatrix that promotes endothelial cell proliferation but limits platelet adhesion and smooth muscle cell proliferation. Effectively, this nanomatrix possesses the potential to promote endothelialization but limit intimal hyperplasia, restenosis and thrombosis. The naomatrix also promotes the adhesion and differentiation of EPCs, which is critical for rapid endothelialization of the stent. Preliminary rabbit studies showed that the nanomatrix coating displayed stability under blood flow. Endothelialization was evident and neointimal proliferation and thrombosis were limited. Therefore, the nanomatrix could have a great potential to improve clinical patency of DES as a coating by enhancing endothelialization while reducing restenosis and thrombosis.

**References:**

1. Kushwaha *et al*, Biomaterials 2010 ; 31 :1502-1508
2. Andukuri *et al*, Tissue Engineering, Part C 2012

**Acknowledgments:** Wallace H. Coulter Foundation, Caroline P. Ireland Research Scholarship, and AHA pre-doctoral Fellowship.