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Nanomatrix Coated Stent Enhances Endothelialization but Reduces Platelet, Smooth Muscle Cell, and Monocyte Adhesion under Physiologic Conditions

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Abstract

Cardiovascular disease is presently the number one cause of death worldwide. Current stents used to treat cardiovascular disease have a litany of unacceptable shortcomings: adverse clinical events including restenosis, neointimal hyperplasia, thrombosis, inflammation, and poor re-endothelialization. We have developed a biocompatible, multifunctional, peptide amphiphile-based nanomatrix coating for stents. In this study, we evaluated the ability of the nanomatrix coated stent to simultaneously address the issues facing current stents under physiological flow conditions in vitro. We found that the nanomatrix coated stent could increase endothelial cell migration, adhesion, and proliferation (potential for re-endothelialization), discourage smooth muscle cell migration and adhesion (potential to reduce neointimal hyperplasia and restenosis), and decrease both platelet activation and adhesion (potential to prevent thrombosis) as well as monocyte adhesion (potential to attenuate inflammatory responses) under physiological flow conditions in vitro. These promising results demonstrate the potential clinical utility of this nanomatrix stent coating, and highlight the importance of biocompatibility, multifunctionality, and bioactivity in cardiovascular device design.

Keywords

pro-healing; multifunctional; endothelium; nanomatrix; stent; bioreactor

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1. INTRODUCTION

Cardiovascular disease is currently one of the leading causes of death, both in the United States and worldwide. Presently, metal stents are used to maintain blood vessel patency in arteries that have weakened or narrowed over time due to plaque deposition. Bare metal stents (BMS) are plagued by restenosis, a renarrowing of the blood vessel after stent deployment.[1–3] The underlying mechanism behind restenosis is neointimal hyperplasia: the proliferation and migration of smooth muscle cells (SMCs) over the stents and the accompanying extracellular matrix deposition, which narrows the blood vessel once more after stent deployment.[4,5] Thus, drug eluting stents (DES) were developed to address and diminish restenosis; although these efforts have been largely successful, DES have their own set of shortcomings, including late stent thrombosis (blood clots), inflammatory responses, and delayed re-endothelialization.[6–10] Moreover, the antiproliferative drugs and antiplatelet regimens required after stent procedures, and which could be necessary for many months, may be associated with additional adverse clinical events.[11,12]

Although much effort has been expended targeting singular goals (DES to prevent restenosis by inhibition of neointimal hyperplasia; coatings to reduce platelet activation and adhesion; advances in biocompatibility to preclude or attenuate inflammation; and strategies involving cell adhesive ligands, antibodies, and endothelial progenitor cell capture methodologies for re-endothelialization), thus far no technology has achieved all of these aims.[13–18] In light of all of this, it has become apparent that stent and coating design must move from focusing on singular targets and objectives and develop into more multifaceted, multifunctional platforms. Stents must simultaneously address neointimal hyperplasia, thrombosis, inflammation, and re-endothelialization. Current stent technologies may focus on one or two of these modalities (e.g., reducing neointimal hyperplasia or encouraging re-endothelialization), but to date it has been difficult to realize multiple targets concurrently.

Furthermore, the stent coating must exhibit high biocompatibility and multifunctionality. Because the endothelium is damaged or disrupted during stent deployment, the stent coating should also confer bioactivity to supply the lost functions of the endothelium until re-endothelialization is complete.[19,20] This can include mimicry of the extracellular matrix anchor sites for cell attachment and proliferation, release of small signaling molecules such as nitric oxide (NO) that are typically produced endogenously by the endothelium, and prevention of platelet adhesion and inflammatory re-sponses.[17,21,22]

Therefore, we have developed an innovative, biocompatible, peptide-based nanomatrix. This nanomatrix is composed of peptide-amphiphiles (PAs), which self-assemble into nanofibers, which in turn deposit in hundreds of layers forming the endothelium-mimicking nanomatrix; critically, this self-assembly of the nanomatrix is achieved without use of potentially inflammatory organic solvents.[23–27] The PA nanofibers include laminin-derived cell adhesive ligands for endothelial cell attachment and proliferation, enzyme-mediated degradable sites for remodeling of the nanomatrix, and polylysine NO donors in optimized ratios for a pro-healing paradigm.[24,25,27] These PA nanofibers that contain both the cell adhesive ligands and NO donors (termed PA-YK-NO) compose the endothelium-mimicking

nanomatrix. Our previous studies have demonstrated that this nanomatrix simultaneously enhances endothelialization while reducing both platelet adhesion and smooth muscle cell proliferation under static conditions in vitro.[24,27] Additionally, the nanomatrix is stable under physiological shear stresses, prevents and attenuates inflammatory responses, and enhances endothelial progenitor cell adhesion and differentiation.[25,26,28] In consideration of these results, it is essential to investigate the effects of nanomatrix-coated stents under physiological flow on endothelialization, smooth muscle cell migration, inflammation, and platelet adhesion.

Thus, this present work examines the effect of nanomatrix coated stents under physiological flow conditions in vitro on endothelialization, smooth muscle cell migration, and inflammatory cell (monocyte) and platelet adhesion (Figure 1). Notably, this goes beyond our previous studies in that we utilize nanomatrix coated stents exposed to pulsatile flow-induced shear stress to better recapitulate in vivo circumstances, rather than static or simple geometric surfaces (e.g., tissue culture plates or glass slides). The synergistic effects of the endothelial cell adhesive ligands for cell adhesion and proliferation, enzyme-mediated degradable sites for remodeling, and polylysine NO donors within the PA nanofibers endow the nanomatrix with multifunctionality, biocompatibility, and bioactivity to simultaneously address the multifaceted requirements for stent coatings. Pro-healing PA-YK-NO nanomatrix coated stents can thus promote endothelialization, suppress neointimal hyperplasia by reducing smooth muscle cell proliferation, and prevent and mitigate inflammation and platelet adhesion, all of which are desirable after stent deployment. Coupled with our previous data, these studies pave the way to evaluation within in vivo animal models.

2. MATERIALS AND METHODS

2.1 Preparation of the Self-Assembled Peptide Amphiphile Nanomatrix.

The peptide amphiphile (PA) nanomatrix was synthesized as previously described.[24–28] Briefly, two distinct PAs were synthesized through Fluorenylmethoxycarbonyl (Fmoc) chemistry. The first PA consisted of an endothelial cell adhesive ligand (YIGSR) coupled with a matrix metalloprotease-2 (MMP-2) degradable sequence (GTAGLIGQ) to form PA-YIGSR. The second PA was composed of the nitric oxide (NO) donor polylysine (KKKKK) linked to the MMP-2 degradable sequence, forming PA-KKKKK. These two distinct PAs were mixed in a 9:1 ratio (YIGSR:KKKKK) to form PA-YK, which was then reacted with NO to form PA-YK-NO, the endothelium-mimicking nanomatrix. Selfassembly of PA-YK-NO was achieved by a water evaporation method as described below.[24–28]

2.2 Rotational Coating Method for Stents.

A rotational coating technique to uniformly coat stents with PA-YK-NO through water evaporation-based self-assembly was performed as previously described.[25,26] First, the stents (generic 15 mm stainless steel stents, Pulse Systems, CA) were cut down the long axis and flattened, allowing them to be utilized within the parallel plate bioreactor chamber. Stents were then mounted on a rotating mandrel attached to a motor and immersed within PA-YK-NO solution contained in an open top reservoir for 12 h. The rotation ensured

uniform coating of the nanomatrix on all stent strut faces; the open top of the reservoir facilitated evaporation. After rotation in PA-YK-NO solution for 12 h, the stents were allowed to continue rotating out of solution to dry for a further 24 h. Stents were then washed twice with sterile DI water and UV sterilized for 2 h prior to all experiments.

2.3 Bioreactor Design.

The bioreactor was assembled as previously described.[25,26,29] Stents were cut down the long axis, flattened, either left uncoated or coated with PA-YK-NO as described above, and placed within the parallel plate flow chamber. The flow chamber was connected to a media reservoir and a peristaltic pump which perfused cell culture media at 10 dyn/cm^2, and placed within an incubator (37°C, 5% CO_2). The flow rate was determined based on physiological shear stress, which ranges from 5 to 10 dyn/cm^2 for arteries, and was calculated on the basis of the Navier–Stokes and continuity equations for the parallel plate flow chamber.[30,31] This assumed fully developed laminar flow of the media as an incompressible fluid throughout the parallel plate flow chamber.

2.4 Endothelialization.

To evaluate the ability of the stents to promote endothelialization, we performed a critical concern after stent deployment, an endothelialization assay by evaluation of endothelial cell migration onto the stents. Human aortic endothelial cells (HAECs; Lonza) were seeded on fibronectin-coated (50 μg/mL) glass slides at a cell density of 30 000/cm^2 and allowed to attach and grow for 24 h in order to create a stable confluent monolayer of endothelial cells (hereafter the “endothelium”). After the old media with any remaining unattached cells was aspirated, stents (uncoated or PA-YK-NO coated) were deployed on top of the endothelium, the bioreactor was assembled, and fresh media (EGM-2, Lonza) was perfused through the bioreactor at 10 dyn/cm^2. At 1, 4, and 7 days, stents were gently removed from the bioreactor, washed twice with warm sterile phosphate buffered saline (PBS), stained with Live/Dead reagent (calcein AM/ethidium homodimer-1) for 15 min in the dark, and imaged with a fluorescent microscope using Nikon NIS Elements imaging software (Melville, NY) to observe migration of HAECs form the endothelium onto the stents. The percent of the stent strut face area covered by cells was also calculated using ImageJ software (NIH).

2.5 Neointimal Hyperplasia.

The growth and proliferation of smooth muscle cells (“neointimal hyperplasia”) over stents after deployment is another key concern. Therefore, similar to the endothelialization assay, a neointimal hyperplasia assay was performed through evaluation of smooth muscle cell migration onto the stents. Human aortic smooth muscle cells (AoSMCs; Lonza) were seeded on fibronectin-coated (50 μg/mL) glass slides at a cell density of 30 000/cm^2 and allowed to attach and grow for 24 h in order to create a stable confluent layer of smooth muscle cells. After the old media with any unattached cells was aspirated, stents (uncoated or PA-YK-NO coated) were deployed on top of the smooth muscle cells, the bioreactor was assembled, and fresh media (SmGM-2, Lonza) was perfused through the bioreactor at 10 dyn/cm^2. At 1, 4, and 7 days, stents were gently removed from the bioreactor, washed twice with warm sterile PBS, stained with Live/Dead reagent for 15 min in the dark, and imaged with a fluorescent microscope using Nikon NIS Elements imaging software (Melville, NY) to observe

migration of AoSMCs onto the stents. The percent of the stent strut face area covered by cells was also calculated using ImageJ software (NIH).

2.6 Thrombosis.

Another crucial concern for stents after deployment is the adhesion and activation of platelets, which can lead to thrombosis (clots). Thus, a thrombosis assay to evaluate platelet adhesion was performed. Platelet rich plasma (PRP) was purchased from Innovative Research. Platelets were counted and diluted in Tyrode's solution to a concentration of 6×10^8 platelets/mL. Platelets were then perfused over PA-YK-NO coated and uncoated control stents at 10 dyn/cm^2. At 30 min and 24 h, stents were collected, gently washed three times with PBS to remove any unattached platelets, stained with calcein AM, and imaged with a fluorescent microscope using Nikon NIS Elements imaging software (Melville, NY) to observe platelet adhesion onto the stents. Percent area of stents covered with attached platelets was then calculated using ImageJ software (NIH).

2.7 Inflammation.

Inflammation is a further consideration to take into account after stent deployment. Nanomatrix coated stainless steel strips demonstrated excellent attenuation of inflammatory responses as previously reported.[25] Here, stents were utilized to better recapitulate clinical conditions. Stents were coated with PA-YK-NO or left uncoated as described above. U937 monocytes (1×10^6 cells/mL) labeled with CellTracker Green CMFDA (Life Technologies) were perfused over the stents at 10 dyn/cm^2 along with TNF-α (10 ng/mL) for 4 h to stimulate inflammatory conditions. After 4 h, stents were removed from the bioreactor and washed twice with warm sterile PBS to remove loosely bound monocytes. Monocyte adhesion was analyzed using Nikon NIS Elements imaging software (Melville, NY), and the percent of the stent strut face area covered by adherent monocytes was calculated using ImageJ (NIH).

2.8 Statistical Analysis.

All experiments were performed at least 3 times. All values are expressed as mean ± standard deviation. Statistical analysis was performed using one-way ANOVA with Tukey post-test using SPSS software with $p < 0.05$ considered significant.

3. RESULTS AND DISCUSSION

3.1 Peptide Amphiphile Nanomatrix Synthesis.

As previously described, the two PAs were successfully synthesized, mixed in a 9:1 ratio, reacted with NO gas, and allowed to self-assemble on the stents by the rotating water evaporation method.[24–28]

3.2 Nanomatrix-Coated Stent Increases Endothelialization.

Rapid re-endothelialization is a highly desired outcome after stents are deployed. The endothelium is a monolayer of endothelial cells along the lumen of the blood vessel that carries out a variety of critical functions, including as a semipermeable barrier, as a

nonthrombogenic surface to prevent unnecessary blood clots, and as a blood vessel modulator, constricting or dilating the blood vessel as needed through release of soluble factors such as nitric oxide (NO).[20,32,33] The endothelium inevitably suffers damage during percutaneous coronary intervention and stent deployment.[34,35] The reformation of the endothelium over stent struts after percutaneous coronary intervention is known to reduce adverse clinical events, such as restenosis, neointimal hyperplasia, inflammatory responses, platelet activation and adhesion, and thrombosis.[36–39]

Therefore, we investigated the pro-healing ability and reendothelialization capacity of the multifunctional nanomatrix coated stent under physiological shear stress in vitro. After the creation of the HAEC monolayer, PA-YK-NO nanomatrix coated or uncoated stents were placed within the bioreactor and exposed to physiological shear stresses of 10 dyn/cm^2 for 1, 4, and 7 days as described above. HAEC migration from the endothelium onto the stents is shown in Figure 2. After 1 day, only a few cells had started to migrate onto the PA-YK-NO coated stents; no cells had yet migrated onto the bare metal stents (BMS; data not shown). At day 4, however, significant differences emerged: a few HAECs began migrating onto the BMS (Figure 2A, B), but many HAECs migrated onto and covered the sides of the PA-YK-NO coated stent struts; some cells even began to migrate over and on top of the struts (Figure 2C, D), resulting in a significant difference in the percent of stent area covered by cells (Figure 2E; BMS = 1.28 ± 0.22%, PA-YK-NO = 23.92 ± 1.92%; $p < 0.001$). By day 7, the difference in endothelialization became more pronounced: although more cells had migrated onto the BMS strut sides (Figure 2F, G), the PA-YK-NO coated stents displayed almost complete HAEC coverage (Figure 2H, I), yielding a significant difference in the percent of stent area covered by cells (Figure 2J; BMS = 8.59 ± 1.56%, PA-YK-NO = 86.14 ± 6.67%; $p < 0.001$). These results corroborate previous studies showing the effect of NO which is known to encourage endothelial cell proliferation and migration and can recruit endothelial cells from surrounding tissues after stent deployment, as well as the endothelial cell adhesive ligand YIGSR, which provides attachment sites for endothelial cells on the stent, enhancing re-endothelialization.[24,26,27,40,41] These results demonstrate the pro-healing ability and endothelialization capability of the PA-YK-NO nanomatrix-coated stent under physiological flow, indicating its potential for rapid re-endothelialization.

3.3. Nanomatrix-Coated Stent Reduces Smooth Muscle Cell Migration.

A major adverse clinical event that occurs after stent deployment is neointimal hyperplasia, the proliferation and migration of smooth muscle cells over the stents, which leads to restenosis.[4,5] Hence, a key consideration in stent design is to slow or halt the neointimal response after percutaneous coronary intervention. NO is known to reduce and slow smooth muscle cell proliferation and can limit neointimal hyperplasia and restenosis.[21,42] Thus, we next investigated whether the PA-YK-NO nanomatrix coated stent could reduce smooth muscle cell (AoSMC) growth and migration onto the stent under physiological flow conditions in vitro.

Once the AoSMC layer had been created, nanomatrix coated and uncoated stents were placed within the bioreactor and exposed to 10 dyn/cm^2 shear stress flow for 1, 4, and 7 days as described above; AoSMC migration results are depicted in Figure 3. After 1 day, there

were no cells present on either the BMS control or the PA-YK-NO coated stents (data not shown). At day 4, a few cells began migrating onto the BMS (Figure 3A), whereas still no cells migrated onto the nanomatrix-coated stents (Figure 3B); the percent of stent area covered by cells is shown in Figure 3C (BMS = 1.05 ± 0.36%, PA-YK-NO = 0.0%, $p < 0.001$). Day 7 resulted in more AoSMC migration and percent of stent area covered by cells for the BMS control (Figure 3D, E, H); the PA-YK-NO coated stent, however, still displayed no AoSMC migration or attachment on the stents (Figure 3F, G, H; BMS = 8.84 ± 0.96%, PA-YK-NO = 0.0%, $p < 0.001$). These significant differences indicate that the NO released from the nanomatrix discourages AoSMC migration and adhesion onto the stents while the BMS controls do not have any such bioactivity; these results are also consistent with previous studies, demonstrating the ability of NO to reduce smooth muscle cell growth and proliferation and keep them quiescent.[24,43] Mechanistically, it has been shown that NO acts largely through the cGMP pathway, inhibiting smooth muscle cell proliferation and migration.[44,45] Thus, the PA-YK-NO nanomatrix coated stents may reduce neointimal hyperplasia, and consequently prevent or greatly mitigate restenosis after stent deployment.

3.4 Nanomatrix-Coated Stent Reduces Platelet Adhesion.

Another significant adverse clinical event with potentially disastrous consequences is thrombosis, the formation of a clot over the stent struts, which can lead to restenosis or may break away leading to an embolism.[46,47] NO is known to be a potent antithrombogenic molecule which can prevent platelet activation and adhesion.[21,48] As such, we tested the potential antithrombogenicity of the PA-YK-NO nanomatrix coated stent under physiological flow in vitro with a platelet adhesion assay as described above.

PA-YK-NO-coated and uncoated BMS were deployed within the bioreactor as described above while platelets were perfused for 30 min and 24 h. Within 30 min of perfusion, a significant amount of platelets adhered to BMS controls; moreover, fibrin strands began to form, localized especially around stent bends and joints (Figure 4A, B, E). The PA-YK-NO coated stents, on the other hand, displayed no fibrin strands and only very few platelets attached to the stents; these were also largely limited to the strut bends (Figure 4C–E). This resulted in 23.54 ± 4.21% stent area covered for the BMS control, compared to only 1.09 ± 0.37% area covered for the nanomatrix coated stents (Figure 4E, $p < 0.001$). By 24 h, the fibrin strands on the BMS controls had coagulated to form mature clots which covered 47.51 ± 6.12% of the BMS stent area (Figure 4F, G, J). In sharp contrast, the PA-YK-NO nanomatrix coated stents still displayed few adherent platelets, absence of fibrin strands or clots, and only 2.51 ± 0.72% stent area covered (Figure 4H, I, J; $p < 0.001$). This dramatic reduction in platelet adhesion, fibrin strand formation, and coagulation highlights the antithrombogenicity of the PA-YK-NO coating; this is likely largely due to the released NO from the nanomatrix, which is known to be a considerable antithrombogenic molecule that prevents platelet activation, adhesion, and coagulation.[21,48,49] Similar to smooth muscle cells, NO acts on platelets largely through the cGMP pathway, reducing platelet aggregation and adhesion.[50,51] Therefore, the PA-YK-NO-coated stents may reduce thrombosis, a significant issue after percutaneous coronary intervention.

3.5 Nanomatrix-Coated Stent Reduces TNF-α-Activated Monocyte Adhesion.

Finally, a third adverse clinical event that occurs after stent deployment, and especially following DES use in response to the polymer coating, is inflammation.[52,53] Prevention or reduction of inflammation is a key element of biocompatible implant design, especially for cardiovascular devices such as stents that are exposed to constant blood flow and immune surveillance. Our previous study examined the anti-inflammatory potential and effects of our PA-YK-NO nanomatrix coating on stainless steel strips in detail; therefore, in this study, we evaluated the nanomatrix coating on actual stents, utilizing monocytes stimulated by TNF-α.[25]

BMS control and PA-YK-NO coated stents were deployed within the bioreactor as described above; monocytes along with inflammatory TNF-α were then perfused for 4 h. After 4 h, TNF-α activated monocyte aggregates formed in the BMS controls; these were concentrated in the strut bends, resulting in 19.01 ± 3.97% of the stent area covered (Figure 5A, B, E). PA-YK-NO-coated stents had only a few monocytes attached; similar to the platelet experiments, these were localized to the strut bends, and only 0.21 ± 0.07% of the nanomatrix coated stent area was covered (Figure 5C, D, E; $p < 0.001$). Thus, the nanomatrix coated stents could prevent adhesion of TNF-α activated monocytes, a major component of the acute inflammatory phase that occurs after stent deployment. These results demonstrate the efficacy of the nanomatrix coating applied to stents; coupled with our previous results, they indicate that the PA-YK-NO nanomatrix coating is anti-inflammatory, thus addressing a critical component of biocompatibility for cardiovascular stents.[25]

Taken together, these studies demonstrate the potential clinical utility of this pro-healing nanomatrix coating for stents: rapid re-endothelialization, reduction of smooth muscle cell migration, and decrease in platelet and inflammatory cell adhesion. These results also help explain the efficacy of the nanomatrix coated stent we observed in our previous preliminary in vivo rabbit iliac artery study, in which the nanomatrix-coated stent displayed reduced neointimal hyperplasia, reduced restenosis, and no evidence of thrombosis or fibrin deposition after 4 weeks.[26] Thus, these results could be translatable to in vivo settings.

In light of these promising results, we expect the following course of events after PA-YK-NO nanomatrix-coated stent implantation in vivo: (1) Within the first few hours following stent implantation, NO released from the biocompatible nanomatrix will prevent both platelet activation and adhesion (i.e., thrombosis) as well as immediate inflammatory responses to the stent.[24,25] (2) During the first few days, the pro-healing nanomatrix will continuously release NO, which will maintain the antithrombogenic and anti-inflammatory effects; simultaneously, the NO and YIGSR cell adhesive ligands will encourage the healing process: recruitment and proliferation of surrounding endothelial cells over the stent surface, adhesion, and differentiation of endothelial progenitor cells to aid in the reformation of the endothelium, and quiescence of smooth muscle cells (i.e., reduce and prevent restenosis).[24–28] (3) As the endothelial cells migrate and proliferate over the nanomatrix coated stent, they will reform the complete endothelium, begin to degrade the nanomatrix, and replace it with native extracellular matrix. (4) Once the healing process is complete, the biocompatible pro-healing nanomatrix will be completely replaced by the mature endothelium, resulting in a normal, functional vessel lumen once more. This will prevent neointimal hyperplasia,

restenosis, and thrombosis, and therefore also reduce the need for repeat percutaneous coronary interventions in the future. Thus, the pro-healing multifunctional nanomatrix-coated stent can address the current challenges of stents; prior to clinical evaluation, we will perform large-scale in vivo studies within appropriate animal models.

4. CONCLUSION

In this study, we evaluated the nanomatrix-coated stent under physiological flow conditions in vitro for re-endothelialization through an endothelial cell migration assay, prevention of neointimal hyperplasia through a smooth muscle cell migration assay, antithrombogenicity by platelet adhesion, and antiinflammatory effects by monocyte adhesion with TNF-α (Figure 1). These four elements (re-endothelialization, prevention of neointimal hyperplasia, reduction of thrombosis, and reduction of inflammation) are of paramount importance in cardiovascular stent design, given the shortcomings of both BMS and DES.[5,9,46,54] In light of this, we investigated a novel, pro-healing, peptide amphiphile-based nanomatrix stent coating that can simultaneously address all of these considerations, thereby offering a multifunctional platform that is both biocompatible and bioactive. The nanomatrix-coated stent promoted endothelial cell migration, adhesion, and proliferation, resulting in over 86% stent area coverage by endothelial cells after only 7 days, which indicates its potential for re-endothelialization after stent deployment. Furthermore, it discouraged smooth muscle cell migration and significantly reduced both platelet and TNF-α activated monocyte adhesion, all desirable effects to prevent restenosis, thrombosis, and inflammation. In combination with our previous studies, these promising results suggest that the nanomatrix can offer a multifaceted solution to the drawbacks of current BMS and DES; these in vitro physiological shear stress experiments are an excellent precursor to future in vivo studies within a suitable animal model.

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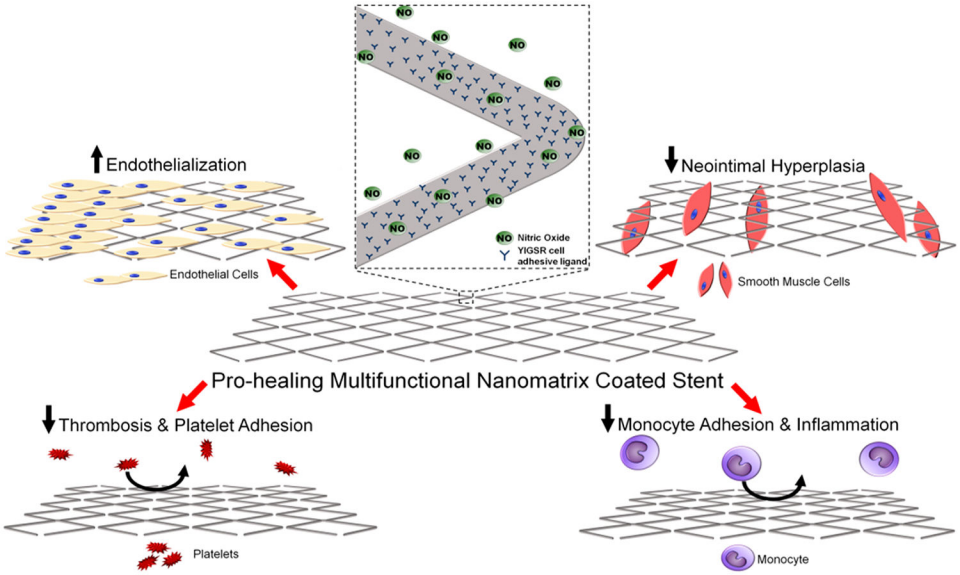

Figure 1.

Pro-healing multifunctional nanomatrix coated stent can enhance endothelialization, suppress neointimal hyperplasia, and reduce or prevent thrombosis and inflammation.

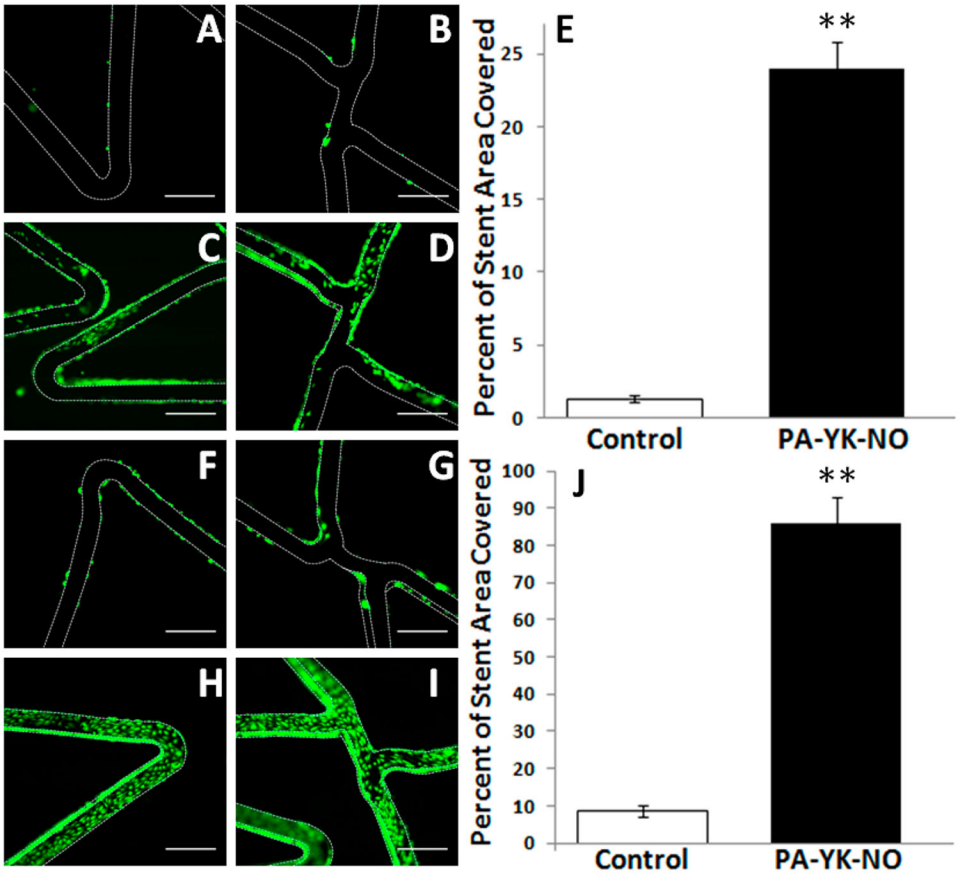

Figure 2.

HAEC migration on (A, B) BMS Control and (C, D) PA-YK-NO coated stents at 4 days. (E) Percent stent area covered by HAECs at 4 days. HAEC migration on (F, G) BMS Control and (H, I) PA-YK-NO coated stents at 7 days. (J) Percent stent area covered by HAECs at 7 days. Scale bar = 300 μm. Bars show mean ± standard deviation. **$p < 0.001$.

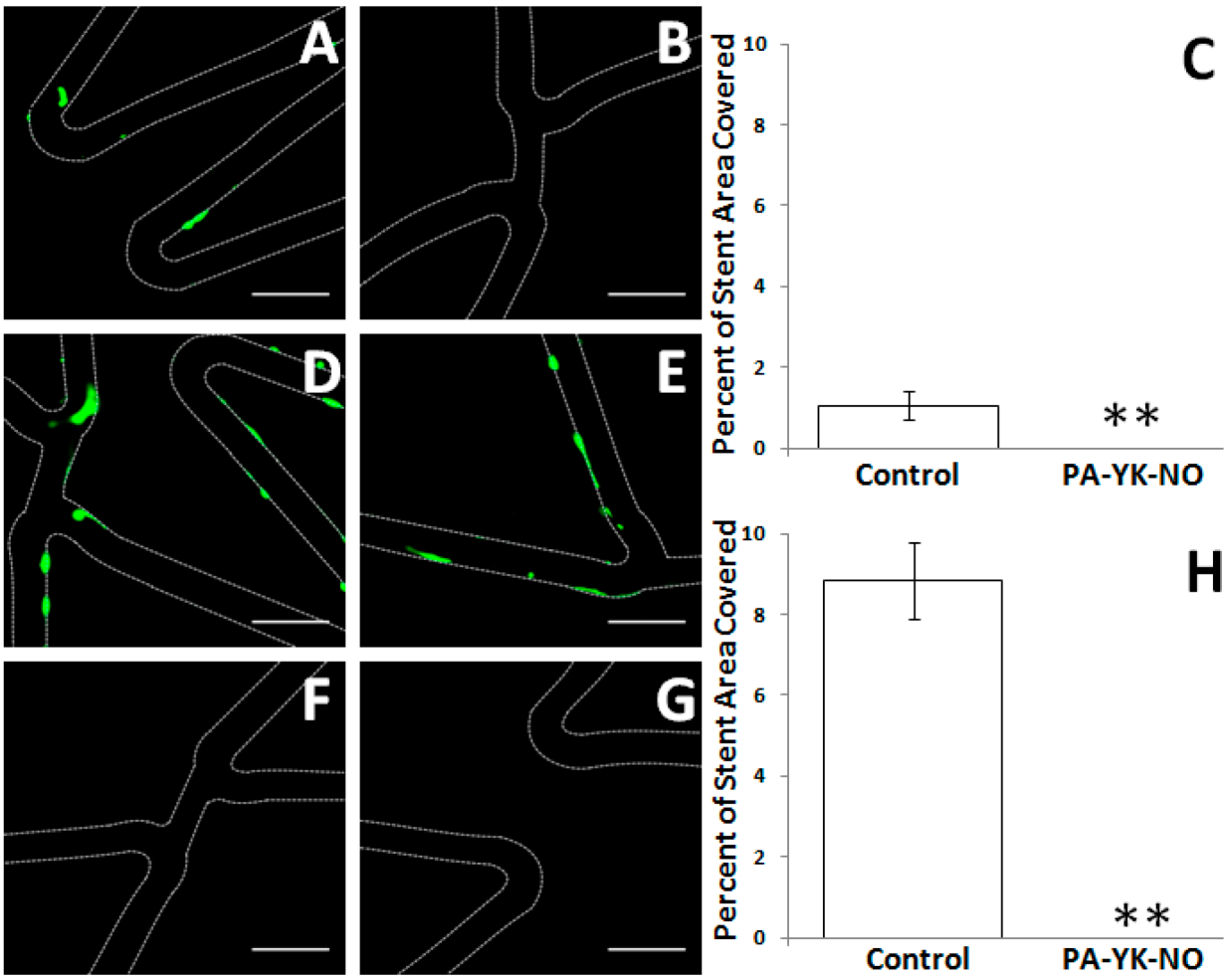

Figure 3.

AoSMC migration on (A) BMS Control and (B) PA-YK-NO coated stent at 4 days. (C) Percent stent area covered by AoSMCs at 4 days. AoSMC migration on (D, E) BMS Control and (F, G) PA-YK-NO coated stent at 7 days. H) Percent stent area covered by AoSMCs at 7 days. Scale bar = 300 μm. Bars show mean ± standard deviation. $^{**}p < 0.001$.

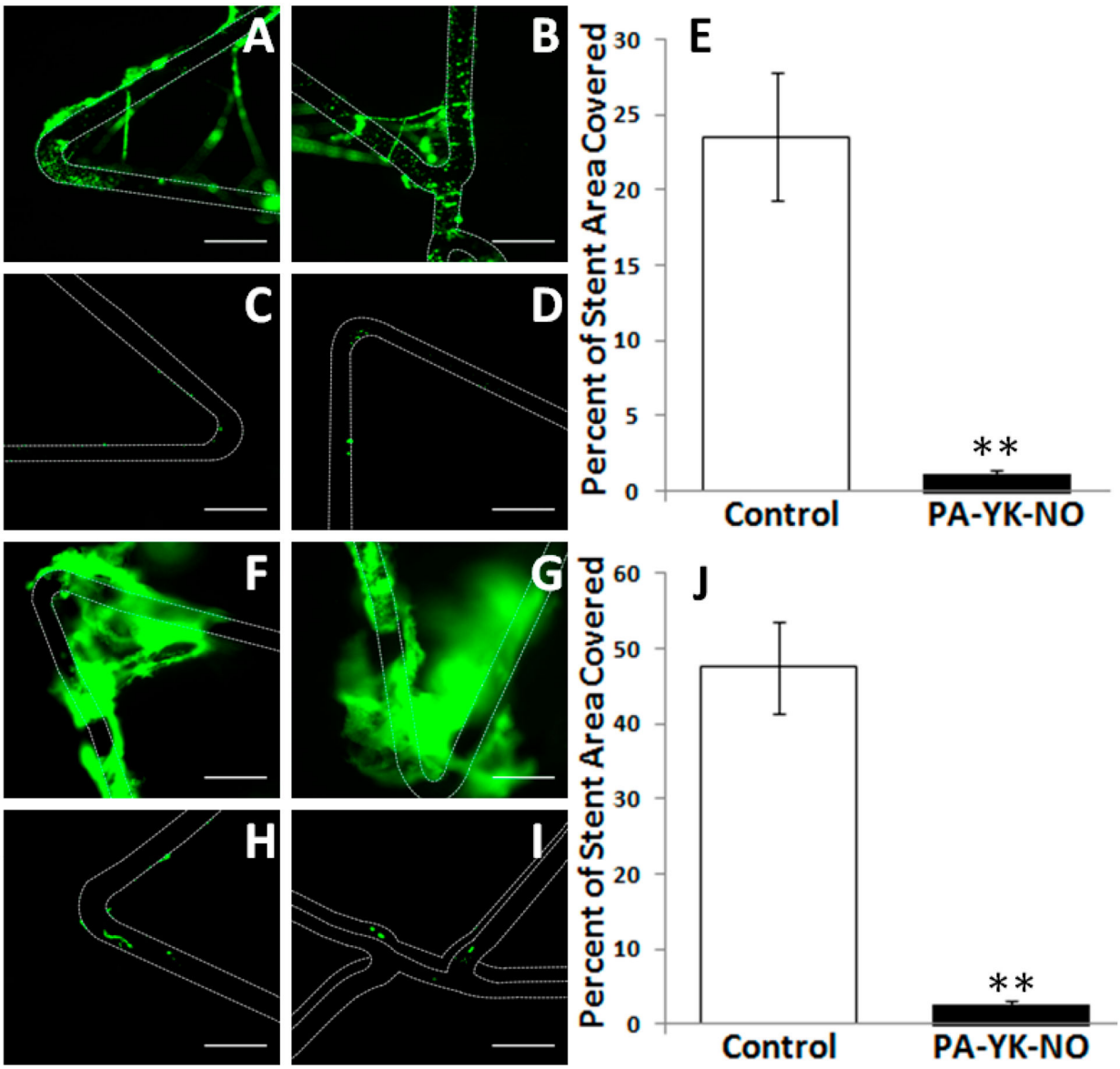

Figure 4.

Platelet adhesion on (A, B) BMS Control and (C, D) PA-YK-NO coated stent at 30 min. (E) Percent stent area covered by platelets at 30 min. Platelet adhesion on (F, G) BMS Control and (H, I) PA-YK-NO coated stent at 24 h. (J) Percent stent area covered by platelets at 24 h. Scale bar = 300 μm. Bars show mean ± standard deviation. **$p < 0.001$.

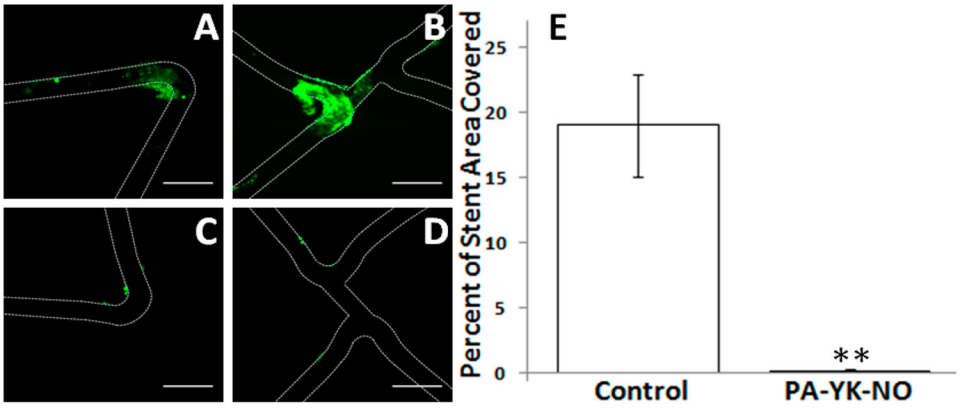

Figure 5.

Monocyte adhesion with TNF-α stimulation on (A, B) BMS Control and (C, D) PA-YK-NO coated stent at 4 h. (E) Percent stent area covered by monocytes at 4 h. Scale bar = 300 μm. Bars show mean ± standard deviation. **$p < 0.001$.