Published in final edited form as:

ASAIO J. 2015 ; 61(5): 589–595. doi:10.1097/MAT.0000000000000257.

A nitric oxide releasing self-assembled peptide amphiphile nanomatrix for improving the biocompatibility of microporous hollow fibers

George T El-Ferzli^{*,†}, Adinarayana Andukuri[&], Grant Alexander[&], Michaela Scopel[†], Namasivayam Ambalavanan^{*}, Rakesh P Patel[†], and Ho-Wook Jun[&]

^{*}Department of Pediatrics, University of Alabama at Birmingham, Birmingham, AL, 35205 (USA)

[†]Center for Free Radical Biology, University of Alabama at Birmingham, Birmingham, AL, 35205 (USA)

[&]Department of Biomedical Engineering, University of Alabama at Birmingham, Birmingham, AL, 35205 (USA)

Abstract

Oxygenators are critical components of extracorporeal circuits used frequently in cardiopulmonary bypass and intensive care, but platelet activation and induction of a complex inflammatory response are usually observed with their use. To improve the biocompatibility of oxygenators, we developed a nitric oxide (NO)-releasing, self-assembled peptide amphiphile nanomatrix. The nanomatrix formed a homogenous coating over the microporous hollow fibers as demonstrated by scanning electron microscopy. We quantitated platelet adhesion to the artificial fibers by measuring absorbance/area of platelets (Abs/A; nm/m²) using acid phosphatase assay. There was a 17-fold decrease in platelet adhesion to the nanomatrix (Abs/A = 0.125) compared to collagen controls (Abs/A = 2.07; $p < 0.05$) and a 22-fold decrease compared to uncoated fibers (Abs/A = 2.75; $p < 0.05$). Importantly, the nanomatrix coating did not impede oxygen transfer in water through coated and uncoated fiber modules ($p > 0.05$) in a bench top test circuit at different flow rates as estimated by change in partial pressure of oxygen in relation to water velocity through fibers. These findings demonstrate the feasibility of coating microporous hollow fibers with a NO-releasing, self-assembled amphiphile nanomatrix that may improve the biocompatibility of the hollow fibers without affecting their gas exchange capacity.

Keywords

nitric oxide; amphiphile; nanomatrix; biocompatibility; oxygenator; hollow fibers

Corresponding author: George T El-Ferzli, M.D., Division of Neonatology, Department of Pediatrics, Women and Infants Center, University of Alabama at Birmingham, 1700 6th Avenue South, Suite 9380, Birmingham, Alabama 35249 (gferzli@peds.uab.edu).

Conflicts of Interest: The authors declare no conflict of interest

Introduction

Extracorporeal circuits (ECCs) are critical to a number of medical procedures including hemodialysis, heart-lung bypass, and extracorporeal membrane oxygenation (ECMO).^{1–4} When blood is exposed to the material of the ECC, platelet activation and adhesion are immediately initiated leading to blood clot formation.^{5–7} In addition, a systemic inflammatory response syndrome (SIRS) is nearly always present during initiation of extracorporeal life support (ECLS). A crucial factor in triggering the inflammatory response to ECLS is exposure of blood to nonphysiologic surfaces and conditions.

Elimination of the need for systemic anticoagulation and SIRS are therefore desirable in ECLS. Different approaches have been taken to develop ECC materials that are more biocompatible. One approach is to develop materials that release therapeutics, such as heparin, at the site of blood contact therefore controlling the initial bioresponse of the material.⁸ Another approach is the use of coating materials that mimic endothelial cells. In the normal blood vessel, the endothelial lining provides a thromboresistive surface by releasing molecules such as nitric oxide (NO).^{9,10} NO serves as an anti-platelet agent to limit coagulation, reduces adsorption of blood clotting proteins to the material surface, and prevents infection and inflammation.^{8,11–14} Previous *in vitro* studies have employed polymeric coatings of artificial surfaces that slowly release NO to mimic endothelial NO-formation. However the major drawback of these systems was the prevalence of significant donor leaching from the material into the soaking solution.^{8,14–19} Despite attempts to coat the ECC with NO releasing materials, there have been limited attempts to coat the oxygenator which is an essential and integral element of the circuit. We have previously developed NO-releasing biomimetic nanomatrix comprising poly lysine peptide amphiphiles (PAs). The nanomatrix has been shown to slowly release NO and mediate NO-dependent signaling in endothelial cells, smooth muscle cells, and endothelial progenitor cells, in addition to limiting platelet adhesion.²⁰ In this study, we tested the feasibility of coating microporous hollow fibers with an NO-releasing biomimetic nanomatrix and determined if this could attenuate platelet adhesion without inhibiting gas exchange.

Materials and Methods

Development of the biomimetic nanomatrix

Two PAs were developed: PA-YIGSR [$\text{CH}_3(\text{CH}_2)_{14}\text{CONH-GTAGLIGQ-YIGSR}$] and PA-KKKKK [$\text{CH}_3(\text{CH}_2)_{14}\text{CONH-GTAGLIGQ-KKKKK}$]. The peptides were synthesized in solid phase using Fmoc chemistry in an Apptech Apex 396 Peptide synthesizer as previously described.²⁰ The PAs contain enzyme-mediated degradable MMP-2 sensitive sequences along with YIGSR or a polylysine (KKKKK) group to form NO donating residues. PA-YKs were designed by mixing 90:10 mol/mol ratios of PA-YIGSR and PA-KKKKK by a water evaporation method. To produce NO-releasing PAs from the biomimetic nanomatrix, PA-YK was reacted with excess NO gas at room temperature for 12 hours, at 5 atm pressure, under anaerobic conditions to form PA-YK-NO and allowed to form a self-assembled coating by water evaporation, which was characterized as previously done.²⁰

Fiber coating

A sample from a Celgard polypropylene hollow fiber mat was cut to $2.5\text{ cm} \times 1.2\text{ cm}$, mounted by running a steel wire through the pores at the edge of the fibers at two different sites. The steel wire was rotated by a motor as the fibers were immersed in PA solution in an open top container. After coating for 12 hours, the fibers were dried for a further 12 hours at room temperature.

Scanning electron microscopy (SEM)

Morphology of the nanomatrix coated on the hollow fibers was observed under a Philips SEM 510 at an accelerating voltage of 20 kV at 3 different magnifications (100 $\times$, 400 $\times$, and 30,000 $\times$). As a control, uncoated fibers were similarly characterized by SEM.

Test Modules

Test modules were constructed from both coated and uncoated control samples of Celgard polypropylene hollow fiber mat ($n=5$ modules / group). Each sample was rolled into a bundle, and each end was then potted into male luer connectors using polyurethane potting compound (WC-753, BJB Enterprises, Tustin, CA). The module was then inserted into the test circuit by inserting each end into a female luer.

Test Circuit

A test circuit (Fig. 1) was constructed with the test module, connectors and tubing. The test module was connected to a syringe pump (New Era Pump System, NE1000) for precise control of water flow through the device. The outlet of the test module was connected to a syringe for sampling. The test module was attached to $3/8''$ connector, which was connected to gas flow through a $3/8''$ Y connector to create a 100% oxygen (O_2) environment. The gas circuit consisted of a 100% O_2 tank with a pressure regulator and several gas flow controller components. Positioned distal to the tank, a mass flow controller (MFC) valve (Sierra Instruments Side-Trak 840-M), rated for 20 standard liters per minute (SLM) with air was installed. The valve was connected via 20-lead ribbon cable to a custom-built MFC Power and Control Unit, which contains a power entry module, a $\pm 15\text{VDC}$ power, as well as valve off and valve purge switches. The mass flow controller valve flow setting was controlled and visualized by a custom Lab VIEW code, and with use of a National Instruments data acquisition (DAQ) hardware (NI cDAQ-9172 chassis and 4 modules: two NI9219 analog input modules, NI9237 bridge module and NI 9263 analog output module). The gas flow units were SLM (standard conditions defined to be at 21°C and 760 mm Hg; 70 F and 1 atm). The gas flow rate was set at 2 LPM.

Circuit Preparation, samples and gas transport measurement

The syringe pump was filled with 5 ml of distilled water. The O_2 gas was opened and the test apparatus was filled with 100% O_2 . A 1 ml syringe was attached to the outlet of the test module for sample collection. Flow through the syringe pump was initiated. The outlet water was collected in the syringe and immediately analyzed on the blood gas machine. Capillary flow (m/s), partial pressure of O_2 ($p\text{O}_2$; mm Hg), change in $p\text{O}_2$ ($\Delta p\text{O}_2$; mm Hg), change in $p\text{O}_2$ per surface area of each module ($\Delta p\text{O}_2/\text{SA}$; mm Hg/ m^2) were measured at baseline and

at water flow rates of 0.1, 0.5 and 1 LPM. All data were recorded manually on designated data sheets. The results from the gas measurements were recorded directly on the individual data sheets and then transcribed on to an Excel spreadsheet for analysis of O₂ transfer. The sequence was repeated for each device. Data was collected from three independent experiments on each test module (n=5 test modules/group).

Nitrite and nitrate formation sample collection and measurement

PA-YK-NO coated and uncoated 1 cm × 1 cm hollow fiber mats (n = 4/group) were placed individually into a 24 well plate. The mats were covered with 500 µL PBS and incubated at 37° C. At 0, 4, 8, 12 hours, 1 day, 3 days, 5 days, and 7 days, the PBS was removed from each well and frozen in liquid nitrogen. 500 µL fresh PBS was added to the wells after each time point. For analysis, nitrite and nitrate levels were measured by the Griess assay coupled with HPLC detection using the ENO-20 (EiCOM) as previously described.²¹ Nitrite and nitrate concentrations were determined by comparison to the nitrite and nitrate standard curves.

Platelet adhesion

Platelet adhesion on the hollow fibers was evaluated by assaying for acid phosphatase²² by incubation of platelet rich plasma (PRP) on uncoated, and PA-YK-NO-coated hollow fibers. PA-YK and PA-YK-NO nanofibrous matrix coatings were prepared on Celgard polypropylene hollow fiber surface (1×1 cm²). A solution of 2.5 mg/ml collagen I was prepared in 3% glacial acetic acid to serve as a positive control and cast into films. Whole blood from a healthy volunteer was collected in BD Vacutainer® Heparin Tubes (BD, NJ) containing 1 ml citrate buffer. This blood was centrifuged at 200g for 20 minutes. The supernatant which contains platelets was collected used for the experiments. 1 cm² PA-YK, PA-YK-NO, collagen films, and uncoated hollow fibers were incubated individually with 500 µl supernatant at 37°C for 90 minutes and then rinsed with PBS. Platelet adhesion was evaluated by incubating with acid phosphatase substrate (5 uM pNitrophenylphosphate, 0.1% Triton X) for one hour. The reaction was stopped and color was developed by adding 2M NaOH, and absorbance was read at 410 nm. The protocol used for collecting blood from volunteer was approved by the Institutional Review Board.

Statistical analysis

All experiments were performed at least three independent times. All data were evaluated using one-way ANOVA to evaluate statistical significance between groups using SPSS software. If significant differences were noted by ANOVA, Tukey multiple comparisons test was performed to find significant differences between pairs. A value of $p < 0.05$ was considered to be statistically significant.

Results

Development of the biomimetic matrix and coating of hollow fibers

We first tested the feasibility of coating microporous hollow fibers with PA's. Two PAs, PA-YIGSR and PA-KKKKK were successfully synthesized and mixed at 90:10 mol/mol ratios of PA-YIGSR to PA-KKKKK to form PA-YK which was subsequently reacted with

NO gas to form PA-YK-NO. The PA-YK-NO self-assembled on the surface of the hollow fibers forming a nanomatrix which was evaluated by SEM. SEM at 100× and 400× (Fig. 2a) showed the uniform coating of the microporous fibers with the self-assembling nanomatrix compared to uncoated fibers, while higher magnification SEM (Fig. 2b) showed that the PAs assembled into a porous nanomatrix on the surface of individual fibers.

Gas exchange

To evaluate the effect of the coating on gas transfer across the microporous hollow fibers, we compared change of pO_2 across fibers between coated and uncoated fiber modules in a test circuit. Change of pO_2 was slightly higher, although not statistically significant, across the coated fibers compared to the uncoated fibers at different water velocities ($y = -14.9x + 477$ for coated fiber modules versus $y = -11.3x + 500$ for uncoated fiber modules; $p > 0.05$) (Fig. 3).

Nitrite and nitrate formation

Nitrite and nitrate were measured as an index of NO-release. As previously observed, nitrite and nitrate formation were characterized by an initial burst in the first 24 hours followed by sustained release thereafter. Nitrite and nitrate release from the PA-YK-NO coated fibers was significantly higher than that measured from uncoated fibers ($p < 0.005$) (Fig. 4a and 4b).

Platelet adhesion

There was no significant difference in platelet adhesion to uncoated fibers compared to collagen controls ($Abs/A = 2.75 \text{ nm/m}^2$ versus $Abs/A = 2.07 \text{ nm/m}^2$, respectively; $p > 0.05$), while coating the fibers with PA-YKNO reduced platelet adhesion by 17-fold ($Abs/A = 0.125 \text{ nm/m}^2$) compared to the collagen controls ($p < 0.05$) and 22-fold compared to uncoated fibers ($p < 0.05$) (Fig. 5).

Discussion

The overall hypothesis of this study was that coating the microporous hollow fibers with a NO-releasing biomimetic nanomatrix would improve the biocompatibility of the hollow fibers by reducing platelet adhesion without affecting their gas exchange capability. Endothelial cells are known to produce over 12 different molecules that affect platelet function, the coagulation cascade, or both processes. The major inhibitors of platelet function are NO, prostacyclin, and matrix metalloproteinases (MMPs).⁵ This inhibition is transient, so that the platelets resume normal function once they are no longer exposed to these inhibitors. Of these inhibitors, NO has been the agent most often studied for incorporation into polymers, due to its very short half-life, its ability to be present in both liquid and gas states, while it can be incorporated into the sweep gas of gas exchange devices if the biocompatible surface cannot be applied to the surface of these devices without jeopardizing their function.^{5,15}

Prior work by other investigators has mainly focused on material synthesis optimization methods for controlled and sustained NO concentration at the blood/polymer interface. The

artificial surfaces were made biocompatible either by entrapping NO donors within their bulk or incorporating catalysts to generate NO from NO-donors. Using the first method to improve biocompatibility of pediatric catheters was limited by short NO release duration while the second method has not been applied to large surface-area devices such as oxygenators.^{23–27}

Previously, we showed that NO releasing polyurethane had great potential for vascular grafts and stents.^{28–31} However, the use of these materials has so far been limited due to (1) the use of organic solvents causing loss of NO during the reaction and coating processes, (2) inflammation from residual synthetic polymers, (3) difficulty in providing long-term sustained release of NO due to limited diffusion through materials, and (4) delayed or limited endothelialization without endothelial cell binding moieties. To overcome these problems, we developed a NO-releasing biomimetic nanomatrix by utilizing a bottom-up method to achieve unique synergistic effects from multiple bioactive functions.^{32,33} The biomimetic nanomatrix has unique features including composition of an exclusively biocompatible peptide-based material that may reduce concerns regarding inflammatory responses and development of a self-assembled coating on the hollow fibers by a water evaporation method without organic solvents. It significantly differs from other NO releasing materials as NO bound to lysine (K) peptides is entrapped within self-assembled PA-KKKKK nanofibers with uniform diameter between 7–8 nm and several microns in length. Therefore, NO can be released by multistage kinetics such as from the top-layered surface of the nanomatrix coating, by diffusion through the several hundred layers of the nanomatrix coating, and from inside each PA-KKKKK nanofiber. These characteristics permit long and sustained release of NO from the biomimetic nanomatrix.^{20,34}

We previously demonstrated that successful NO release occurred as a burst in the first 48 hours, followed by a slower sustained release over a period of 30 days, resulting in recovery of 90.8% of available NO. The initial burst release is possibly explained by NO release from the surface of PA-YK-NO nanomatrix. The subsequent sustained slower release may be attributed to NO release from the inside of each nanofiber and the bulk of the nanomatrix by diffusion.³² This trend was confirmed in our current study as we showed that there was a sustained formation of nitrite and nitrate over 7 days after an initial burst during the first 24 hours (Fig. 4a and 4b).

In our previous studies, we calculated that the rate of NO release was of the same order of magnitude as cumulative NO released by endothelial cells at a rate of $1 \times 10^{-10} \text{ mol cm}^{-2} \text{ min}^{-1}$. This flux demonstrates the ability to functionalize polymers to release NO at levels comparable to that produced by the endothelium ($0.4 - 5 \times 10^{-10} \text{ mol cm}^{-2} \text{ min}^{-1}$).^{8,15,35–38} In our current study, we showed that there was significant decrease in platelet adhesion in the group of fibers coated with the biomimetic nanomatrix compared to the uncoated fibers or collagen controls. These results are in accordance with the studies that showed that surfaces releasing $1 \times 10^{-10} \text{ mol cm}^{-2} \text{ min}^{-1}$ of NO showed a significant inhibition of clot formation compared to non-NO releasing controls in *in vivo* animal studies^{7,15,39} although other studies showed that platelet consumption was proportional to NO release in the absence of systemic anticoagulation with optimal level of NO needed to preserve platelet consumption was $13.7 \times 10^{-10} \text{ mol cm}^{-2} \text{ min}^{-1}$ in their animal model.⁵

While decreases in platelet adhesion and thrombus formation in the presence of NO-releasing polymeric coatings *in vitro* and *in vivo* have been observed in several studies, the leaching of potentially harmful amine byproducts from these coatings has also been observed.^{8,15} The NO-producing materials developed by our group contain covalently bound NO donors which are derivatives of natural amino acids, and have been shown to decrease platelet adhesion. We have shown previously that the addition of an NO-releasing peptide into the main chain of a polyurethane can improve thromboresistance while retaining the mechanical properties of commercially available vascular graft materials.³⁹ Similarly, we have demonstrated that the PA-YK-NO was stable under physiologic conditions with very little flaking or cracking, even when compared to commercial medical device coatings.⁴⁰ In this study, we showed that the biomimetic nanomatrix did not affect the gas exchange properties of the microporous hollow fibers in bench-top studies.

Conclusion

Taken together, we have demonstrated the feasibility of coating microporous hollow fibers with a self-assembling NO-releasing biomimetic nanomatrix using utilizing a bottom-up methodology. The coating did not affect the gas exchange efficacy of the fibers while it significantly reduced platelet adhesion. Future studies evaluating the effect of shear stress on the release of NO and the stability of the coating as well as the effect of NO on the multitude of biological systems are warranted.

Acknowledgments

This project was supported by the Center for Clinical and Translational Science, the Department of Biomedical Engineering, and the Center for Free Radical Biology at the University of Alabama at Birmingham, and funded by UL1 TR000165, National Science Foundation Career Award (CBET-0952974), National Institute of Health (1R01HL125391 and 1R03EB017344-01), and in part by a grant to the University of Alabama at Birmingham from the Howard Hughes Medical Institute through the Med into Grad Initiative.

We thank MC3 Corp (Ann Arbor, MI, USA) for providing us with the hollow fibers and assisting in the bench top experiments and data analysis.

References

1. Kumar VA, Ledezma ML, Rasgon SA. Daily home hemodialysis at a health maintenance organization: three-year experience. *Hemodial Int.* 2007; 11:225–230. [PubMed: 17403175]
2. Lowry AW, Morales DL, Graves DE, et al. Characterization of Extracorporeal Membrane Oxygenation for Pediatric Cardiac Arrest in the United States: Analysis of the Kids' Inpatient Database. *Pediatr Cardiol.* 2013; 34:1422–1430. [PubMed: 23503928]
3. Lin YJ, Chung SY, Liang CD, et al. Impact of extracorporeal membrane oxygenation support on clinical outcome of pediatric patients with acute cardiopulmonary failure: a single-center experience. *Biomed J.* 2013; 36:28–34. [PubMed: 23515152]
4. Bréchet N, Luyt CE, Schmidt M, et al. Venoarterial extracorporeal membrane oxygenation support for refractory cardiovascular dysfunction during severe bacterial septic shock. *Crit Care Med.* 2013; 41:1616–1626. [PubMed: 23563585]
5. Reynolds MR, Annich GM. The artificial endothelium. *Organogenesis.* 2011; 7:42–49. [PubMed: 21289481]
6. Sefton MV, Gemmell CH, Gorbet MB. What really is blood compatible? *J Biomater Sci Polymer Edn.* 2000; 11:1165–1182.

7. Zhang H, Annich GM, Miskulin J, et al. Nitric oxide releasing silicone rubbers with improved blood compatibility: preparation, characterization, and in vivo evaluation. *Biomaterials*. 2002; 23:1485–1494. [PubMed: 11829445]
8. Joslin JM, Lantvit SM, Reynolds NN. Nitric oxide releasing tygon materials: studies in donor leaching and localized nitric oxide release at a polymer-buffer interface. *ACS Appl Mater Interfaces*. 2013; 9:9285–9294. [PubMed: 23957655]
9. Gorbet MB, Sefton MV. Biomaterials–associated thrombosis: roles of coagulation factors, complement, platelets, and leukocytes. *Biomaterials*. 2004; 25:5681–5703. [PubMed: 15147815]
10. Baumgartner HR, Muggli R, Tschopp TB, Tiritto VT. Platelet adhesion, release and aggregation in flowing blood: effects of surface properties and platelet function. *Thromb Haemost*. 1976; 35:124–138. [PubMed: 989184]
11. Radomski MW, Palmer RM, Moncada S. The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. *Br J Pharmacol*. 1987; 92:639–646. [PubMed: 3322462]
12. Lantvit SM, Barrett BJ, Reynolds MM. Nitric oxide releasing material adsorbs more fibrinogen. *J Biomed Mater Res A*. 2013; 101:3201–3210. [PubMed: 23554300]
13. Ialenti A, Iannaro A, Moncada S, Di Rosa M. Modulation of acute inflammation by endogenous nitric oxide. *Eur J Pharmacol*. 1992; 211:177–182. [PubMed: 1612108]
14. Bogdan C. Nitric oxide and the immune response. *Nat Immunol*. 2001; 2:907–916. [PubMed: 11577346]
15. Reynolds MM, Frost MC, Meyerhoff ME. Nitric oxide-releasing hydrophobic polymers: preparation, characterization, and potential biomedical applications. *Free Radic Biol Med*. 2004; 37:926–936. [PubMed: 15336308]
16. Mowery KA, Schoenfish MH, Saavedra JE, Keefer LK, Meyerhoff ME. Preparation and characterization of hydrophobic polymeric films that are thromboresistant via nitric oxide release. *Biomaterials*. 2000; 21:9–21. [PubMed: 10619674]
17. Seabra AB, De Oliveira MG. Poly(vinyl alcohol) and poly(vinyl pyrrolidone) blended films for local nitric oxide release. *Biomaterials*. 2004; 25:3773–3782. [PubMed: 15020153]
18. Seabra AB, da Rocha LL, Eberlin MN, de Oliveira MG. Solid films of blended poly(vinyl alcohol)/poly(vinyl pyrrolidone) for topical S-nitrosoglutathione and nitric oxide release. *J Pharm Sci*. 2005; 94:994–1003. [PubMed: 15793801]
19. Simões MM, de Oliveira MG. Poly(vinyl alcohol) films for topical delivery of S-nitrosoglutathione: effect of freezing-thawing on the diffusion properties. *J Biomed Mater Res B Appl Biomater*. 2010; 93:416–424. [PubMed: 20101734]
20. Kushwaha M, Anderson J, Minor W, et al. A nitric oxide releasing, self assembled peptide amphiphile matrix that mimics native endothelium for coating implantable cardiovascular devices. *Biomaterials*. 2010; 31:1502–1508. [PubMed: 19913295]
21. Samal AA, Honavar J, Brandon A, et al. Administration of nitrite after chlorine gas exposure prevents lung injury: effect of administration modality. *Free Radic Biol Med*. 2012; 53:1431–1439. [PubMed: 22917977]
22. Miller MW, Bara S, Kulp DW, et al. Small-molecule inhibitors of integrin $\alpha 2 \beta 1$ that prevent pathological thrombus formation via allosteric mechanism. *Proc Natl Acad Sci USA*. 2009; 106:719–724. [PubMed: 19141632]
23. Maragos CM, Morley D, Wink DA, et al. Complexes of NO with nucleophiles as agents for the controlled biological release of nitric oxide vasorelaxant effects. *J Med Chem*. 1991; 34:3242–3247. [PubMed: 1956043]
24. Smith DJ, Chakravarthy D, Pulfer S, Simmons ML, Hrabie JA, Citro ML, et al. Nitric oxide-releasing polymers containing the [N(O)NO]-group. *J Med Chem*. 1996; 39:1148–1156. [PubMed: 8676352]
25. Annich GM, Meinhardt JP, Mowery KA, et al. Reduced platelet activation and thrombosis in extracorporeal circuits coated with nitric oxide release polymers. *Crit Care Med*. 2000; 28:915–920. [PubMed: 10809259]

26. Zhang H, Annich GM, Miskulin J, et al. Nitric oxide releasing silicone rubbers with improved blood compatibility: preparation, characterization, and in vivo evaluation. *Biomaterials*. 2002; 23:1485–1494. [PubMed: 11829445]
27. Batchelor MM, Reoma SL, Fleser PS, et al. More lipophilic dialkyldiamine-based diazeniumdiolates: synthesis, characterization, and application in preparing thromboresistant nitric oxide release polymeric coatings. *J Med Chem*. 2003; 46:5153–5161. [PubMed: 14613318]
28. Taite LJ, Yang P, Jun HW, West JL. Nitric oxide-releasing polyurethane-PEG copolymer containing the YIGSR peptide promotes endothelialization with decreased platelet adhesion. *J Biomed Mater Res B Appl Biomater*. 2008; 84:108–116. [PubMed: 17497680]
29. Jun HW, West JL. Modification of polyurethaneurea with PEG and YIGSR peptide to enhance endothelialization without platelet adhesion. *J Biomed Mater Res B Appl Biomater*. 2005; 72:131–139. [PubMed: 15389489]
30. Jun HW, West J. Development of a YIGSR-peptide-modified polyurethaneurea to enhance endothelialization. *J Biomater Sci Polym Ed*. 2004; 15:73–94. [PubMed: 15027844]
31. Jun HW, Taite LJ, West JL. Nitric oxide-producing polyurethanes. *Biomacromolecules*. 2005; 6:838–844. [PubMed: 15762649]
32. Kushwaha M, Anderson JM, Bosworth CA, et al. A nitric oxide releasing, self assembled peptide amphiphile matrix that mimics native endothelium for coating implantable cardiovascular devices. *Biomaterials*. 2010; 31:1502–1508. [PubMed: 19913295]
33. Andukuri A, Minor WP, Kushwaha M, Anderson JM, Jun HW. Effect of endothelium mimicking self-assembled nanomatrices on cell adhesion and spreading of human endothelial cells and smooth muscle cells. *Nanomedicine*. 2010; 6:289–297. [PubMed: 19800987]
34. Andukuri A, Kushwaha M, Tambralli A, et al. A hybrid biomimetic nanomatrix composed Of electrospun polycaprolactone and bioactive peptide amphiphiles for cardiovascular implants. *Acta Biomaterialia*. 2011; 7:225–233. [PubMed: 20728588]
35. Vaughn MW, Kuo L, Liao JC. Estimation of nitric oxide production and reaction rates in tissue by use of a mathematical model. *Am J Physiol*. 1998; 274:H2163–176. [PubMed: 9841542]
36. Radomski MW, Palmer RM, Moncada S. The role of nitric oxide and cGMP in platelet adhesion to vascular endothelium. *Biochem Biophys Res Commun*. 1987; 148:1482–1489. [PubMed: 2825688]
37. Aktas B, Utz A, Hoenig-Liedl P, Walter U, Geiger J. Dipyridamole enhances NO/cGMP-mediated vasodilator-stimulated phosphoprotein phosphorylation and signaling in human platelets: in vitro and in vivo/ex vivo studies. *Stroke*. 2003; 34:764–769. [PubMed: 12624305]
38. Nguyen BL, Saitoh M, Ware JA. Interaction of nitric oxide and cGMP with signal transduction in activated platelets. *Am J Physiol*. 1991; 261:H1043–1052. [PubMed: 1656783]
39. Jun HW, West JL. Endothelialization of microporous YIGSR/PEG-modified polyurethaneurea. *Tissue Eng*. 2005; 11:1133–1140. [PubMed: 16144449]
40. Andukuri A, Min I, Hwang P, et al. Evaluation of the effect of expansion and shear stress on a self-assembled endothelium mimicking nanomatrix coating for drug eluting stents in vitro and in vivo. *Biofabrication*. 2014; 6:035019. [PubMed: 25048693]

Fig. 1.

A test circuit was constructed with the test module, connectors and tubing. The test module was connected to a syringe pump for precise control of the water flow through the device and to another syringe for sampling. The gas circuit consisted of a 100% O₂ tank with a pressure regulator.

Fig. 2.
Fig. 2a. Scanning electron microscopy (SEM) of self-assembled nanomatrix over Celgard polypropylene microporous hollow fibers. Magnification 100 $\times$ and 400 $\times$.

Fig. 2b. Scanning electron microscopy (SEM) of self-assembled nanomatrix over Celgard polypropylene microporous hollow fibers. Magnification 30000 $\times$.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 3. Oxygen transfer across the Celgard polypropylene microporous hollow fibers: coated (♦ Coated) and uncoated (▲ Control) (n=5 /group). Results are expressed as change in partial pressure of oxygen (pO₂) in relation to water velocity through fibers. ($p>0.05$)

Fig. 4.

Fig. 4a. Cumulative nitrite formation from PA-YK-NO coated (■NO) and uncoated (◆control) polypropylene microporous hollow fibers over time ($n = 4/\text{group}$). Error bars denote mean $\pm$ standard deviation. ($p < 0.005$)

Fig. 4b. Cumulative nitrate formation from PA-YK-NO coated (■NO) and uncoated (◆control) polypropylene microporous hollow fibers over time ($n = 4/\text{group}$). Error bars denote mean $\pm$ standard deviation. ($p < 0.005$)

Fig. 5. Platelet adhesion on collagen controls (Collagen), uncoated polypropylene microporous hollow fibers (Uncoated) and fibers coated with PA-YKNO (YKNO). Results are expressed as absorbance per unit area (nm/m^2). Data represent the mean of 6 samples. Error bars represent mean $\pm$ standard deviation (*: $p < 0.05$; YKNO compared to collagen and uncoated)