



University of
New Haven

University of New Haven
Digital Commons @ New Haven

Mechanical Engineering Faculty Publications

Mechanical Engineering

11-2014

Multi-Modal, Surface-Focused Anticoagulation Using Poly-2-methoxyethylacrylate Polymer Grafts and Surface Nitric Oxide Release

Surbhi Gupta

University of Michigan - Ann Arbor

Kagya Amoako

University of New Haven, kamoako@newhaven.edu

Ahmed Suhaib

University of Michigan - Ann Arbor

Keith E. Cook

Carnegie Mellon University

Follow this and additional works at: <http://digitalcommons.newhaven.edu/mechanicalengineering-facpubs>



Part of the [Biomedical Engineering and Bioengineering Commons](#), and the [Mechanical Engineering Commons](#)

Publisher Citation

Surbhi Gupta, Kagya A. Amoako, Ahmed Suhaib, Keith E. Cook. Multi-modal, surface focused anticoagulation using poly-2-methoxyethylacrylate polymer grafts and surface nitric oxide release. *Advanced Material Interfaces*. Volume 1, Issue 8, November, 2014, 1:1400012. doi: 10.1002/admi.201400012.

Comments

This is the accepted version of the following article: Gupta, S., Amoako, K. A., Suhaib, A., Cook, K. E. (2014). Multi-Modal, Surface-Focused Anticoagulation Using Poly-2-methoxyethylacrylate Polymer Grafts and Surface Nitric Oxide Release. *Adv. Mater. Interfaces*, 1: 1400012. which has been published in final form at <http://onlinelibrary.wiley.com/doi/10.1002/admi.201400012/abstract>

Advanced Materials Interfaces

Multi-modal, surface focused anticoagulation using poly-2-methoxyethylacrylate polymer grafts and surface nitric oxide release --Manuscript Draft--

Manuscript Number:	
Full Title:	Multi-modal, surface focused anticoagulation using poly-2-methoxyethylacrylate polymer grafts and surface nitric oxide release
Article Type:	Full Paper
Keywords:	Functional biomaterial; Blood-contacting surfaces; low-fouling coating; biomimetics; anti-platelet nitric oxide
Corresponding Author:	Kagya Amoako, Ph.D. University of Michigan Ann Arbor, MI UNITED STATES
Additional Information:	
Question	Response
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Michigan
Corresponding Author's Secondary Institution:	
First Author:	Surbhi Gupta, MS
First Author Secondary Information:	
Order of Authors:	Surbhi Gupta, MS Kagya Amoako, Ph.D. Ahmed Suhaib, BS Keith E Cook, Ph.D.
Order of Authors Secondary Information:	
Abstract:	This study examines platelet adhesion on surfaces that combine coatings to limit protein adsorption along with "anti-platelet" nitric oxide (NO) release. Uncoated and poly-2-methoxyethylacrylate (PMEA) coated, gas permeable polypropylene (PP) membranes were placed in a bioreactor to separate plasma and gas flows. Nitrogen with 100/500/1000 ppm of NO was supplied to the gas side as a proof of concept. On the plasma side, platelet rich plasma (PRP, 1x10 ⁸ cell/mL) was recirculated at low (60)/high (300) flows (ml/min). After 8 hours, adsorbed platelets on PP was quantified via a lactate dehydrogenase assay. Compared to plain PP, the PMEA coating alone reduced adsorption by 17.4±9.2% and 29.6±16.6% at low and high flow (p<0.05), respectively. NO was more effective at low plasma flow. At 100 and 500 ppm of NO, adsorption fell by 37.9±6.1% and 100±4.7%, (p<0.001), on plain PP. At high flow with 100, 500, and 1000 ppm of NO, adsorption reduced by 17.9±17.8%, 46.4±23.2%, and 100±4.8%, (p<0.001), respectively. On PMEA-coated PP with only 100 ppm, adsorption fell by 69.7±6.8 and 65.6%±16.9%, (p< 0.001), at low and high flows respectively. Therefore, the combination of an anti-adsorptive coating with NO has great potential to reduce platelet adhesion and coagulation at biomaterial surfaces.

Multi-modal, surface focused anticoagulation using poly-2-methoxyethylacrylate polymer grafts and surface nitric oxide release

Surbhi Gupta*, Kagya A Amoako^{#@}, Ahmed Suhaib*, Keith E. Cook⁺

College of Human Medicine*, Michigan State University, E Lansing, MI 48824, USA, Departments of Internal Medicine, Cardiology[#] and Biomedical Engineering[@] University of Michigan, Ann Arbor, MI 48109, USA and Department of Biomedical Engineering⁺, Carnegie Mellon University, Pittsburgh, PA 15219, USA

Corresponding Author: Keith E. Cook, PhD

Department of Biomedical Engineering

3321 PTC

700 Technology Drive

Pittsburgh, PA 15219

Email: keicook@andrew.cmu.edu

Keywords: Functional biomaterial; Blood-contacting surfaces; low-fouling coating; biomimetics; anti-platelet nitric oxide.

1. Abstract

This study examines platelet adhesion on surfaces that combine coatings to limit protein adsorption along with "anti-platelet" nitric oxide (NO) release. Uncoated and poly-2-methoxyethylacrylate (PMEA) coated, gas permeable polypropylene (PP) membranes were placed in a bioreactor to separate plasma and gas flows. Nitrogen with 100/500/1000 ppm of NO was supplied to the gas side as a proof of concept. On the plasma side, platelet rich plasma (PRP, 1×10^8 cell/mL) was recirculated at low (60)/high (300) flows (ml/min). After 8 hours, adsorbed platelets on PP was quantified via a lactate dehydrogenase assay. Compared to plain PP, the PMEA coating alone reduced adsorption by $17.4 \pm 9.2\%$ and $29.6 \pm 16.6\%$ at low and high flow ($p < 0.05$), respectively. NO was more effective at low plasma flow. At 100 and 500 ppm of NO, adsorption fell by $37.9 \pm 6.1\%$ and $100 \pm 4.7\%$, ($p < 0.001$), on plain PP. At high flow with 100, 500, and 1000 ppm of NO, adsorption reduced by $17.9 \pm 17.8\%$, $46.4 \pm 23.2\%$, and $100 \pm 4.8\%$, ($p < 0.001$), respectively. On PMEA-coated PP with only 100 ppm, adsorption fell by 69.7 ± 6.8 and $65.6 \pm 16.9\%$, ($p < 0.001$), at low and high flows respectively. Therefore, the combination of an anti-adsorptive coating with NO has great potential to reduce platelet adhesion and coagulation at biomaterial surfaces.

2. Introduction

Various artificial organs, catheters, stents, and other medical devices operate under long-term blood contact. Protein adsorption and platelet activation on the surfaces of these devices lead to clot formation. This, in turn, causes device failure and thromboembolic complications. The most common solution to this problem is systemic anticoagulation, but this leads to bleeding complications that can also contribute to patient morbidity and mortality.^[1-3] Anticoagulation that is limited only upon device surfaces could remedy these shortcomings.

Two methods of surface focused anticoagulation have demonstrated some promise in reducing coagulation: surface coatings designed to limit nonspecific protein adsorption and anti-platelet surface NO release. Surface coatings only reduce coagulation in the device and thus have no systemic anticoagulant effect. Various commercial anti-thrombogenic coatings have shown better preservation of platelet counts than the uncoated control surface^[4,5] during short-

term applications. However, these surface coatings have not yet proven sufficient to allow the elimination of systemic anticoagulation or long-term use of high surface area artificial organs without significant decrease in systemic platelet concentration. [6, 7]

Nitric oxide is released from biomaterials into flowing blood, [8-12] but has a short half-life of 2-5 seconds [8] in blood prior to being scavenged. Thus, its systemic effects are minor, and it has been examined as a means to focus anticoagulation at biomaterials' surfaces rather than systemically. Several means of supplying surface NO flux have been tested. They include NO release from a stored pool in the biomaterial, NO generation from endogenous sources in blood, and NO delivery via the gas flow in artificial lungs. [9-12] Early studies examining the latter approach were largely unsuccessful. These studies did not quantify surface NO flux, and it was likely insufficient. [13] More recent studies with proven, endothelial levels of NO flux ($> 2 \times 10^{-10}$ mol/min/cm²) have been successful, reducing platelet adhesion in tubing and catheters by approximately 40%, [9, 10] and markedly reducing coagulation and increasing longevity in artificial lungs. [12] Although positive, these studies were all for a period of 4 hours. Longer-term effectiveness in these settings is unknown. Additionally, use of NO in high surface area artificial organs could lead to excessive generation of methemoglobin in the blood, limiting the possible flux rates, and anticoagulation.

In this study, we hypothesize that the combination of anti-adsorptive coatings and surface NO flux will lead to more effective surface-focused anticoagulation. Anti-adsorptive coatings would limit protein adsorption, thereby reducing platelet activation induced via surface adhesion or surface-generation of agonists. In theory, a lower level of NO could then be used to anesthetize the limited number of platelets that adhere to the surface or contact the lower levels of soluble agonists. In this way, the surface would operate more like the endothelium, which uses several simultaneous methods to control coagulation. [14 - 16]

To examine this hypothesis, bioreactors were constructed with gas permeable polypropylene membranes. The bioreactors were then used to determine the relationship between sweep gas concentration and NO flux. Platelet rich plasma was recirculated over polypropylene membranes that were either uncoated or coated with PMEA. Various levels of NO

flux at varying plasma flow rates were then examined with each type of membrane, and the number of adsorbed platelets was used as the primary index of biocompatibility.

3. Materials and methods

3.1 Bioreactor Test Circuits

Several means of surface NO delivery are possible.^[9, 11-13] For simplicity, custom-built, polycarbonate bioreactors were created in order to simulate surface NO flux (Figure 1). Each bioreactor consisted of a two-piece flow cell that sandwiches a gas permeable polypropylene membrane with a surface area of 7.7 cm². The membrane partitions the flow cell into a blood plasma flow side and a gas flow side. The bioreactor was then used to study NO flux from the surface as well as surface platelet deposition under a variety of conditions.

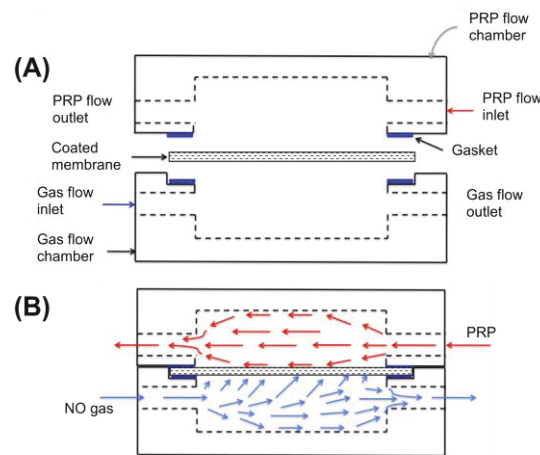


Figure 1. Bioreactor flow cell; exploded view of flow cell showing platelet rich plasma (PRP) flow chamber, test membrane, and sweep gas flow chamber (A) and assembled bioreactor showing PRP and NO gas counter-current flow (B).

3.2 Nitric Oxide (NO) Flux Measurements

Prior to examining platelet adhesion, the relationship between sweep gas NO concentration and bioreactor membrane NO flux was determined using PBS within the bioreactor circuits. This is a conservative estimate of NO flux during our plasma experiments, below. In plasma, some NO would bind to proteins, leading to a slightly higher effective diffusivity. A total of 55 mL of PBS (25 ml to fill bioreactor + 30 ml reservoir) was recirculated on one side of the membrane at high flow

(300 ml/min) and at low flow (60 mL/min), while N₂ with 100 ppm and 500ppm of NO flowed over the opposite surface. PBS samples were taken before initiating flow and at 3min, 5min, 10min, 20min and 30min of recirculation. PBS NO concentrations were immediately measured with a Sievers Nitric Oxide Analyzer 280i (GE Instruments, Boulder, CO) using standard wet-phase measurement methods. The NO flux at each sampling time, $J_{NO\ t=x}$ (mol/min/cm²), was first calculated as the change in NO concentration between current and previous samples divided by time elapsed between sampling times and membrane surface area. J_{NO} at each time point was then used to calculate NO mass transfer coefficient, K_c (cm/min), for each time point. K_c was calculated as $K_c = J_{NO}/([NO]_{max} - [NO]_{measured})$, where $[NO]_{max}$ (mol/ml) is the maximum achievable [NO] in PBS at the temperature and pressure from the NO gas concentration. We used $1.94 \times 10^{-3} \text{ mol L}^{-1} \text{ atm}^{-1}$ as a reference value for the solubility of NO in PBS.^[17] The K_c values at all sampling times were plotted and a linear regression line was used to estimate NO's mass transfer coefficient at the onset of NO gas flow $K_c(t=0)$. Having determined $K_c(t=0)$ and $[NO]_{max}$, the J_{NO} through the tested PP membranes was calculated as $J_{NO} = K_c(t=0) \times [NO]_{max}$.

3.3 Platelet Adhesion Studies: Effect of NO Flux and Surface Coating

All platelet adhesion studies utilized freshly drawn sheep plasma. The animal handling and surgical procedures were approved by the University Committee on the Use and Care of Animals in accordance with University of Michigan and federal regulations. Sheep whole blood was gravity drawn into a blood transfer blood bag (1:10 acid citrate dextrose 4% (ACD) anticoagulant, Sigma Aldrich St Louis MO). Whole blood from the bag was then carefully transferred to 50 mL centrifuge tubes and centrifuged at 730 rpm for 20 min to generate PRP. Platelet rich plasma was then recovered, and the remaining blood was centrifuged at 2750 rpm for 15 min to obtain platelet poor plasma (PPP). A Beckman-Coulter particle counter (Z1 Coulter Particle Counter, Indianapolis IN) was then used to determine platelet counts of the PRP and PPP. The PRP and PPP were then mixed to obtain 1×10^8 cells/mL.

Twenty-five millimeters of this mixture was injected into each of the three bioreactors. Each bioreactor was fitted with a 3" x 0.5" gas permeable PP membrane with an average pore

size of 0.1 μm (Sterlitech, Kent, WA) that was uncoated or coated with PMEA using standard procedures (Terumo Cardiovascular, Ann Arbor, MI). PMEA coating on PP membranes was confirmed (See Figure 2) with energy dispersive x-ray spectroscopy (EDAX Inc., Mahwah NJ) as previously described.^[18]

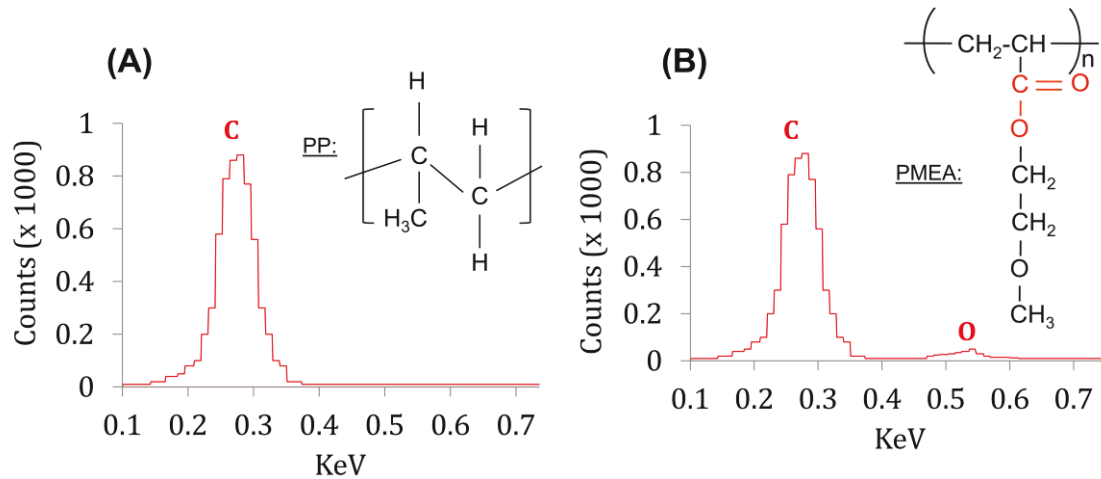


Figure 2. Surface chemical composition and chemical spectra of plain polypropylene (A), and PMEA-coated polypropylene (B).

The bioreactors were incorporated into a circuit consisting solely of 3/16" inner diameter Tygon tubing (Fisher Scientific, Pittsburg, PA). The tubing was then placed within a roller pump (Stockert multiframe, Baxter Century Baxter Healthcare Cooperation Irvine, CA). The PRP flow rate was set to either 300 ml/min (high flow) or 60 ml/min (low flow) using transonic flow probes (Transonic Systems, Ithaca, NY). These flows are equivalent to Reynolds numbers of 1.4 ($Pe = 5384$) and 7.1 ($Pe = 27307$), respectively.^[19] Lastly, the sweep gas was nitrogen with either 100, 500, or 1000 ppm of NO set at a flow rate twice the PRP flow rate.

To reduce biases created by run-to-run variability, three circuits were always run simultaneously such that three experimental conditions could be run with the same batch of plasma. In each run, three conditions were randomly chosen from all possible combinations of surface (PP, PMEA-coated PP, and PMEA-coated PP) and NO concentration (0, 100, 500, or 1000). After 8 hours of PRP recirculation, a 1 mL aliquot of PRP was obtained to test for platelet aggregation. The membranes were then carefully removed from the bioreactors and rinsed 3x in PBS using a 3mL pipette to wash away non-adhered blood plasma elements. The membrane

was then sectioned lengthwise into two halves. One half was assayed for LDH, and the other half was fixed for SEM analysis (see sections 3.6 and 3.7). Experimental runs with three circuits were repeated until five experiments were performed for each coating, NO concentration, and PRP flow rate combination.

3.4 Lactate Dehydrogenase (LDH) Assay

A lactate dehydrogenase (LDH) assay kit (Cayman Chemicals Ann Arbor, 10008882) was used to quantify platelets adhered on the surface of each membrane after 8 hours of PRP exposure in the bioreactor. Each membrane section was incubated at 37°C in two mL of lysing reagent (1% Triton-X100, 0.75% Bovine Albumin Serum in phosphate buffered saline) for 1hr, with occasional agitation. The suspension was then assayed for LDH according to the kit manufacturer's protocol. To convert LDH concentration to the number of adhered cells, a calibration curve was first constructed. PRP (7×10^8 platelets/mL) was serially diluted with PPP to achieve platelet concentrations of 4.7×10^8 , 2.4×10^8 , 1.2×10^8 , 6.0×10^7 , 3.0×10^7 , 1.5×10^7 , and 7.5×10^6 cell/ml and were assayed for LDH. A linear curve fit was then used to determine the relationship between absorbance from LDH and number of cells. This curve was found to be $\text{LDH}_{\text{absorbance}} = 0.12 \times \text{platelets (x10}^6 \text{ cells/mL)} + 0.01$, ($R^2 = 0.99$).

3.5 SEM Analysis

One half of each tested membrane was fixed with 2% glutaraldehyde (Sigma Aldrich, ST Louis MO) overnight and then dehydrated in a series of ethanol solutions and completely dried. The mid-section of the membrane was cut, sputter-coated with gold, and imaged by a Hitachi S-3200N scanning electron microscope (15 kV).

3.6 Data and Statistical Analysis

Raw LDH data obtained from coated and uncoated samples were adjusted to remove background LDH levels. To do so, a control study was run using PBS only in the circuit and assaying these membranes for LDH in the described manner (n=4). The average background

LDH data was then subtracted from the LDH concentrations measured in all studies. Any results giving a number below this value were given an adjusted LDH value of zero. Adjusted LDH data of test samples was then normalized by expressing the data as a percentage of LDH data obtained from uncoated control PP. Lastly, the calibration curve between LDH and platelet count was used to determine the number of adhered platelets, which was then divided by the surface area of test sample to give platelets/cm². Statistics was performed on normalized LDH data using the Origin statistical package (OriginLab, Northampton MA). Specifically, one-way analysis of variance (ANOVA) with a Tukey's post hoc analysis was used to compare normalized data. Statistical significance was set to p<0.05. Numerical values are presented as mean +/- standard deviation.

4. Results

Nitric oxide concentration in water over time exhibited the characteristic curve shown in Figure 3. The gas' concentration in water steadily increased during recirculation of PBS and flow of NO gas. After reaching the solubility limit, NO concentration remained fairly constant at the temperature and pressure for each test condition. At low PBS flow, J_{NO} was calculated to be $(2.40 \pm 1.63) \times 10^{-10}$ mol/min/cm² from 100 ppm sweep gas NO and $(11.17 \pm 2.98) \times 10^{-10}$ mol/min/cm² from 500 ppm sweep gas NO. At high flow, the same J_{NO} were $1.70 \pm 0.56 \times 10^{-10}$ mol/min/cm² and $21.13 \pm 1.97 \times 10^{-10}$ mol/min/cm². See Figure 4. These values are similar to NO flux levels reported to significantly inhibit clot formation.^[9-11]

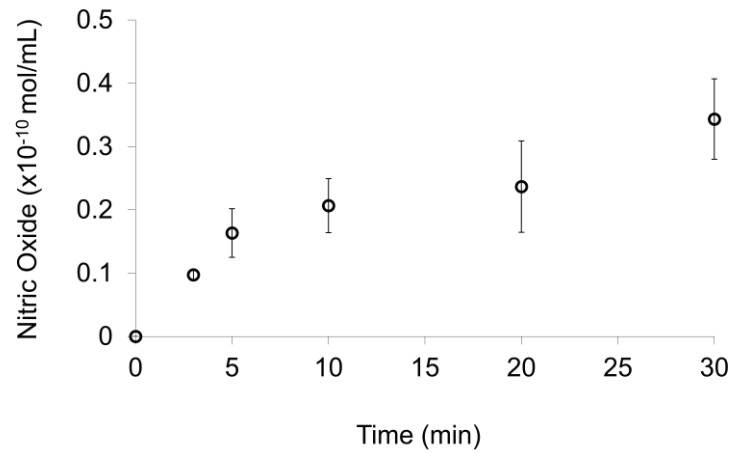


Figure 3. Nitric oxide (NO) concentration in water during recirculation of water at 120 ml/min and 500 ppm NO gas flow at 300 ml/min.

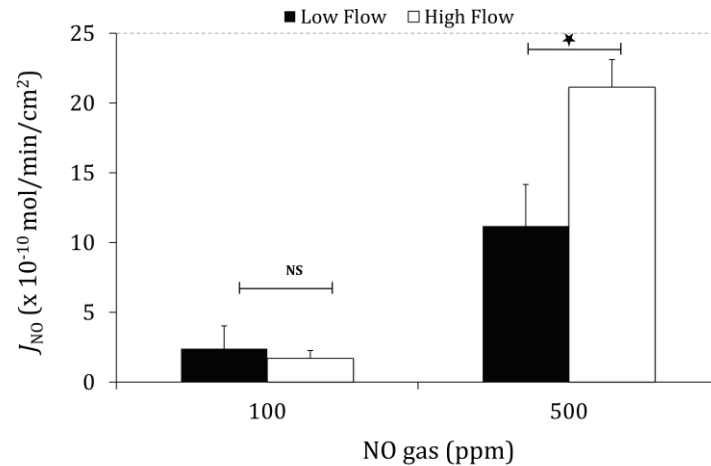


Figure 4. NO flux, J_{NO} , across gas permeable PP membrane after 10 minutes of low (60ml/min) and high (300ml/min) PBS flows.

Platelet adsorption, (% control), on plain PP and PMEA-coated PP at 60 ml/min PRP flow using several sweep gas NO concentrations is shown in Figure 5(B). Platelet adsorption on PMEA-coated PP without NO was $82.6 \pm 9.2\%$ of the control PP surface ($p < 0.05$). Platelet adhesion on plain PP with sweep gas NO at 100 and 500 was $62.1 \pm 6.1\%$ ($p < 0.001$) and $0 \pm 4.5\%$ ($p < 0.001$). Together, PMEA and NO act synergistically to reduce platelet adhesion when the NO concentration is low and thus not completely eliminating platelet adhesion. At 100 ppm of NO, adhesion on PMEA coated surfaces was $30.3 \pm 6.8\%$ of the control. The reduction at 100

ppm is thus 69.7%, which is greater than the additive effects of PMEA (17.4% reduction) and NO (27.9% reduction) alone.

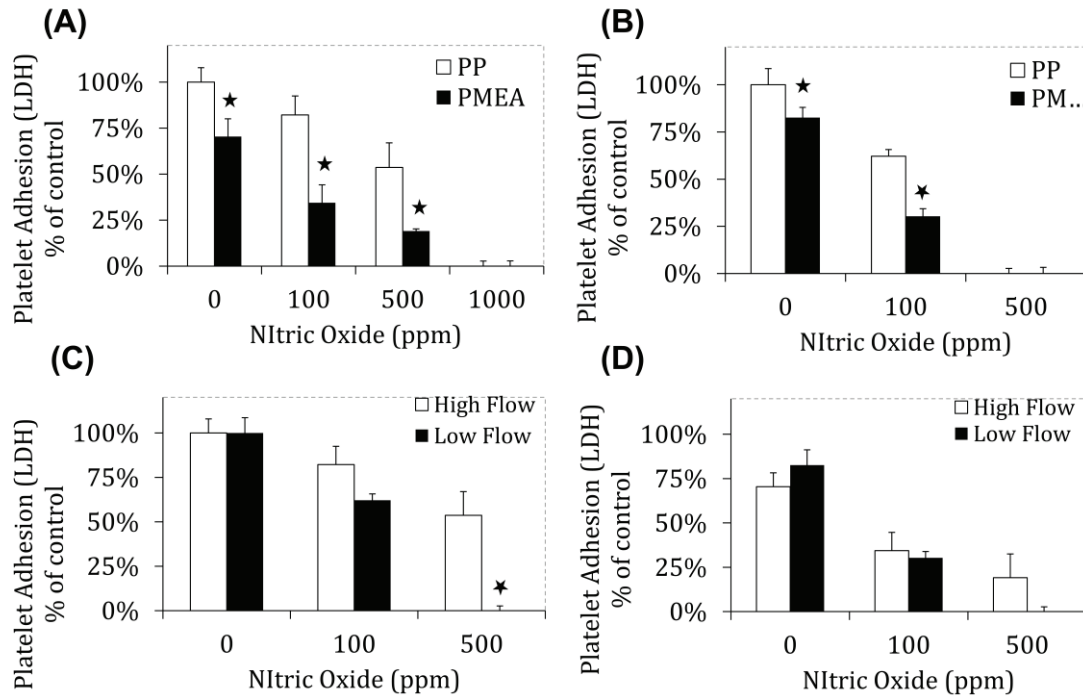


Figure 5. Platelet adsorption at high flow, 300 ml/min (A) and low flow, 60 ml/min (B) on PP and PMEA-coated PP membranes. Other panels show platelet adsorption at high and low flows on PP (C) and on PMEA-coated PP (D) membranes. * Indicates ($p < 0.05$) significance.

Figure 5(A) shows the same experiments at high PRP flow (300 ml/min). High flow results showed similar qualitative PMEA and coating effects, but reduced effectiveness of NO. Platelet adsorption on PMEA-coated PP without NO was $70.4 \pm 9.5\%$ of the control ($p < 0.05$). Platelet adhesion on plain PP with sweep gas NO at 100, 500, and 1000 ppm was $82.1 \pm 17.8\%$ ($p < 0.05$), $53.6 \pm 23.2\%$ ($p < 0.05$), and $0 \pm 4.6\%$ ($p < 0.001$). Thus, at these higher flows, a greater amount of NO is necessary to create the same level of platelet inhibition. The combined effect of PMEA and NO at low NO concentrations at high flows again appears greater than additive. With a PMEA coating and 100 ppm of NO, platelet adhesion was $34.4 \pm 17.0\%$ of the control. The reduction in adhesion is thus 65.6%, which is again greater than the additive effects of PMEA (29.6% reduction) and NO (17.9% reduction) alone.

Figures 5C and 5D reorganize the data to more clearly demonstrate the effect of PRP flow rate. Flow rate does not cause a consistent effect on plain PP or PMEA-coated surfaces. However, higher flow does consistently reduce the effectiveness of NO. Surface fouling as analyzed with scanning electron microscopy, is shown in Figures 6 and 7 for low and high flows, respectively. The left panels show platelet adsorption on plain PP and the right panels similarly show adsorption on PMEA-coated PP. In both flow conditions, platelet deposition was reduced by PMEA coating as well as increasing sweep gas NO concentration. In the low flow case, there is very little platelet adhesion when PMEA surfaces are combined with greater than 100 ppm of NO. In the high flow case, however, there is still significant platelet adhesion at 500 ppm with or without the PMEA coating. At high flow, 1000 ppm is needed to largely eliminate platelet adhesion.

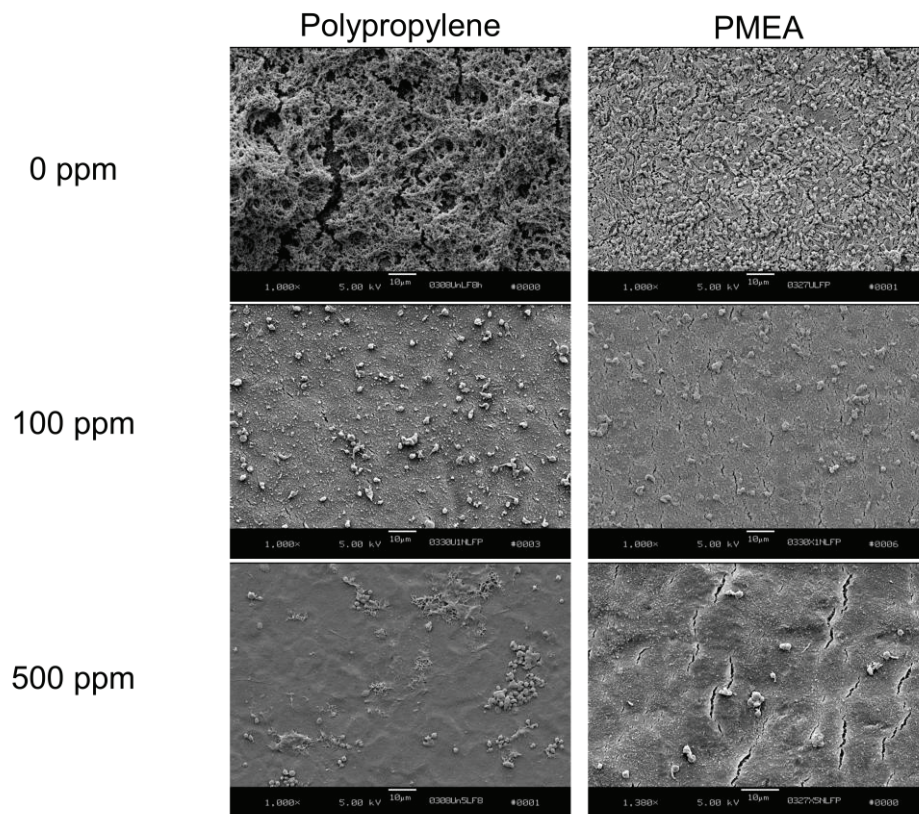


Figure 6. Scanning electron micrographs of fouling levels on PP and PMEA-coated PP membranes with 0 -500 ppm NO gas flows and 60 ml/min blood plasma flow.

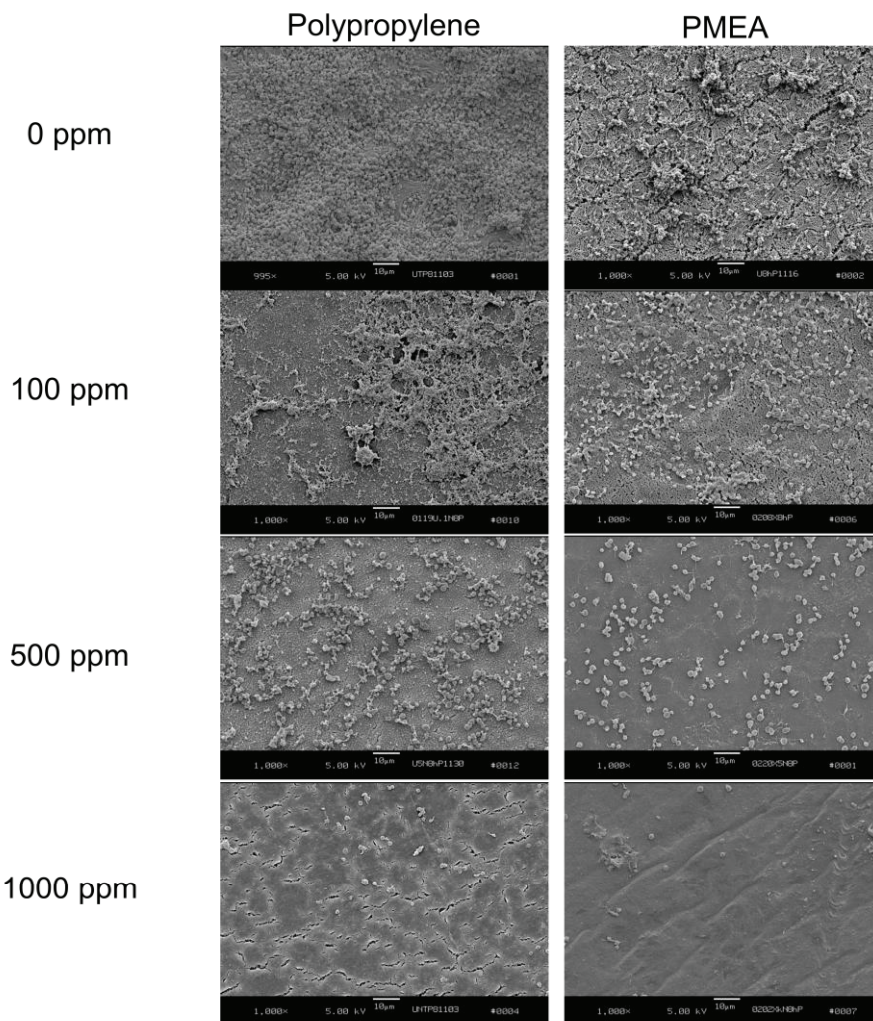


Figure 7. Scanning electron micrographs of fouling levels on PP and PMEA-coated PP membranes with 0 -1000 ppm NO gas flows and 300 ml/min blood plasma flow.

The platelet surface coverage, (cells/cm²), on tested PP membranes (Table I) was calculated using the calibration curve. It shows PMEA coating and NO gas resistance to platelet adsorption. Platelet adhesion on uncoated PP was $6.01 \pm 0.88 \times 10^6$ platelets/cm² and $6.49 \pm 0.87 \times 10^6$ platelets/cm² at low and high flows respectively. This was reduced to below the sensitivity of the LDH assay with the PMEA coating combined with 500 ppm of NO at low flow or 1000 ppm of NO at high flow.

Table I. Platelet surface coverage on unmodified and modified PP membranes.

Surface	Low flow		High Flow	
	Absorbance	Surface Coverage ($\times 10^6$ cells/cm 2) \pm SD	Absorbance	Surface Coverage ($\times 10^6$ cells/cm 2) \pm SD
PP	3.5 \pm 0.51	6.01 \pm 0.88	3.78 \pm 0.51	6.49 \pm 0.87
PMEA	2.89 \pm 0.26	4.95 \pm 0.45	2.66 \pm 0.44	4.56 \pm 0.75
PP+100ppm NO	2.17 \pm 0.21	3.72 \pm 0.36	3.1 \pm 0.68	5.32 \pm 1.16
PP+500ppm NO	0.0 \pm 0.16	0.0 \pm 0.01	2.02 \pm 0.88	3.46 \pm 1.51
PP+1000ppm NO	---	---	---	---
PMEA+100ppm NO	0.66 \pm 0.19	1.12 \pm 0.32	1.3 \pm 0.45	2.22 \pm 0.76
PMEA+500ppm NO	0.01 \pm 0.16	0.0 \pm 0.01	0.72 \pm 0.05	1.22 \pm 0.59
PMEA+1000ppm NO	---	---	---	---

5. Discussion

These results confirm that platelet adhesion on a biomaterial is synergistically reduced by combining a coating intended to reduce protein adsorption with NO flux. In this study, the reduction in platelet adhesion by the coating alone was modest (18-30%). The effect of NO alone increased with NO concentration, ranging from a modest 18% or 38% (high and low flow, respectively) at 100 ppm to complete elimination of platelet binding at 500-1000 ppm. Although the effects of PMEA and low concentration NO are modest separately, together their effects are amplified. With 100 ppm of NO and PMEA at low flow, platelet binding was reduced 70%, greater than the sum of the reductions caused by PMEA and 100 ppm of NO alone (45%). With 100 ppm of NO and PMEA at high flow, platelet adhesion was reduced 66%, once again greater than the effect of PMEA and NO alone (48%). Thus, the combined effect is 18-25% greater than if their effects were additive, suggesting that the presence of a protein adsorption resistant coating may potentiate the effect of NO. Local fluid mechanics is also shown to play a significant role in the ability of NO to inhibit platelet activation. At high flow rates, Peclet numbers are increased, and local transport of both surface generated procoagulants (e.g. thrombin) and NO are increased at the biomaterial surface. On a normal surface, without NO transport, increased flow typically slows clot formation due to higher procoagulant transport from the surface. In this study, however, the effectiveness of NO is diminished by increased convective transport of NO. At 60 ml/min and 300 ml/min the Peclet numbers are 5380 and 27300, respectively.

1
2
3
4 This suggests two things. First, NO should be more effective in stagnant regions where
5 clots typical form in medical devices. Local Peclet numbers will be small, nitric oxide will
6 accumulate, and this should have a greater effect than local procoagulant accumulation. Second,
7 NO will be less effective in whole blood. At the same Reynolds number, the Peclet number will be
8 larger because of 1) higher blood viscosity ($\approx 4\times$ water) and 2) a smaller effective NO diffusivity
9 due to NO scavenging by red blood cells (RBCs).^[20] The extent of this effect, however, will vary
10 from application to application. In all situations, there will be a cell-free, exclusionary layer near
11 the surface that contains only platelet rich plasma, which may moderate the effect of RBCs. The
12 thickness of this zone in laminar flows is on the order of the RBC diameter, 8 μm , but will vary
13 with local fluid mechanics.^[21-23] Further studies are thus necessary to examine the effect of RBCs
14 on NO inhibition of platelets in various whole blood flow conditions.
15
16
17
18
19
20
21
22
23
24
25
26

27 Several other limitations should be considered in the interpretation of these results. First,
28 the test system was a closed *in-vitro* circuit. Thus, both NO concentration and surface-generated
29 procoagulant molecules accumulate over time. If used *in-vivo* with whole blood, such
30 accumulation would not occur due to RBC binding and by clearance of procoagulant molecules.
31
32
33
34
35
36

37 Second, NO delivery was provided in this study via diffusion from an N_2 and NO mixture.
38 This served as a relatively simple means to approximate NO release from NO donors in polymers
39^[24] and estimate NO fluxes. However, as mentioned previously, NO flux rates and local
40 concentrations will be different in blood due to the presence of NO scavengers. Furthermore, the
41 effect of NO will vary slightly if NO is catalytically generated from donors in blood^[25], or if NO is
42 mixed with O_2 immediately prior to delivery to artificial lungs. In the former case, the effect of local
43 flow rate will be different, as increased flow rate will also increase transport of NO donors to the
44 polymer surface. In the latter, NO will be transported in part as nitrite or nitrate, which could
45 reduce platelet inhibition.
46
47
48
49
50
51
52
53

54 On the positive side, this technique could be attempted with other polymer coatings.
55 PMEA has been determined to be the most effective anti-protein coating of the acrylate polymers,
56 but it's also one of many low-fouling polymers available^[26]. Another, promising type of non-
57
58
59
60
61
62
63
64
65

fouling coating are those composed of zwitterionic polymers. This class of polymers demonstrates far lower protein adsorption ($<0.3 \text{ pg/cm}^2$) after incubation with 100% plasma and whole blood under static conditions^[27 - 36]. Protein adsorption on PMEA coatings, however, has been reported as 300 pg/cm^2 ^[37]. Thus, zwitterionic coatings may prove even more effective when combined with NO, allowing for even greater platelet inhibition at low NO concentrations. This, in turn, would help avoid the metHb generation by nitric oxide.

6. Conclusion

Alone, PMEA, protein-adsorption resistant coatings and surface NO flux both reduce platelet adhesion on polypropylene membranes. When PMEA coatings and NO release were combined, platelet adhesion was significantly lower. Furthermore, the reduction in platelet adhesion was greater than what would be predicted by the addition of reductions in adhesion caused by PMEA and NO alone. Thus, combining non-fouling surface coatings with surface NO release acts synergistically. Future work should test new, ultra-low fouling coatings with different means of surface NO delivery.

Acknowledgements

This work was supported by NIH grant R01 HL089043. We also thank Dr. Robert Bartlett and the University of Michigan Extracorporeal Life Support Laboratory for their assistance.

Disclosure

The authors have no disclosures to make.

Reference

- [1] J. Murphy, C. Savage, S. Alpard, and D. Deyo, *Perfusion*, **2001**, 16(6), 460.
- [2] A. Ali, M. Hashem, H.S. Rosman, L. Moser, A. Rehan, T. Davis, 1020 patients. *Journal of Clinical Pharmacology*, **2004**, 44(11), 1328.
- [3] R.H. Bartlett, D.W. Rolo, J.R. Custer, J.G. Younger, R.B. Hirschl, *JAMA*, **2000**, 283(7), 904.
- [4] J.O. Defraigne, J.I. Pincemail, G. Dekoster, R. Larbuisson, M. Dujardin, F. Blaart, J.L. David, and R. Limet, *Ann Thorac Surg*, **2000**; 70, 2075.

- [5] T.N. Hoel, V. Videm, S.T. Baksaas, T.E. Mollnes, F. Brosstad, and J.L. Svennevig, *Perfuaion*, **2004**, 19,177.
- [6] R.L. Korn, C.A. Fisher, E.R. Livingston, N. Stenach, S.J. Fishman, V. Jeevanandam, and V.P. Addonizio, *The Journal of Thoracic and Cardiovascular Surgery*, **1996**, 111, 1073.
- [7] Y. Kitano, M. Takata, K. Miyasaka, N. Sasaki, Q. Zhang, D. Liu, and Y. Tsuchida, *Journal of Pediatric Surgery*, **1997**, 32(5), 691.
- [8] M.M. Reynolds, M.C. Frost, and M.E. Meyerhoff, *Free Radical Biology & Medicine*, **2004**, 37, 926.
- [9] K.A. Amoako, C. Archangeli, T.C. Major, M.E. Meyerhoff, G.M. Annich, R.H. Bartlett, *ASAIO Journal*, **2012**, 58, 238.
- [10] T.C. Major, D.O. Brant, C. P. Burney, K.A. Amoako, G.M. Annich, M.E. Meyerhoff, H. Handa, and R.H. Bartlett, *Biomaterials*, **2011**, 32, 5957.
- [11] H. Zhang, G. M. Annich, J. Miskulin, K. Osterholzer, S. I. Merz, R.H. Bartlett, and M.E. Meyerhoff, *Biomaterials*, **2002**, 23,1485.
- [12] K.A. Amoako, P.J. Montoya, T.C. Major, A.B. Suhaib, H. Handa, D.O. Brant, M.E. Meyerhoff, R. H. Bartlett, K.E.Cook, **2013**, DOI: 10.1002/jbm.a.34655.
- [13] K.A. Amoako, M.E. Kreuz, R.H. Bartlett, K.E. Cook, *ASAIO Journal*, **2011**, 57, 113.
- [14] F. Murad, *Bioscience Reports*, **1999**, 19, 453.
- [15] D.M. Whelan, W.J. van der Giessen, S.C. Krabbendam, E.A. van Vliet, P.D. Verdouw, P.W. Serruys, and H.M. Mvan Beusekom, *Heart*, **2000**, 83, 338.
- [15] K.R. Veil, N.A.F Chronos, S.J. Palmer, *Circulation*, **1995**, 92, 2337.
- [16] I.G. Zacharia, and W.M. Deen, *Ann Biomed Eng*, **2005**, 33(2), 214.
- [17] K.A. Amoako, K.E Cook, *ASAIO*, **2012**, 58, 539.
- [18] E. L. Cussler, *Diffusion: Mass Transfer in Fluid Systems* (2nd ed.), Cambridge University Press, New York, USA **1997**.
- [19] B.S. Massey, J. O. H. N. Ward-Smith. *Mechanics of Fluids* (Ninth ed.) Spon Press, New York, USA **2011**.

- [20] B.B. Gupta, K.M. Nigam, and M.Y. Jaffrin, *Journal of Biomechanical Engineering*, **1982**, 104, 129.
- [21] G. Bugliarello, and J. Sevilla, *Velocity, Biorheology*, **1970**, 7, 85.
- [22] R.N. Das, and V. Seshadri, *Bulletin of Mathematical Biology*, **1975**, 37, 459.
- [23] Z. Zhou and M.E. Meyerho, *Biomaterials*, **2005**, 26, 6506.
- [24] Y. Wu, A. P. Rojas, G.W. Griffith, A.M. Skrzypchak, N. Lafayette, R.H. Bartlett, and M.E. Meyerho, *Sensors and Actuators B Chemical*, **2007**, 121, 36.
- [25] M. Tanaka, T. Motomura, M. Kawada, T. Anzai, Y. Kasori, T. Shiroya, K. Shimura, M. Onishi and A. Mochizuki, *Biomaterials*, **2000**, 21, 1471.
- [26] V. Gaberc-Porekar, I. Zore, B. Podobnik, V. Menart, *Curr. Opin, Curr Opin Drug Discov Devel*, **2008**, 11(2), 242.
- [27] S. Jiang, Z. Cao, *Advanced Materials*, **2010**, 22(9), 920.
- [28] S.F. Chen, J. Zheng, L.Y. Li, S.Y. Jiang, *J. Am. Chem. Soc.*, **2005**, 127, 14473.
- [29] K. Ishihara, N.P. Ziats, B.P. Tierney, N. Nakabayashi, J.M. Anderson, *J. Biomed. Mater. Res.*, **1991**, 25(11), 1397.
- [30] A.L. Lewis, *Colloids Surf. B*, **2000**, 18(3-4), 261.
- [31] J.H. Silver, J.C. Lin, F. Lim, V.A. Tegoulia, M.K. Chaudhury, S.L. Cooper, *Biomaterials*, **1999**, 20(17), 1533.
- [32] Y.C. Chung, Y.H. Chiu, Y.W. Wu, Y.t. Tao, *Biomaterials*, **2005**, 26(15), 2313.
- [33] W. Feng, S.P. Zhu, K. Ishihara, J.L. Brash, *Langmuir*, **2005**, 21, 5980.
- [34] W. Feng, J.L. Brash, S. Zhu, *Biomaterials*, **2006**, 27(6), 847.
- [35] S.F. Chen, L.Y. Liu, S.Y. Jiang, *Langmuir*, **2006**, 22, 2418.
- [36] V. Vijay, K. McCusker, *Perfusion*, **2003**, 18(1), 41.

Figure Captions

Figure 1. Bioreactor flow cell; exploded view of flow cell showing platelet rich plasma (PRP) flow chamber, test membrane, and sweep gas flow chamber (A) and assembled bioreactor showing PRP and NO gas counter-current flow (B).

Figure 2. Surface chemical composition and chemical spectra of plain polypropylene (A), and

PMEA-coated polypropylene (B).

Figure 3. Nitric oxide (NO) concentration in water during recirculation of water at 120 ml/min and 500 ppm NO gas flow at 300 ml/min.

Figure 4. NO flux, J_{NO} , across gas permeable PP membrane after 10 minutes of low (60ml/min) and high (300ml/min) PBS flows.

Figure 5. Platelet adsorption at high flow, 300 ml/min (A) and low flow, 60 ml/min (B) on PP and PMEA-coated PP membranes. Other panels show platelet adsorption at high and low flows on PP (C) and on PMEA-coated PP (D) membranes. * Indicates ($p < 0.05$) significance.

Figure 6. Scanning electron micrographs of fouling levels on PP and PMEA-coated PP membranes with 0 -500 ppm NO gas flows and 60 ml/min blood plasma flow.

Figure 7. Scanning electron micrographs of fouling levels on PP and PMEA-coated PP membranes with 0 -1000 ppm NO gas flows and 300 ml/min blood plasma flow.