A Simple Flow Cell System for Evaluating the Stability of non-Fouling Medical Coatings

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Abstract

A flow system, comprising flow cells and tygon tubing circuits, suitable for studying flow effects on non-fouling coatings was developed and used to evaluate dopamine-polycarboxybetaine (DOPApCB) coating known to be highly resistant to protein fouling. Typically, blood-contacting devices with commercial coatings begin to clot after about 2 weeks of implantation/attachment. The failure modes of many of these non-fouling coatings may be due 1) slow-paced protein fouling and 2) coating erosion due to sheer stress. Therefore a flow system was used to evaluate the effect of coating erosion due to sheer stress on coating biocompatibility. The system imposed sheer stresses typically present on blood-contacting devices. The acrylic flow cells featured dual chambers (top and bottom), which assembles to affix coated 8.89cm x 2.54 cm polydimethylsiloxane (PDMS) membranes to allow variable flow rates over the coated surface. A blood perfusion pump recirculated blood simulant through Tygon tubing that were connected to flow cells. In this study, uncoated and DOPApCB-coated PDMS membranes (N=5/membrane) were first be tested for fibrinogen adsorption using standard enzyme linked immunosorbent assay. The ELISA was then repeated for coated PDMS membranes that were exposed to 1 dyne/cm^2, 6 dynes/cm^2 and 10 dynes/cm^2 shear stresses for 8 hrs. Fibrinogen fouling is reported as percent of fouling on uncoated PDMS. Fibrinogen fouling on coated PDMS (5.73% ± 1.97%) was significantly lower than uncoated PDMS (100%, $p < 0.001$). Fouling on coated PDMS unexposed to shear stress, although lower, was not significantly different from coated PDMS membranes that were exposed to 1 dynes/cm^2 (9.55% ± 0.09%, $p = 0.23$), 6 dynes/cm^2 (15.92% ± 10.88%, $p = 0.14$), and 10 dynes/cm^2 (21.62% ± 13.68%, $p = 0.08$). Our initial results show that DOPApCB coating are stable under shear stresses tested with minimal erosion.

Introduction

Artificial materials in medicine are increasingly being used in ways that warrant their interaction with cells, bacteria, blood, tissue, and sometimes a combination of these complex living systems. In devices such as artificial lungs, catheters, stents, and dialysis membranes, the fate of their artificial surface interaction with blood mainly determines device function and hematologic complications. As blood interacts with an artificial surface, clotting factors in blood auto-adsorb and activate the blood coagulation cascade leading to clot formation[1-5]. Clots on these devices can cause embolism when flowing blood dislodges them into systemic circulation. The devices can also fail due to clots coverage on their surfaces. For example gas-transfer across gas-exchange membranes of artificial lungs becomes poorer with clot coverage [6, 7]. In general majority of materials that make up blood-contacting devices are procoagulant, and lack any of the normal anti-colagulation properties that healthy blood vessels use to maintain hemostasis. There is a big push to add anti-coagulant properties of the endothelium, the layer of cells that line internal surface of the blood vessels, to limit clot formation on blood-contacting devices[8-12]. The endothelium secretes and expresses many compounds including anti-platelet nitric oxide (NO), Heparan sulfate, and Thrombomodulin[1]. Heparan sulfate and Thrombomodulin are respectively an anti-clotting polysaccharide and protein found on membrane of endothelial cells. These cells also secret NO gas to inhibit clot formation via preventing platelet activation[1-4]. Thus far artificial materials have been modified with heparan sulfate, nitric oxide release/generation, and with a host of hydrophilic functional groups in the quest to inhibit clot formation only at the interacting surface of medical devices. Advances towards achieving local anti-clotting surfaces are still being made. Such surfaces will inhibit clot formation locally and elicit no systemic effects typical to systemic anticoagulation. Systemic infusion of heparin, the main clinical protocol for controlling clot formation induced by artificial materials, is effective but can lead to bleeding [4].

Locating blood anti-coagulation methods using surface coatings have shown great benefits in the clinical setting. Nevertheless, blood-contacting devices with commercial coatings typically begin to clot after about 2 weeks of implantation/attachment. The failure modes of many of these non-fouling coatings may be due 1) slow-paced protein fouling and 2) coating erosion due to sheer stress. It is our hypothesis that current limitations of surface coatings to remain effective over long term may not only be due to poor coating coverage or poor hydration, but also coating erosion from coated devices. To test our hypothesis, a relatively new coating material that has shown ultra-low auto-adsorption of pro-coagulant proteins comparable to commercial medical coatings polycarboxybetaine [1,10,11] was used to study coating erosion effect on biocompatibility of coated surfaces. The pCB coating material is well studied to show high protein fouling resistance and thus was a good candidate for studying erosion effect on biocompatibility. For example, blood glucose sensors coated with pCB accurately detected glucose at levels identical to measurements obtained from disposable glucose test strips for 42 days whereas the uncoated glucose sensor controls failed after 1.5 days [10].

To evaluate the ability of pCB coating to withstand shear stresses from normal blood flow [7], we simulated physiological flows and shear stresses over pCB coated PDMS. The metric used for evaluating the stability of the

coating was fibrinogen fouling using a standard ELISA protocol.

Methods

The acrylic (Custom Creative Plastics, FL) flow cell shown in **Figure 1** had dimensions Length = 11.43 cm, width = 5.87 cm, height = 1.60 cm. The flow cells were attached to a recirculation circuit, shown in **Figure 2**, using polycarbonate connectors (Qosina, NY)**.** The entire circuit consists of 3/16" I.D. and 5/16" O.D. tygon tubing (Fisher Scientific, MA), Acrylic flow cell, a Stöckert Shirley multiflow roller blood perfusion pump, Transonic flow meter (Transonic Inc. Cambridge MA). Each pump was calibrated to determine volumetric flow rate, mL/min, as a function of pump output rpm readout. The flow cell chamber measures a 1cm x 6.35cm x 0.5cm with a hydrodynamic diameter = 0.73 cm, entrance area = 0.37 cm^2. An 8.89cm x 2.54 cm PDMS membrane (NuSil Tech. CA) were casted (Figure 2C) via two-part polymerization process. Cured PDMS membranes were coated with DOPApCB (Figure 2D) using a dip-coating process previously described [11]. Coated and uncoated membranes (N=5/sample) were inserted into flow cells (Figure 2A, 2E) and the circuit was primed with phosphate buffered saline, pH= 7, (Sigma Aldrich, MO) was recirculated through the flow cells at 60 mL/min, 150 mL/min and 230 mL/min for 8 hours. These flows yield physiologically relevant shear stress of 1 dyne/cm^2, 6 dynes/cm^2 and 10 dynes/cm^2. The membranes were carefully removed, stored in PBS overnight. 1 cm x 1cm pieces of the PDMS samples were sectioned and prepared for standard fibrinogen adsorption ELISA as previously described [11]. Briefly, squares were placed into a 24-well plate and incubated in 1 mL of 1mg/mL fibrinogen for 90 minutes at room temperature. The disks are then washed five times with PBS and incubated with 1mL of 1 mg/mL BSA (Sigma Aldrich) for 90 minutes at room temperature. The samples were again washed five times with PBS. Next, the samples were transferred into new wells and incubated in 1:1000 dilution of HRP (Sigma Aldrich) anti fibrinogen in PBS for 30 minutes, followed by another wash in PBS. The samples were then transferred to a new set of wells. The solution is then incubated in 500 uL of 1 mg/mL OPD (Sigma Aldrich) in 0.1 M citrate-phosphate buffer containing 0.03% hydrogen peroxide. This reaction was then quenched after 30 minutes by the addition of 500 uL of 1N HCL (Sigma Aldrich). The supernatant was then removed from each sample and transferred into cuvettes. The absorbance of each supernatant was then measured at 492 nm using UV-vis spectrophotometer (Beckman Coulter, CT).

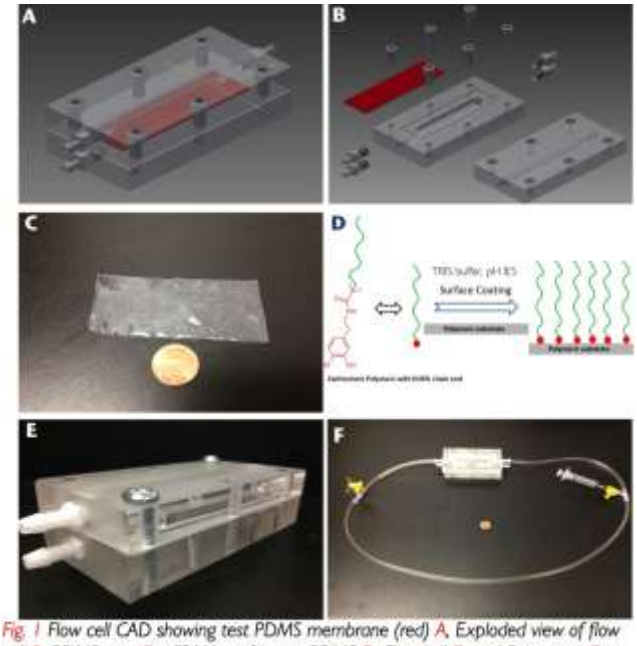


Fig. 1 Flow cell CAD showing test PDMS membrane (red) A, Exploded view of flow cell B, PDMS cast C, pCBMA grafting on PDMS D, Flow cell E, and flow circuit F.

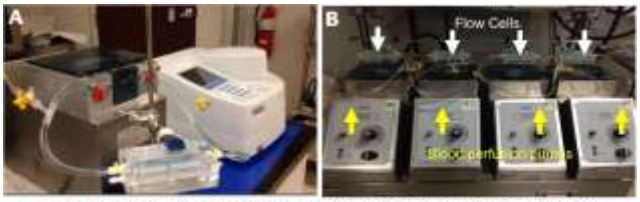
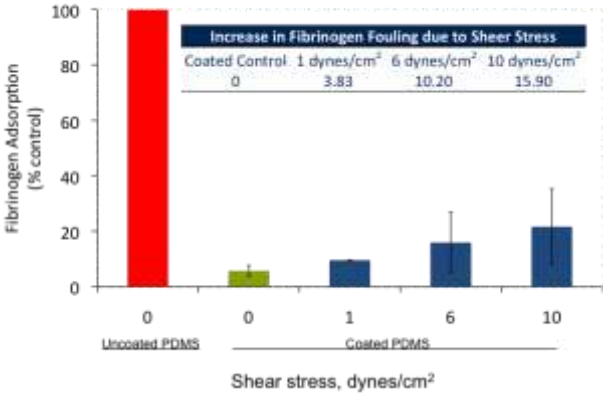


Fig. 2 Flow circuit-pump system A, and Multi flow circuit-pump systems B.

It was expected that uncoated PDMS samples would have higher absorption of fibrinogen and thus higher UV-vis absorbance levels. The effect of coating erosion on biocompatibility was determined as the percent increase in fibrinogen adsorption compared to appropriate controls. Less than 10% increment was considered highly stable, between 10 – 30% increase was considered stable and 30% or greater was considered unstable. A single factor ANOVA was run to determine statistical differences between controls (uncoated PDMS, and DOPApCB coated PDMS with no flow) and coated PDMS exposed to 1 dynes/cm^2, 6 dynes/cm^2 and 10 dynes/cm^2 shear stresses.

Results

Fibrinogen fouling on coated PDMS (5.73 ± 1.97%) was significantly lower than uncoated PDMS (100%, $p < 0.001$). See **Figure 3**. Fouling on coated PDMS unexposed to shear stress, although lower, was not significantly different from coated PDMS membranes that were exposed to 1 dynes/cm^2 (9.55% ± 0.09%, $p = 0.23$), 6 dynes/cm^2 (15.92% ± 10.88%, $p = 0.14$), and 10 dynes/cm^2 (21.62% ± 13.68%, $p = 0.08$). Our initial results show that DOPApCB coating are stable under the shear stresses tested and only minimal coating erosion was observed. Compared to the coated PDMS no shear stress case, coated surfaces that were exposed to 1, 6, and 10 dynes/cm^2 of shear stress, adsorbed 3.83%, 10.20%, and 15.90% more fibrinogen respectively.



Discussion

An in-vitro flow cell test system was developed to examine the stability of zwitterionic polycarboxybetaine anti-clotting coating on polydimethylsiloxane. The artificial surfaces of blood-contacting devices rapidly activate blood into clots during their interaction. In fact over 200 million of these devices including catheters, stents, and artificial lungs are used annually in the US alone [5]. Clot formation on their surfaces can lead to embolism in the heart, lungs, brain, peripheral veins as well as device failure. Anti-clotting surface coatings show the promise of inhibiting clot formation locally on devices without systemic anti-coagulation that is typically seen with heparin infusion (the clinical gold standard). As newer and more robust anti-clotting coatings get developed, a simple and easy-to-use in-vitro flow cell system becomes a greatly important screening tool for determining coating stability under flow before conducting animal and human testings. The results here support our hypothesis that coating erosion may play a significant role in failure of coated blood-contacting medical devices due to clot formation. More data is however needed before the study is ready for publication. This flow system can be used to evaluate many coatings and materials used in drug delivery, implantable medical devices. Other biomarkers of blood coagulation can easily be studied with the flow biological media (whole blood plasma and blood). The effect of pH, temperature on many substrates can also be studied with this platform. The gas transfer properties of polymeric materials could also be studied taking advantage of the dual chamber design of the flow cell.

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