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Hyaluronan and Dextran Modified Tubes Resist Cellular Activation With Blood Contact

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Abstract

This study was undertaken to evaluate the effects of thin film hyaluronic acid and dextran surface coatings to blunt cellular activation in a laboratory model of extracorporeal blood circulation. The inner lumen surface of polyurethane (PU) and poly(vinyl) chloride (PVC) tubing was grafted with hyaluronic acid and dextran. Surfaces were characterized for the presence of the grafted layer using ellipsometry, atomic force microscopy (AFM), and X-ray photoelectron spectroscopy (XPS). Persistence of the surface layer was maintained for up to 5 days of continuous exposure to shear flow using a Chandler loop apparatus. The Chandler loop method was used to study human whole blood activation activity. Whole blood aggregometry and flow cytometry measures of CD18, CD62L, CD62P, Annexin V and myeloperoxidase performed on blood samples exposed to the tubing for up to three hours were complemented by scanning electron microscopy (SEM) analysis of adherent cells and state of activation. In these studies commercial hospital products and uncoated PVC and PU tubes were used as controls. We found that hyaluranized PU and PVC conferred the greatest resistance to blood activation and that dextranization of the PU and PU tubing also provided significant diminution of the bioresponses measured. Based on our findings, we suggest that surface coating with hyaluronic acid or dextran acts as a potent shield from blood cellular activation during forms of extracorporeal circulation.

Keywords

biocompatible surfaces; dextran; hyaluronate; platelet aggregometry; neutrophil activation; flow cytometry

Introduction

Enormous numbers of vascular implants and medical devices that contact blood, such as vascular stents, grafts, catheters as well as cardiopulmonary bypass and hemodialysis tubing are used daily in clinical healthcare practice. Patients are at continual risk of having associated complications that include blood clotting, local systemic inflammatory responses and infection as a result [43]. The surfaces of vascular catheters, stents and extracorporeal

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circuits commonly induce blood cell activation represented by platelet and neutrophil activation as signs of thrombosis and inflammation. These cell-based bioresponses are one significant aspect of the compendium of biological events that are used to frame biomaterial biocompatibility [13;26;44].

As of the present, development of ideal biocompatible materials has remained elusive. There still exists a need for patients to undergo antiplatelet and anticoagulant therapies when receiving implantable cardiovascular devices. The appropriate pharmacological therapies carry their own risks and complications, including bleeding and stroke risks [15]. To achieve more true biocompatibility of new biomaterials for vascular applications requires deeper understanding of the relationship between constituents of the material surface, the types of biological responses evoked by blood contact (e.g., neutrophil activation, platelet aggregation), and the magnitude of those associated biological responses (e.g., degree of attenuation of response achieved).

Blood normally contacts the vascular endothelium, which has on its luminal surface the glycocalyx, or endothelial surface layer. This polysaccharide rich thin (0.5–1 μm) layer contains membrane-bound proteoglycans and glycoproteins. It has several critical biological functions: it serves to regulate vascular permeability [34;45], it acts as a mechanotransducer [19;47], and it balances locally chemical signaling events in the microenvironment [33]. When tissue inflammation occurs, the glycocalyx layer is known to degrade, which facilitates leukocyte recruitment [8;46]. In its native, healthy state the glycocalyx serves a protective role by inhibiting onset of the cascade of inflammatory and thrombotic events that occur when the glycocalyx is damaged [17]. It is therefore implicitly evident that the molecular constituents of the glycocalyx and their topography play important roles in vascular thrombogenicity and inflammatory responses. As a result, we surmise that strategies to mimic the endothelial glycocalyx surface in producing the next generation of blood-contacting biomaterials is an obvious route to reducing undesirable clotting and inflammation attributable to medical implants and devices [5].

Few experiments examining how surface coating containing the molecular constituents of the endothelial glycocalyx can reduce inflammatory and clotting responses provoked by biomaterials are represented in the literature. In this study, we have grafted dextran and hyaluronic acid (HA) onto polyurethane (PU) and poly(vinyl) chloride (PVC) surfaces to investigate human whole blood cell activation responses resulting from controlled blood-biomaterial interactions. We have chosen to use dextran and HA as a relatively simple model approach because these carbohydrates are well represented in the backbone of the endothelial glycocalyx layer.

Several research groups have demonstrated some key features related to blood cell and macromolecule (e.g., protein) interactions with grafted material surfaces. Previously, our laboratories have demonstrated that silicon coated with either dextran or HA inhibits protein adsorption [28;30]. We have also shown that surfaces coated homogeneously or patterned with either dextran or HA, or coated with a binary pattern of dextran and HA, have differential effects for adhesion of human macrophages (THP-1 cell line) [41] and that cell morphology and secretory responses a substrate dependent [21]. In other studies, surface-bound platelet adhesion was reduced on polycarbonate surfaces treated with a surfactant polymer coating (covalently linked poly (vinyl amine) with dextran) [14] and material surfaces grafted with hyaluronic acid or coated with heparin yielded improved biocompatibility and hemocompatibility profiles [16;18;20;37]. A variety of chemistry approaches have been utilized to graft biomacromolecules to synthetic polymeric surfaces. One method commonly used is immobilization by gas-plasma-activated polymer surfaces. Polysaccharides have been covalently linked to the surfaces of Teflon and poly-1-

trimethylsilyl-1-propyne by plasma treatment using ammonia gas [9]. Surfaces have also been functionalized with amine groups by diamines [3] or silanes [10] which were then coupled to the acid groups in the particular biomacromolecules used [11;23].

In this work, we covalently linked dextran and hyaluronic acid onto the surfaces of tubes made of PVC and PU, two base materials that are both commonly utilized in medical applications. PVC tubing is a major component of cardiopulmonary bypass extracorporeal circuits and other blood contacting medical device surfaces. PU is used to coat the blood contacting surfaces of left ventricular assist devices [39;48] and is often used for long-term vascular implants such as cardiac pacemaker lead insulation [38]. The two coating molecules, dextran and HA, are both FDA approved, and thus, each is an excellent biopolymer to use in studies directed at incorporating macromolecular components of the endothelial glycocalyx into a biomimetic surface construct. The grafted surface layers were characterized using ellipsometry, fluorescence microscopy and atomic force microscopy. PU and PVC tubes coated on the luminal surface were subjected to controlled exposure to human blood, from which samples were drawn periodically for analysis of platelet activation, platelet aggregation and markers of neutrophil activation. Conduit samples were also examined for cell adhesion and state of cell activation following blood exposure. Control experiments were also conducted using tubing segments from commercially available cardiopulmonary bypass circuits. The duration of our experiments was designed to simulate the typical duration of clinical hemodialysis or a cardiopulmonary bypass pump run during cardiac surgery for coronary artery bypass grafting or a heart valve replacement operation. We hypothesized that dextranized and hyaluronized surfaces would resist activation of cellular blood components and that differences in the specific activation profiles would relate directly to distinct compositional and structural features of the grafted dextran and hyaluronic acid layers on the different substrates.

Materials and Methods

Materials

We utilized all reagents and solvents as received. 3-Aminopropyl triethoxysilane (APTES), sodium periodate (NaIO_4), sodium cyanoborohydrate (NaBH_3CN), 1,6-Hexanediamine 60 wt. % aqueous solution, Acros Organics and HEPES were purchased from Sigma-Aldrich Co. and Fisher Scientific. Dextran, derived from *Leuconostoc mesenteroides* with molecular weight of 64,000–76,000, was from Sigma (# D4751), as was hyaluronic acid having a molecular weight of 750,000, (Sigma #H1504). For fluorescence imaging, rhodamine B isothiocyanate-dextran (Sigma #R9379, MW=70,000) and fluorescein hyaluronic acid (Sigma #F1177, MW= 800,000) were used. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, #22980) and sulfo-NHS (#24510) were purchased from Thermo Fisher Scientific. Polyester-based polyurethane (PU; Nalgene 280 Polyurethane Tubing #14-176-174) and poly(vinyl chloride) (PVC; PVC Tubing #14-169-1J) tubing was obtained from Fisher Scientific. Blood tubing modified with Poly(2-methoxy-ethylacrylate) (PMEA), and marketed as Terumo-XTM coated tubing as well as unmodified PVC tubing (referred to herein as Terumo) was acquired from Terumo Cardiovascular Systems (Ann Arbor, MI). For platelet aggregometry, ADP (1 mmol/L; Chrono-Par® reagent no. 070212), cuvettes, and siliconized stir bars were purchased from Chrono-Log Corporation (Havertown, PA). Antibodies for flow cytometry experiments were purchased from BD Pharmingen, San Jose, CA or BD Biosciences, Franklin Lakes, NJ.

Dextran and hyaluronic acid modification of polyurethane and poly(vinyl chloride) tubes

Tubing was cut into 33 cm lengths for Chandler Loop experiments described below. The inner lumen of PU and PVC tubes was functionalized using 1,6-Hexanediamine 60 wt. %

aqueous solution at 25 °C for 1 day. The solution was drained from the tubing samples after the grafting reaction, washed with water, and then dried. Oxidized dextran macromolecules were grafted onto the aminated tubes using 0.015 gm of NaBH₃CN for 1 day. Dextran was oxidized using the same procedure as described in our previous publications for silicon wafers [7;24;25;28–32;41;42]. The dextranized surface for this experiment was obtained by oxidizing dextran for 0.5 hr, with additional methodological detail having been given previously [25;30]. Similarly, for hyaluronic acid modification, PU and PVC tubes were first aminated using 1,6-Hexanediamine 60 wt. % aqueous solution. Samples were then drained and rinsed with DI water several times and reacted in hyaluronic acid solution using 2 mg/ml concentration, 0.38 g/ml EDC and 2 mg/ml Sulfo-NHS for one day [30;41]. The tubes were rinsed several times with DI water the succeeding day. In some experiments, either Rhodamine B isothiocyanate-Dextran or fluorescein-hyaluronic acid was used to verify by fluorescence imaging achieved with an Olympus BX50 microscope the success of layer grafting on the tubes and for subsequent surface visualization in wear testing analysis.

Dextran and Hyaluronic Acid Modified Surface Characterization

Surface characterization of dextranized and hyaluronized tubes was performed using ellipsometry, atomic force microscopy (AFM), and X-ray photoelectron spectroscopy (XPS) as described below. Results of surface characterization including these types of measures as well as zeta potential analysis of planar dextranized and hyaluronized surfaces have previously been reported [25;30;41;42].

Ellipsometry measurement—Sections of the tubes whose surfaces we coated with APTES, dextran and hyaluronic acid were assessed by ellipsometry. All measurements were made in triplicate at a minimum using a Rudolph Research/AutoEL-II ellipsometer.

AFM—Surface topography and roughness of sections cut from coated tubes were determined by AFM (Digital Instruments, Santa Barbara, CA: Dimension 3000 AFM). Tapping mode was used in air with a single crystal Si tip having a spring constant of 48 N/m, a radius of curvature of approximately 10 nm and a resonance frequency of about 190 kHz. Images were analyzed offline using Picoview 1.6 software (Agilent Technologies) to determine feature size and roughness.

XPS—Both PVC and PU surfaces were evaluated for confirmation of dextran immobilization using XPS with a Physical Electronics Quantum 2000 spectrometer with a 200 µm spot size and monochromatic Al K α radiation (1486.68 eV). The X-ray source was operated at 50 W and 15 kV. Core-level signals were obtained at photoelectron takeoff angles of 45° (2.5 nm detection depths) with respect to the sample surface.

Blood-Tubing Interaction Experiments

We assessed the potential of functionalized tubing surfaces to diminish cell activation responses (e.g., neutrophil and platelet activation, neutrophil-platelet interactions, platelet aggregation) initiated by blood contact with either experimental or clinically employed polymeric blood conduits using an ex vivo model. The Chandler Loop Apparatus enables examination of modified surfaces with respect to blood-surface interactions [6;27;35] by flowing blood within a circular closed loop of tubing. We used the Chandler Loop to analyze the effects of whole blood interactions with native and modified (dextran, HA) PVC and PU tubes as well as Terumo and Terumo-XTM commercial blood tubing under flow conditions. Briefly, following an approved Institutional Review Board protocol, 10 ml of freshly drawn whole human blood, supplemented with Sodium Citrate, was introduced into a closed tubing loop. The tubing was rotated continuously while partially submerged in a water bath at 37 °C. At time zero, 1 hr, 2 hr and 3 hr, 0.5 ml samples of blood were acquired

from each tube, placed in a stop solution and stored for further analyses by flow cytometry. In an identical parallel set of experiments conducted using the same tubing materials, 0.5 ml samples of blood were acquired from each tube at the specified time points for platelet aggregometry measurements. At the termination of the protocol, SEM imaging was performed on the inner lumen of the conduit sections.

Platelet Aggregometry Measurements

For assessment of platelet activation, electrical aggregometry measurement was used. In this technique the impedance between a pair of metal electrodes immersed in diluted whole blood is continuously quantified [4]. Increases in impedance correlate with the amount of platelet aggregates that deposit onto the electrodes following addition of a platelet agonist. A ChronoLog Whole Blood Analyzer Model 592A was used with samples maintained at 37 °C and stirred constantly at a stir bar speed of 1000 rpm. The 0.5 ml citrate-anticoagulated whole blood sample was diluted with 0.5 ml of 0.9% saline solution warmed to 37 °C in a polycarbonate cuvette. The electrodes were immediately submerged in the sample and the mixture was incubated for 2 min. Following a short electrical calibration, aggregation was initiated by the addition of ADP at a final concentration of 5 $\mu\text{mol/l}$. The impedance (Ω) was recorded for 7 min, although just the impedance values at the 6 min time point were used for data analyses.

Flow Cytometry Measurements

Flow cytometry was performed with a 4-color, dual laser analog FACSCalibur (Becton Dickinson, San Jose, CA) using standard acquisition software. Analysis of leukocytes was performed on fixed blood. Erythrocytes were not removed in order to guard against cell activation that might occur with lysis steps. Specific markers utilized were CD18, a marker of leukocyte activation, CD62P (P-selectin) and CD62L (L-selectin), markers for platelet activation and neutrophil activation respectively, myeloperoxidase (MPO), whose presence on the surface of neutrophils is a manifestation of cellular degranulation and an indicator of polymorphonuclear leukocyte auto-activation, and Annexin V, whose surface staining indicates the presence of surface phosphatidylserine, which occurs as an early step toward apoptosis. Assay methods, permeabilization and compensation procedures were as described in a previous publication [40]. Cells were defined as positive for a particular antigen if linear fluorescence intensity was above 10.

Scanning Electron Microscopy

Following completion of the Chandler Loop runs (3 hour exposure), conduit sections were first rinsed gently in PBS and then prepared for SEM analysis as described previously [12]. Cells adherent to the conduit inner lumen were affixed using a solution of 4% glutaraldehyde in Hepes Buffered Saline. Then the samples were dehydrated in ethanol and dried with hexamethyldisilazane before being sputter coated with gold-palladium. The samples were examined with a Phillip/FEI XL20 scanning electron microscope (FEI, Hillsboro, OR).

Surface Wear Characterization

Lengths of fluorescently labeled dextranized and hyaluronized PU and PVC tubes were filled with saline and rotated on the Chandler Loop Apparatus for up to 5 days at 37 °C. At baseline, 24, 48 and 120 hours elapsed time, short segments of the tubes were removed and imaged to demonstrate the presence of the surface coating. The fluorescence images were obtained with an Olympus BX50 microscope. The percentage of surface area demonstrating fluorescence for the specific fluorophores used was analyzed using ImageJ software, downloadable from the National Institutes of Health.

Data analysis

All experiments were conducted in triplicate for each set of experimental conditions. Platelet aggregation was analyzed using analysis of variance (ANOVA). Cellular activation measured by flow cytometry was analyzed by two-way ANOVA followed by the Holm-Sidak test. Single comparisons were performed using Student's t-tests.

Results and Discussion

Ellipsometry measurement

The thickness (mean $\pm$ standard deviation) of the dextran- and hyaluronic acid-modified PVC and PU surfaces appear in Table 1. The dextran and HA layers were present at essentially equal thickness whether present on PVC or PU tubes. These data are consistent with our previous published results [30;41;42] and other published values [22].

AFM—The surfaces of the untreated, dextranized and HA coated PU and PVC tubes were characterized using AFM to quantify their topographic differences. Values for surface roughness (RMS), a singular index of surface topographic distinction, are given in Table 1. Both PU and PVC tubes have relatively smooth surfaces compared to the subsequent dextranization and hyaluronic acid modification. The rougher surface after chemical modification is most likely a result of the amination step. In all samples, the PU tubes, including those bearing dextran and HA, are rougher than their counterpart PVC tubes.

XPS—XPS was used to confirm dextran immobilization. For the PVC tubing, the XPS survey scan showed a clear chlorine peak, attributable to the vinyl chloride, at 45° or 2.5 nm detection depths. Upon amination, the nitrogen peak is distinctly identified, which validates the success of chemical modification of the surface to contain amine groups. Subsequent to dextranization, the nitrogen peak decreased, as is expected. This is further verified with multiplex surveys which showed the ratio of carbon to nitrogen (C:N) to be 7.7 for the aminated surface and 16 after the dextranization step. This result indicates that the dextran layer has covered the amine groups, making them inaccessibly deep for detection. In addition, the ratio of carbon to chlorine also increased upon amination, from 15.4 to 50, indicative of a layer of amine groups covering the polyvinyl chloride. For PU tubing, the XPS multiplex surveys also demonstrated a change in the C:N ratio with a resultant value of 17.3 following the dextranization process, indicating that the dextran layer was present. This is consistent with our previous XPS analysis of dextranized polyurethane in protein adsorption studies [42].

Surface Wear Characterization—In Figure 1 are shown the results for percentage of surface coverage of samples of PVC and PU tubes coated with fluorescently labeled dextran and HA. At baseline, coverage is nearly complete for all surfaces by this measure. For samples assessed after 24, 48 and 120 hours of continuous rotation and shear exposure, the spatial distribution of fluorescence detection indicates that both HA and dextran remain attached to the surface without any decrement in degree of surface coverage. The major finding from these studies is that the underlying polymer tube surface does not become exposed, even after 5 days of continuous shear exposure in the Chandler loop apparatus.

Whole blood aggregometry—Shown in Figure 2 are the data for the whole blood aggregometry measurements plotted for the eight different tubing/coating types studied for the three distinct time points evaluated. At each of the different circulation times, the HA-PU surface generated the greatest aggregation response to ADP, indicating the least thrombogenicity induced by blood exposure to this conduit. The untreated PVC tubing promoted the greatest thrombogenicity and hence generated the smallest residual platelet

response to ADP, followed consistently by the Terumo and Terumo-X tubing and our dextranized PVC tubing, the three of which were essentially indistinguishable by this measure. The platelet aggregation response to our Dex-PU, PU, and HA-PVC tubes was intermediate between our HA-PU tubes and the commercially available Terumo products. For direct numerical comparison, the aggregation response provoked by our HA-PU tubing at 1, 2 and 3 hours of blood circulation time was less than that provoked by either of the Terumo products by approximately 60%, 62% and 52%, respectively, and less than that provoked by PU by 55%, 52%, and 40%, respectively. These findings indicate that significant reductions in the platelet response to material contact occur with hyaluronization of the PU surface in comparison to the other surface coatings examined: HA-PU is more effective than the other surfaces at preventing stimulation of platelet aggregation.

Flow cytometry—The general findings regarding differential effects of the various surfaces on platelet aggregometry are also evident in the flow cytometry measurements. Figures 3, 4 and 5 demonstrate the data for the whole blood flow cytometry measurements of CD18, CD62L and CD62P levels, respectively, for the eight different tubing/coating types studied for the three distinct time points evaluated. Also included with these results are the measurements made with non-circulated blood samples at the same time points as those acquired in the Chandler loop apparatus. A consistent pattern emerges in these data: at each of the different circulation times, the HA-PVC and HA-PU surfaces generated the lowest mean value for CD18, CD62L and CD62P marker positivity. The untreated PVC tubing promoted the greatest amount of CD18, CD62L and CD62P response, followed by the PU, Terumo and Terumo-X tubing. The biomarker responses promoted by our dextranized PU and PVC tubing were intermediate between our hyaluronized tubes and the commercially available Terumo products. For direct numerical comparison, the CD18 positivity response provoked by our HA-PVC tubing at 1, 2 or 3 hours of blood circulation time was less than that provoked by the Terumo and Terumo-X products by approximately 45% to 50%, and less than that provoked by PU tubing by about 40%. The CD62L positivity response provoked by our HA-PVC and HA-PU tubing at 1, 2 or 3 hours of blood circulation time was less than that provoked by the Terumo-X product by approximately 25% to 30%, and less than that provoked by Terumo tubing by about 35%. These results are mirrored in the CD62P responses, with Dex-PU and HA-PU eliciting nearly identical activation responses. It is noteworthy that following the first hour of blood exposure to the surfaces, the CD18, CD62L and CD62P levels for all surfaces have risen significantly above the reference values, with the exception of the hyaluranized PVC and the dextranized and hyaluranized PU. At the conclusion of the second and third hours of exposure, there are only modest changes in the CD62P levels detected. The CD18 levels detected generally did not change much after the first hour, although for HA-PU there was a total increase of 26% after three hours. While the particular increases in CD62P, CD62L and CD18 levels, especially for HA-PU, may appear to be significant, the overall levels still compare quite favorably with the reference levels measured. Taken together, these findings indicate that minimal blood activation is associated with tubing surface hyaluronization, with recognition that there are some differential effects on receptor expression for these three different measures of blood activation.

Figures 6 and 7 demonstrate the data for the whole blood flow cytometry measurements of MPO and Annexin V levels, respectively, for the eight different tubing/coating types studied for the three distinct time points evaluated. Also included with these results are the reference measurements made with blood samples not being circulated in the Chandler loop apparatus. A relatively consistent pattern also emerges in these data: at each of the different circulation times, as the HA-PVC and HA-PU surfaces generated the lowest mean value for MPO and Annexin V levels at each of the time points, while the untreated PVC tubing promoted the greatest amount of MPO and Annexin V response. The Terumo and Terumo-X tubing

responses were basically indistinguishable from each other and from the responses to our dextranized PVC and PU tubes. The effect of exposure duration extending from 1 h to 3 h had only modest additional influence on MPO and Annexin V responses, with total increases of MPO levels ranging from 9% to 16% over that time frame for the various surfaces studied. The lone exception was PVC, for which the MPO level increased by 32% over the last two hours. The net change in the Annexin V levels was in the range of 2% to 10% for all the tubing types over the final two hours. Together these data suggest that the initial cell effects that result from the blood interaction with the biomaterial surface is then buffered or attenuated, perhaps by surface adsorption of blood-borne proteins [28;30;42]. These results further amplify our other findings that, placement of a thin hyaluronic acid layer on the tube surface leads to minimal blood activation effect compared to dextran coating or use of naïve surface materials. Overall, the flow cytometry results are in concurrence with the platelet aggregometry studies to indicate that our hyaluronidate coating serves as the most effective shield from bioreactivity among the different surfaces tested, at least as assessed by these measures of preventing stimulation of blood cellular activation.

Figure 8 demonstrates SEM images from the eight different material sample types studied. On both the naïve PVC conduit as well as the Terumo material were found a large surface density of platelets, consistent with the number of cells identified in another study [12]. Platelets adherent to PVC and Terumo conduits were largely activated as determined by a primarily stellate morphology with pseudopod extensions being present. A small number of erythrocytes along with some platelets that were not highly activated were present on the Terumo-X coated, Dex PU and HA PVC conduits, with a larger number of erythrocytes adherent to the HA PU surface. The PU surface demonstrated adherence of platelets that were present in an activation state apparently less promoted than was evident on the Terumo and PVC sections. This was also the case with the Dex PVC surface, which yielded a high number of adherent (but not strongly activated) platelets along with a small number of erythrocytes. In these cases the platelets appeared to have much less spreading on the surface with far fewer pseudopod extensions than on the most adhesion-stimulating materials studied, as has also been noted previously for platelet-resistant biomaterials [12]. This analysis is also consistent with findings reported in other studies of platelet adhesion to selectively coated PU and PVC surfaces [1;2;12;36].

Conclusions

Grafting methods were used successfully to create coated tubing surfaces bearing two polysaccharides, dextran and hyaluronic acid, which were then characterized using ellipsometry, XPS and AFM. The surfaces, including uncoated controls and commercial blood tubing products were examined further in a biological model of blood activation. Blood cellular activation for platelets and neutrophils in particular was evaluated over a timecourse consistent with clinical hemodialysis and cardiopulmonary bypass using flow cytometry and whole blood aggregometry measures. Hyaluronic acid-modified surfaces resisted platelet and neutrophil activation as well as cell adhesion relative to other surfaces studied, including dextranized PU and PVC. Surface wear analysis of the coated tubing indicated that the grafted layers remain confluent across the entirety of the tube surface and persist for up to 5 days during continuous shear stress exposure. These results demonstrate that our dextranized and hyaluronized surfaces do resist activation of cellular blood components as well as cell adhesion onto the biomaterial surface and thereby limit the embolic potential of the conduits after blood contact as well as the activation of circulating blood components resulting from blood-biomaterial interactions. These differences in the specific activation profiles relate directly to distinct compositional and structural features of the grafted dextran and hyaluronic acid layers on the different substrates.

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Figure 1. Areal surface coverage of dextranized and hyaluranized PU and PVC tubing at baseline and following 24, 48 and 120 h of continuous shear exposure in the Chandler loop assembly.

Figure 2.

Platelet aggregometry with whole blood samples for Chandler loop runs of coated and uncoated tubing at 1, 2 and 3 h of circulation. Experiments were performed in triplicate (n=3) for each tubing type. Error bars represent standard deviation.

Figure 3. CD18 detection in whole blood samples by flow cytometry for Chandler loop runs of coated and uncoated tubing at 1, 2 and 3 h of circulation. Experiments were performed in triplicate (n=3) for each tubing type. Error bars represent standard deviation.

Figure 4. CD62L detection in whole blood samples by flow cytometry for Chandler loop runs of coated and uncoated tubing at 1, 2 and 3 h of circulation. Experiments were performed in triplicate (n=3) for each tubing type. Error bars represent standard deviation.

Figure 5.

CD62P detection in whole blood samples by flow cytometry for Chandler loop runs of coated and uncoated tubing at 1, 2 and 3 h of circulation. Experiments were performed in triplicate (n=3) for each tubing type. Error bars represent standard deviation.

Figure 6. Myeloperoxidase (MPO) detection in whole blood samples by flow cytometry for Chandler loop runs of coated and uncoated tubing at 1, 2 and 3 h of circulation. Experiments were performed in triplicate (n=3) for each tubing type. Error bars represent standard deviation.

Figure 7. Annexin V detection in whole blood samples by flow cytometry for Chandler loop runs of coated and uncoated tubing at 1, 2 and 3 h of circulation. Experiments were performed in triplicate (n=3) for each tubing type. Error bars represent standard deviation.

Figure 8. Scanning electron microscopy fields demonstrating cell adhesion and cell activation state on blood conduits following 3 h of circulation time. Images are all at identical magnification with length bars 50 µm.

Table 1

Atomic Force Microscopy and Ellipsometry Blood Conduit Surface Analysis

Tube Material	RMS roughness (nm)	Thickness (Å), n=3
PVC	6.4	N/A
Dex-PVC	28	10 ± 3
HA-PVC	57	17 ± 4
PU	13	N/A
Dex-PU	67	9 ± 3
HA-PU	129	19 ± 4