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The effects of PEG-based surface modification of PDMS microchannels on long-term hemocompatibility

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Abstract: The current study demonstrates the first surface modification for poly(dimethylsiloxane) (PDMS) microfluidic networks that displays a long shelf life as well as extended hemocompatibility. Uncoated PDMS microchannel networks rapidly adsorb high levels of fibrinogen in blood contacting applications. Fibrinogen adsorption initiates platelet activation, and causes a rapid increase in pressure across microchannel networks, rendering them useless for long term applications. Here, we describe the modification of sealed PDMS microchannels using an oxygen plasma pretreatment and poly(ethylene glycol) grafting approach. We present results regarding the testing of the coated microchannels after extended periods of aging and blood exposure. Our PEG-grafted channels showed significantly reduced fibrinogen adsorption and platelet adhesion up to 28 days after application, highlighting the stability and functionality of the coating over time. Our coated microchannel networks also displayed a significant reduction in the coagulation response under whole blood flow. Further, pressure across coated microchannel networks took over 16 times longer to double than the uncoated controls. Collectively, our data implies the potential for a coating platform for microfluidic devices in many blood-contacting applications. © 2014 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 102A: 4195–4205, 2014.

Key Words: surface modification, hemocompatibility, microfluidics, polydimethylsiloxane, polyethylene glycol

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INTRODUCTION

Poly(dimethylsiloxane) (PDMS) elastomers have found a broad range of biological applications due to a unique combination of physical properties.[1] Such properties include: flexibility, mechanical durability, optical transparency, chemical inertness, nontoxicity, and high gas permeability, as well as ease of microfabrication.[2,3] PDMS may be most recognized for its contribution to several leading biomedical devices including breast implants, pacemaker leads, catheters, shunts, and extracorporeal device membranes.[4] However, the unique properties of PDMS have also made it versatile for application as a construction material for microfluidic devices, especially for biological applications.[1] Microfluidic devices composed (at least in part) of PDMS have been used for biomolecule separation, immunoassays, cell culture, and artificial lungs.[3,5–10] Despite the many advantages of PDMS, the natural hydrophobicity presents problems when used as a blood-contacting surface.

It is well known that hydrophobic surfaces tend to adsorb high levels of plasma proteins, leading to increased platelet activation, and the intrinsic coagulation response.[11–13] Although protein adsorption is less of a problem on PDMS surfaces than with other biomedical polymers, the level of foreign body response remains high enough to cause thrombogenesis.[14] Thrombogenic activity is particularly relevant for devices with microscale features. Platelet aggregation and clotting can rapidly block microscale features and lead to decreased function and device failure. To date, there have been very few studies which report surface modifications of PDMS within an intact microchannel structure that focus on hemocompatiblity testing of the channel.[14–16] Of these, none have tested long-term hemocompatibility (>1 h blood exposure).

Increasing the hydrophilicity of a material is one of the most common approaches to reducing the initial biological response of nonspecific protein adsorption and subsequent cellular events.[17] Well-hydrated surfaces tend to repel protein binding through the combination of steric repulsion and the

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formation of a structured water layer.[18,19] To impart hydrophilicity and other material or biological properties, researchers have used a variety of strategies to modify the PDMS surface.[3,20] One of the most common methods is plasma treatment, where hydroxyl or other functional groups are generated on the PDMS surface by a reactive ionized gas. Oxygen plasma treatments are able to apply a dense, confluent covering of hydroxyl (—OH) functional groups derived from the reacting gas within a short reaction time. The major drawback of this approach is the rapid hydrophobic recovery of the PDMS, as uncured oligomers migrate from the material bulk and rearrange the functional groups away from the material surface.[21,22] To avoid this problem, some modification methods gaining popularity strive to combine multiple strategies, such as the pretreatment of PDMS with plasma or UV energy to introduce reactive groups followed by the application of a polyelectrolyte multilayer or functional molecule to create a more stable, covalently bound surface.[23–27] Specifically, a number of groups have described the reaction of methoxy-silane or other alkoxy-silane functional molecules with plasma-treated PDMS surfaces to form a grafted silane coating (Fig. 1).[25,28–30] Our work also utilizes this silanization reaction scheme in the context of an enclosed microchannel.

Most studies describing PDMS surface modification, particularly those utilizing plasma pretreatment, have developed and characterized their modification methods on planar material samples without microchannel geometries.[23,27,31] The assembly of PDMS devices typically requires the exposure of the inner channel surfaces to an additional plasma step to enable bonding. Therefore, coatings that have been applied before assembly may be destroyed by the reactive plasma.[32] Initial efforts have demonstrated the utility of microchannel-based coatings to optimize properties such as surface contact angle and electro-osmotic flow for protein separation applications.[25,26,33,34] Other groups have tested nonplasma based or nonsilanized coating methods both in microchannels and on planar PDMS surfaces in the context of developing protein resistant and blood contacting materials.[18,35–38] To date however, no study has described the plasma pretreatment-based silanized coating of intact PDMS microchannels and the long-term hemocompatibility testing of those channels.

In the current work, we describe a method to apply a siloxane-bound poly(ethylene glycol) (PEG) coating to an arbitrary, enclosed microchannel network (bed) after assembly. Single microchannels were coated and characterized using contact angle and XPS analyses. These microchannels were then evaluated for stability and basic functionality through physiologic exposure to fibrinogen solutions, platelet-rich plasma (PRP), and whole blood. More complex, branching microchannel networks were coated and exposed to whole blood as a more robust measure of long-term hemocompatibility.

MATERIALS AND METHODS

Materials

SU-8 2035 permanent epoxy negative photoresist and SU-8 developer were purchased from MicroChem (Newton, MA), while 2-isopropanol was obtained from Sigma Aldrich (St. Louis, MO). Glutaraldehyde solution, ethanol, and hexamethyldisilazane (HMDS) were also purchased from Sigma Aldrich. Sylgard 184 silicone elastomer base and curing agent were purchased from Dow Corning (Midland, MI). 2-[methoxy(polyethyleneoxy)propyl]trimethoxysilane (PEG-silane), tech-90, 6–9 C_2H_4O groups was obtained from Gelest (Morrisville, PA). Acetone, 99.8%, extra dry was purchased from Acros Organics (Geel, Belgium). Dulbecco's phosphate-buffered saline (PBS) and Calcein AM dye were purchased from Life Technologies (Grand Island, NY). Fluorescein isothiocyanate-labeled human fibrinogen (FITC-Fg) was obtained from Molecular Innovations (Novi, MI). Heparin sodium injection USP (1,000 USP units/mL) was obtained from Hospira (Lake Forest, IL).

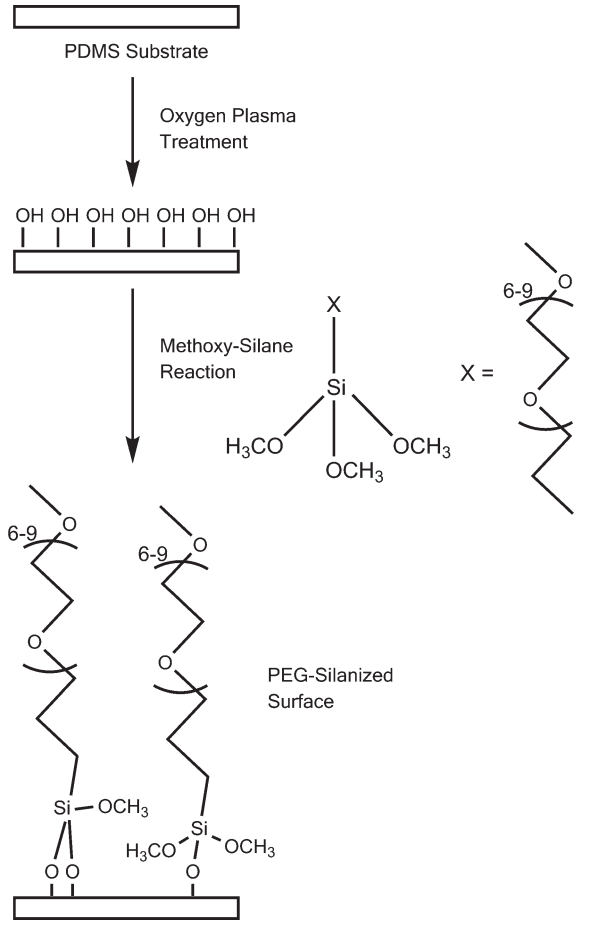

FIGURE 1. Schematic of the silanization reaction between a methoxy-silane molecule and an oxygen plasma treated PDMS surface. In this work, a PEG-functional silane is used.

Microfabrication of PDMS microchannels

To create a pattern for the PDMS microchannel structures, a mold was made on a 4 inch silicon wafer using soft lithography microfabrication techniques.[39] A layer of SU-8 photoresist was spin coated onto the silicon wafer using a Laurell Technologies WS-650S-6NPP/LITE spin coater (North Wales, PA), with the thickness corresponding to the height of the casted channels. Transparency masks were printed using an

inkjet printer with the channel length and width features and a Kinsten KVB-30D UV exposure unit (Australia) was used to crosslink and pattern the photoresist. The same methodology was used to create molds for a single microchannel with a height of 100 μm (length and width of 5 × 1 cm) and a branching microchannel network, with varying channel heights of 225, 60, and 20 μm as well as varying lengths and widths. The branching microchannel network consists of a flow path in which fluid enters the tallest channels, branches off two times into many smaller microchannels (60 and then 20 μm tall), and then exits the network in the opposite way by condensing from many small channels into the largest ones. This configuration allows for the applied surface coating to be tested in many different microfluidic geometries simultaneously.

Sylgard 184 base polymer was thoroughly mixed with curing agent at 10:1 w/w and degassed in a vacuum desiccator for 1 h. The mixture was then poured over the prepatterned silicon wafers and cured at 80°C for 1 h. Additionally, polymer mixture was poured into an empty polystyrene dish to form a flat top covering for the channels. The cured polymer was then peeled from the molds, allowing individual channels or microchannel beds and corresponding top sections to be cut out (Fig. 2). Holes were punched at both ends of the channel tops, and short sections of silicone tubing were bonded to the holes to allow for fluidic access.

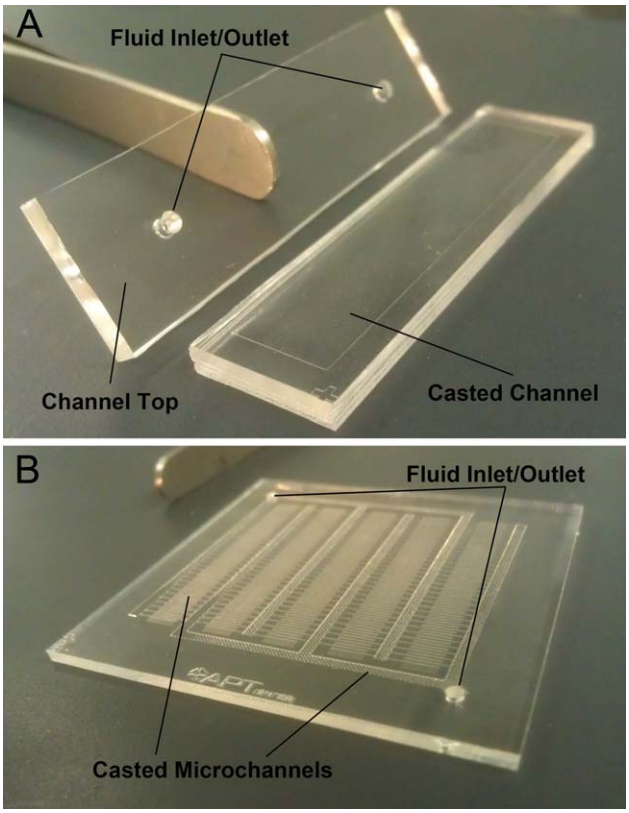

FIGURE 2. Unassembled single PDMS microchannel (A) and branching microchannel network (B). The single microchannel (A) was used in XPS studies, to test coating hydrophilicity over time, to measure protein adsorption after extended periods of aging, and to determine platelet adhesion. The complex microchannel network (B) was used to test long term hemocompatibility under flow with whole blood. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Assembly and application of PEG coating

To assemble the single microchannel structure or complex microchannel bed, one channel bottom and a flat top section were placed in a Nordson MARCH PX-250 Plasma Cleaning System (Concord, CA) powered by an MKS ACG-3B RF Plasma Generator (Andover, MA). The two channel components were exposed to oxygen plasma under vacuum (900 mTorr O_2, 25 W, 25 s).[40] The activated surfaces were then brought into contact and bonded to form an irreversibly sealed microchannel with connected tubing ports at either end. A 50% (v/v) PEG solution was made by diluting 0.5 mL PEG-silane in 0.5 mL acetone. After bonding, the channel was immediately filled with the PEG solution and allowed to react for 1 h at room temperature (Fig. 1). The channel was then thoroughly washed with deionized water, cleared with air, and stored in an ambient environment until use. Uncoated control channels were assembled using the same process without exposure to a PEG-silane solution. These uncoated channels were stored for at least 6 days before use to allow for the complete hydrophobic recovery of the inner channel surfaces.[25] Coated single microchannels were used to validate the application, stability, and basic functionality of the coating. The branching microchannel beds were then used to more rigorously test the long-term performance of the coating under whole blood flow.

Contact angle measurement

The contact angle of single microchannel surfaces was measured using the sessile drop method on a custom-built goniometer (deionized water, 8 μL drop size). Images of the water droplets were taken with a Tucsen TCA 1.31C camera (Fujian, China) and the droplet angles were later measured using a custom Matlab program. Four material samples for each group were initially fabricated and remeasured at each time point throughout the study. The contact angle was measured on at least three different locations on each sample and averaged.

X-ray photoelectron spectroscopy (XPS)

Uncoated PDMS and PEG-coated material samples from single microchannels were analyzed for elemental composition using a PHI VersaProbe XPS Microprobe (Chanhassen, MN) at the Swagelok Center for Surface Analysis of Materials, Case Western Reserve University. Each sample was scanned using a 15° incident angle, averaging the signal over a 0.3 × 0.7 mm area (300 μm ion beam size). Survey scans were performed over 0–1,100 eV with a pass energy of 93.90 eV, while high resolution C1s scans used a 23.50 eV pass energy. Elemental binding energies were referenced to the Si2p binding energy of silicone at 102.0 eV.[41]

Protein adsorption study

To equilibrate the material surfaces to an aqueous environment, PEG-coated and uncoated single microchannels were filled with PBS for 30 min at room temperature and then

emptied immediately prior to the experiment. FITC-labeled human fibrinogen was diluted to a final concentration of 0.5 or 2.0 mg/mL in PBS and warmed to 37°C in a water bath. The protein concentrations were chosen for physiological relevance, with 2.0 mg/mL being approximately the level of fibrinogen found in normal human blood.[42] This solution was then pumped through either a PEG-coated or uncoated control channel at 0.7 mL/min. using a Cole Parmer Masterflex L/S 7550 peristaltic pump (Vernon Hills, IL) for 1 h. This flow rate resulted in a wall shear stress of ~5 dynes/cm^2 within the channels, mimicking the shear experienced by the pulmonary vessels.[43] PEG channels were stored in an ambient environment after coating for various periods before testing, either 0, 5, 10, 14, 21, or 28 days (three separate channels per time point). The 2 mg/mL experiment at time point 0 was also repeated using an extended protein exposure time of 8 h. Following protein flow, the channel was washed briefly with PBS at the same flow rate and cut apart to allow for analysis of the inner protein-exposed surfaces.

For fluorescence measurement, 5 mm-diameter circular samples of both channel surfaces (at least 8 samples/channel) were punched out and placed in a 96-well plate. The fluorescent signal was then measured using a Molecular Devices SpectraMax M2e spectrophotometer (Sunnyvale, CA). To estimate the adsorbed protein mass on the material samples, a serial dilution of FITC-Fg in solution was prepared and measured for fluorescent intensity, creating a standard curve.

Platelet and blood cell adhesion study

Freshly drawn human blood was obtained from consenting, aspirin-refraining donors according to IRB protocol and treated with sodium citrate at 10:1 (v/v) to prevent short-term coagulation. The whole blood was then centrifuged to separate PRP from the white and red blood cell layers. After removal from the cell layers, calcein AM dye was added to half of the PRP volume according to the manufacturer's instructions to fluorescently label the platelets. The remaining PRP volume was left unlabeled to be used for platelet resuspension. Following calcein staining, all PRP was centrifuged a second time to collect the labeled platelets in a pellet. The fluorescent platelets were then resuspended in clean platelet-poor plasma, resulting in a volume of calcein-stained PRP with minimal background fluorescence. An additional volume of nonseparated, nonstained whole blood was also set aside for a separate experiment.

As in the protein adsorption study, the microchannel in single microchannel devices was equilibrated with PBS for 30 min at room temperature and then emptied immediately prior to the experiment. The stained PRP was warmed to 37°C in a water bath and pumped through either a PEG-coated (0 days storage) or uncoated control channel at 0.7 mL/min for 30 min. The channels were then washed briefly with PBS at the same flow rate and cleared with air. The washed channels were immediately imaged using a Zeiss Observer D.1 fluorescence microscope and AxioCam MRm camera (Oberkochen, Germany) with a FITC filter at 10× magnification. At least 16 images per channel were taken, covering the entire channel length and including both the center and edge regions. The images were analyzed using a custom Matlab program, measuring the total fluorescent area per image and averaging the values across all images.

In a separate experiment, the unstained whole blood was flowed through uncoated and PEG-coated microchannels using the protocol described above. Following washing, these microchannels were cut apart and the inner surfaces were prepared for SEM imaging. The material samples were first immersed in a 2.5% glutaraldehyde solution in PBS, allowing the adhered cells to fix overnight at 4°C. After washing with deionized water, the samples were dehydrated in a deionized water/ethanol gradient of 50, 70, 95, and 100% ethanol for 15 min each. Finally, the surfaces were covered with hexamethyldisilazane twice for 15 min and allowed to air dry in a fume hood. The samples were then mounted on SEM stubs and sputter coated with 5 nm of palladium. Images of each channel surface were collected at 3.0 kV using a FEI Nova NanoLab 200 FEG-SEM/FIB scanning electron microscope (Hillsboro, OR) at the Swagelok Center for Surface Analysis of Materials, Case Western Reserve University.

Blood flow through complex microchannel networks

Freshly drawn whole porcine blood was obtained from donor animals according to IACUC protocol and treated with a clinically relevant level of heparin (6.7 U/mL). Prior to the experiment, the flow loop tubing and devices with branching microchannel networks were primed with PBS at room temperature to aid in the complete and even filling of the channels with blood. Forty micrometer Pall blood transfusion filters (Port Washington, NY) were connected to the flow circuit upstream of the microchannel beds to remove any clots that may form due to contact with the blood reservoir or circuit tubing. The blood was heated to 37°C in a water bath and pumped through either a PEG-coated (1 day storage) or uncoated control microchannel bed at 0.7 mL/min, recirculating to the blood reservoir in a closed loop. The blood reservoirs were periodically stirred to prevent settling of the blood and maintain even blood mixing. The pressure drop across the microchannel beds and blood filters was monitored throughout the experiment using Honeywell 26PCCFA6D pressure sensors (Golden Valley, MN). Pressure measurements were taken across the networks every 2 min for the first 30 min, every 5 min from 30 to 60 min, every 10 min from 1 to 2 h, and every 15 min from 2 to 7 h. If the networks were left overnight after 7 h, measurements were automatically taken every minute. The pressure sensors were powered using an Agilent E3620A Dual Output DC Power Supply (Santa Clara, CA) and the voltage signal recorded through a Keithley 2000 Multimeter (Cleveland, OH). The multimeter was computer-controlled via an IEEE-488 GPIB connection, and the digital signal was converted to pressure values using a custom Visual Basic program. The experiment was continued for each microchannel bed at least until the initial pressure drop doubled in magnitude, where a doubling in pressure was defined as the

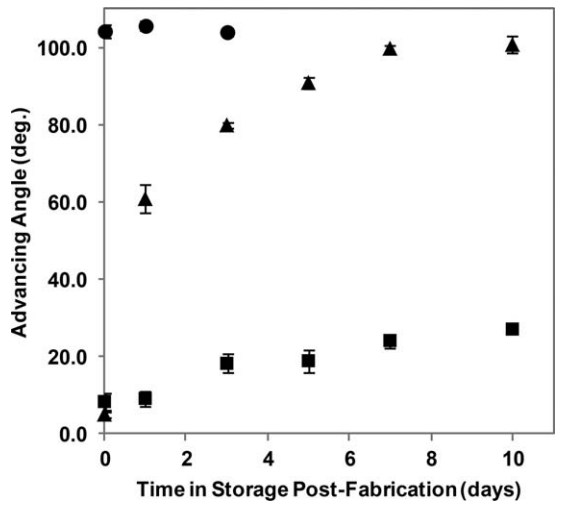

FIGURE 3. Water contact angle on unmodified PDMS (●), plasma-oxidized PDMS (▲), and PEG-grafted microchannel surfaces (■). Data are the mean ± std. error ($n = 4$).

lifetime or the time until failure of the bed. In cases where a microchannel bed lasted longer than 7 h, the experiment was continued overnight to 19 h and the time at which the pressure had doubled was determined from the dataset. The initial pressure drop was defined as the average of the first three pressure measurements obtained for a given bed. Immediately after ending an experiment, the networks were flushed with PBS for 20 min at 0.7 mL/min and imaged to qualitatively observe surface bound clots.

Statistics

Three single microchannels or four microchannel beds for both the PEG-coated and uncoated groups were tested for each experiment. On each single microchannel, at least three measurements for contact angle, protein adsorption, and platelet adhesion were made and averaged. For microchannel beds, each 0–7 h pressure time point is the average of 30–50 measurements taken over a 5 s window. Time points after 7 h are the average of 10 measurements over a 10 min window. Results were analyzed in Excel using a student's *t*-test and ANOVA. Plotted data for contact angle, protein adsorption, platelet adhesion, and microchannel bed lifetime are presented as mean values ± standard error.

RESULTS

Surface characterization

Figure 3 displays the water contact angle of the neat PDMS substrate, plasma-oxidized PDMS substrate, and the PEG-coated microchannel. Contact angle measurements were made for up to 10 days following treatment. Unmodified, hydrophobic PDMS exhibited a characteristically high contact angle of ~105°.[21,25] Plasma-oxidation of the PDMS decreased the hydrophobicity of the surface, as indicated in the drop of contact angle from 104.4° to 5.2° immediately after treatment. Within one day after plasma-oxidation, the resulting PDMS became more hydrophobic (increase in contact angle from 5.2° to 61.0°). Within 5–7 days of plasma-oxidation, the oxidized surfaces displayed contact angles comparable to untreated PDMS. In contrast, the PEG-modified microchannel surface retains a high level of hydrophilicity over a period of 10 days, exhibiting a maximum contact angle of only 27.2°.

The presence of the PEG layer was also confirmed through XPS analysis of the microchannel (Fig. 4). Survey scans of neat PDMS and PEG-coated PDMS highlight the changes in elemental composition after PEG grafting, namely the increase in carbon and oxygen content and corresponding decrease in silicon. A high-resolution scan of the C1s binding energy depicted the C—Si bond peak of PDMS at 284.4 eV on both samples. However, the dual peak signal of the PEG sample included the presence of the characteristic C—O bond[25] at 286.5 eV,[41] not found on unmodified PDMS.

Protein adsorption to PEG-coated microchannels

In addition to general material characterizations, coated microchannels were evaluated in terms of their functionality when exposed to biological fluids. Fluorescently-labeled fibrinogen at physiologically relevant concentrations was flowed through single microchannel structures for one hour at 37°C (Fig. 5). This experiment was performed on PEG-coated microchannels ranging from 0 to 28 days old in order to draw conclusions about the stability and efficacy of the coating after periods of storage in an ambient environment. The PEG-coated microchannels showed significantly reduced adsorption for both protein concentrations as compared to uncoated controls at all time points. To determine whether adsorption would increase after a longer protein exposure time, the experiment was repeated at time point 0 for a duration of 8 h [Fig. 5(A), open symbols]. It was found that the resulting adsorption levels on either uncoated or PEG-coated channels did not significantly change from the 1 h experiment. Most importantly, there is no statistical difference in the protein adsorption levels within the PEG group for either concentration.

Platelet adhesion to PEG-coated microchannels

Freshly coated single microchannels were also exposed to human PRP to investigate the extent of platelet adhesion to the coated surfaces. After 30 min of exposure to PRP flow, fluorescent images were taken of the channel surfaces and the area covered by platelets was measured (Fig. 6). PEG-coated channels exhibited nearly undetectable levels of platelet adhesion. In comparison to uncoated PDMS, PEG-coated microchannels decreased platelet adhesion by more than 50-fold.

A similar difference was seen in the SEM images of whole blood-exposed microchannels (Fig. 7). Extensive platelet adhesion as well as protein adsorption is seen on uncoated microchannel surfaces, while very little of either is visible on PEG-coated channels. Morphological differences between the platelets on each surface are also apparent, with platelets on the uncoated material appearing branched and in an activated state [Fig. 7(C)] as compared to the round, unactivated platelets on the PEG surface [Fig. 7(D)].

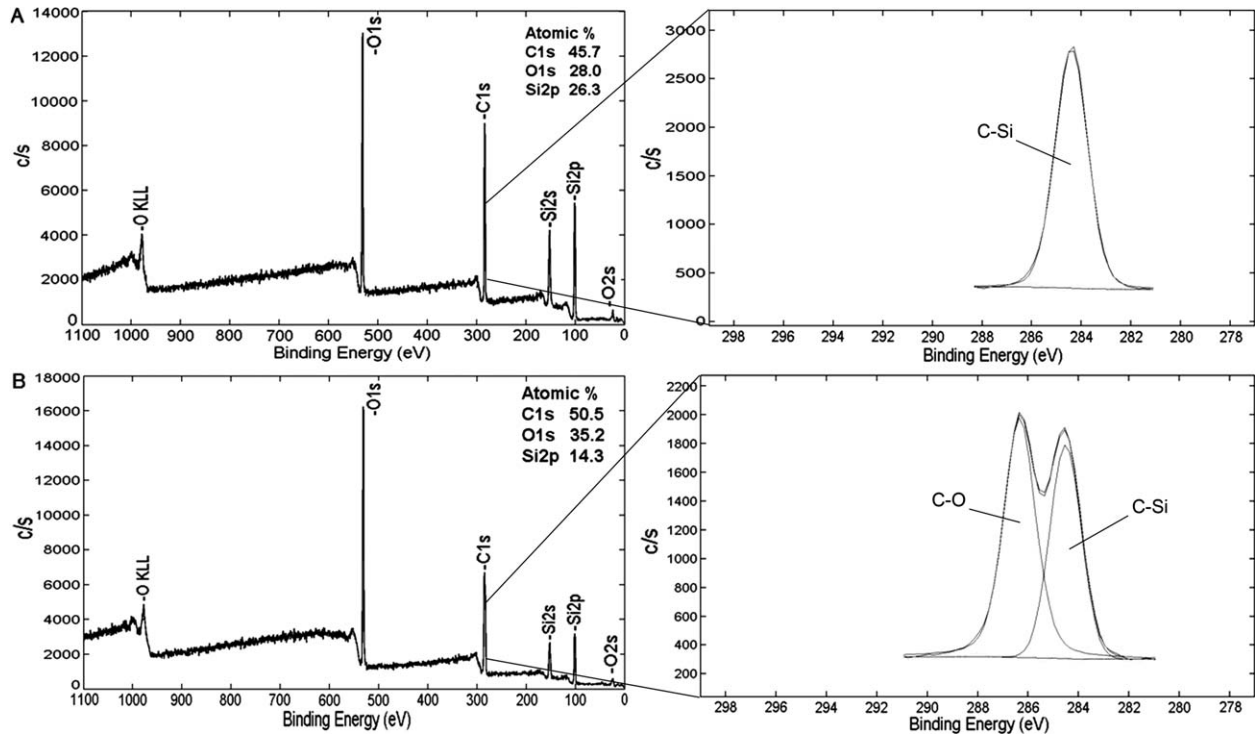

FIGURE 4. XPS spectra of unmodified (A) and PEG-grafted (B) PDMS surfaces. High resolution scans (right insets) of the C1s signal highlight the presence of the C—O bond within the grafted PEG chain.

Long-term thrombogenic response in complex microchannel networks

The trans-device pressure drop of both PEG-coated and uncoated microchannel networks was measured over time while flowing whole heparinized blood through the devices [Fig. 8(A)]. The average time until the initial pressure doubled for each experimental group is displayed in Figure 8(B), with the PEG-coated microchannel networks exhibiting a lifetime of more than 16× longer than uncoated controls. Figure 9(A) is a representative image of a microchannel network filled with blood during the flow experiment. Figure 9(B) contains representative macroscopic and microscopic images of residual clotting within the PEG-coated and uncoated microchannel networks following a postexperiment PBS wash.

DISCUSSION

Coating application

Oxygen plasma treatment. Previous studies investigating oxygen plasma treatment of PDMS have documented the hydrophobic recovery of the oxidized surface, resulting in the rapid degradation of functional groups and hydrophilic character.[21,44] In contrast, the stability of our PEG surface's contact angle over time is an indication of the grafted molecular layer formed during the coating reaction. It is believed that this is because a large PEG chain is able to sterically and electrostatically resist rearrangement into the PDMS bulk as well as bond degradation in ambient storage. The observed stability also suggests that the coating may remain functional for an extended period of time after application, as hydrophilicity plays a major role in biomaterial hemocompatibility.[18,19]

PEG grafting. Following plasma activation, PEG-silane molecules can react with and graft to the PDMS surface. Because of the transient nature of plasma-generated reactive sites on PDMS, procedure timing, PEG chain length, solute concentration, and zero flow during reaction were determined to be key parameters in generating an effective and stable surface coating. Anhydrous acetone was specifically chosen as a solvent for the coating solution due to its low tendency to swell the PDMS substrate as compared with other organic solvents.[45] Furthermore, acetone will not react with the functional methoxy-silane groups on the PEG chain needed for the silanization reaction. Dilution in a solvent is also important as it allows for the increased mobility of PEG molecules in solution, leading to increased interaction with the reactive hydroxyl groups on the PDMS surface.

Other studies have used similar approaches to PDMS modification, although they were performed on planar material samples as opposed to a microchannel model.[23,27,31,37] Coating processes developed in this way may not translate well to microfluidic structures due to the harsh assembly conditions of most PDMS microfluidic devices.[32] Any preapplied molecules would likely be cleaved, altered, or inactivated by oxygen radicals by plasma exposure during device bonding. To solve this problem, our work uses the same plasma activation step to achieve PDMS bonding and activation of the material surface for PEG grafting. PEG functionalization is performed immediately after PDMS bonding on a

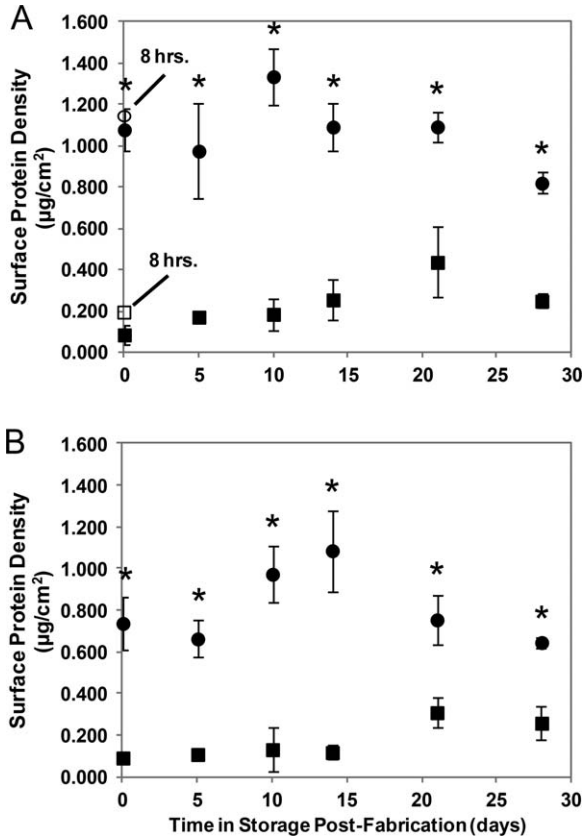

FIGURE 5. Fibrinogen adsorption from 2.0 mg/mL (A) and 0.5 mg/mL (B) solutions to uncoated PDMS (●) and PEG-coated (■) microchannel surfaces following 1 h of protein exposure. An 8 h experiment was also performed at the 0 day time point, highlighted in (A, ○ and □). Data are the mean ± std. error ($n = 3$). *$p < 0.05$.

fully assembled device. In our studies, the timing of this step was discovered to be crucial to coating integrity. Process optimization revealed that the PEG solution must be introduced into the channel within 45 s to 1 min of removal from the plasma system. As time elapses after plasma treatment, hydroxyl bonding sites disappear due to the hydrophobic recovery of PDMS. This phenomenon is largely due to mobile oligomer chains from the PDMS bulk migrating to the surface, decreasing hydrophilic character and increasing the contact angle back to near that of untreated silicone.[21,22] It was observed that excessive hydrophobic recovery results in fewer PEG chains bonding to the surface, a higher initial contact angle, and more rapid degradation of the coating during storage. In addition to coating application issues, the functionality of previous studies planar samples may not be representative of a coating's performance under shear stress and within an enclosed microfluidic geometry.[46,47]

Within the realm of microfluidic modification, much past work has been focused on applications outside of blood-contacting biomaterials and hemocompatibility.[24–26,33,34] Studies that have looked at blood-material interactions have used nonsilanized coating methods,[36] utilized a planar testing model as described above,[48] or have not investigated the degradation of their coating over time.[37] One recent study does describe the coating and thrombogenic evaluation of an intact microchannel, but is limited to a relatively large channel height (350 μm) and short-term blood exposure (1 h).[14] Our work seeks to provide a platform for durable microfluidic surface modification and physiologically relevant evaluation, namely applying a silanized PEG coating to complex assembled microchannel structures and investigating the long-term hemocompatibility of that coating within the microchannel geometry.

Hemocompatibility assessment

Protein and platelet interactions. The physical scale of microfluidic devices lends them directly to applications that mimic or interact with the function of human capillary beds, often necessitating high hemocompatibility. Thus, our protein adsorption and platelet adhesion experiments were designed to mimic physiologic conditions of microscale pulmonary vascular flow. Fibrinogen was chosen as the protein of interest due to its key role in the intrinsic coagulation cascade and relative abundance in human plasma.[49,50] PEG-coated microchannels exhibited drastically decreased fibrinogen adsorption at physiologic temperature, concentration (~2 mg/mL) and shear stress (~5 dynes/cm^2).[43] It was also notable that protein adsorption levels did not significantly change at any time point of channel aging, revealing that our coating process is capable of producing protein resistant surfaces with a "shelf life" of at least several weeks. Overall, this resistance to fibrinogen adsorption is important to long-term hemocompatibility, as surfaces which adhere low levels of fibrinogen have been shown to reduce the coagulation response of blood.[51,52]

We have previously shown that PEG-grafted substrates with reduced protein adsorption also prevent subsequent cell adhesion.[53,54] Therefore, we also examined the ability of our PEG-grafted microchannels to resist platelet adhesion. As anticipated, a significant reduction in platelet adhesion

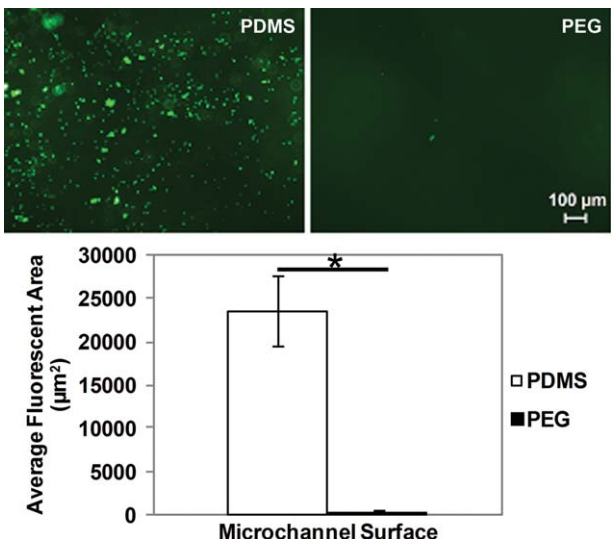

FIGURE 6. Fluorescent platelet adhesion to microchannel surfaces. Data are the mean ± std. error ($n = 3$). *$p < 0.05$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

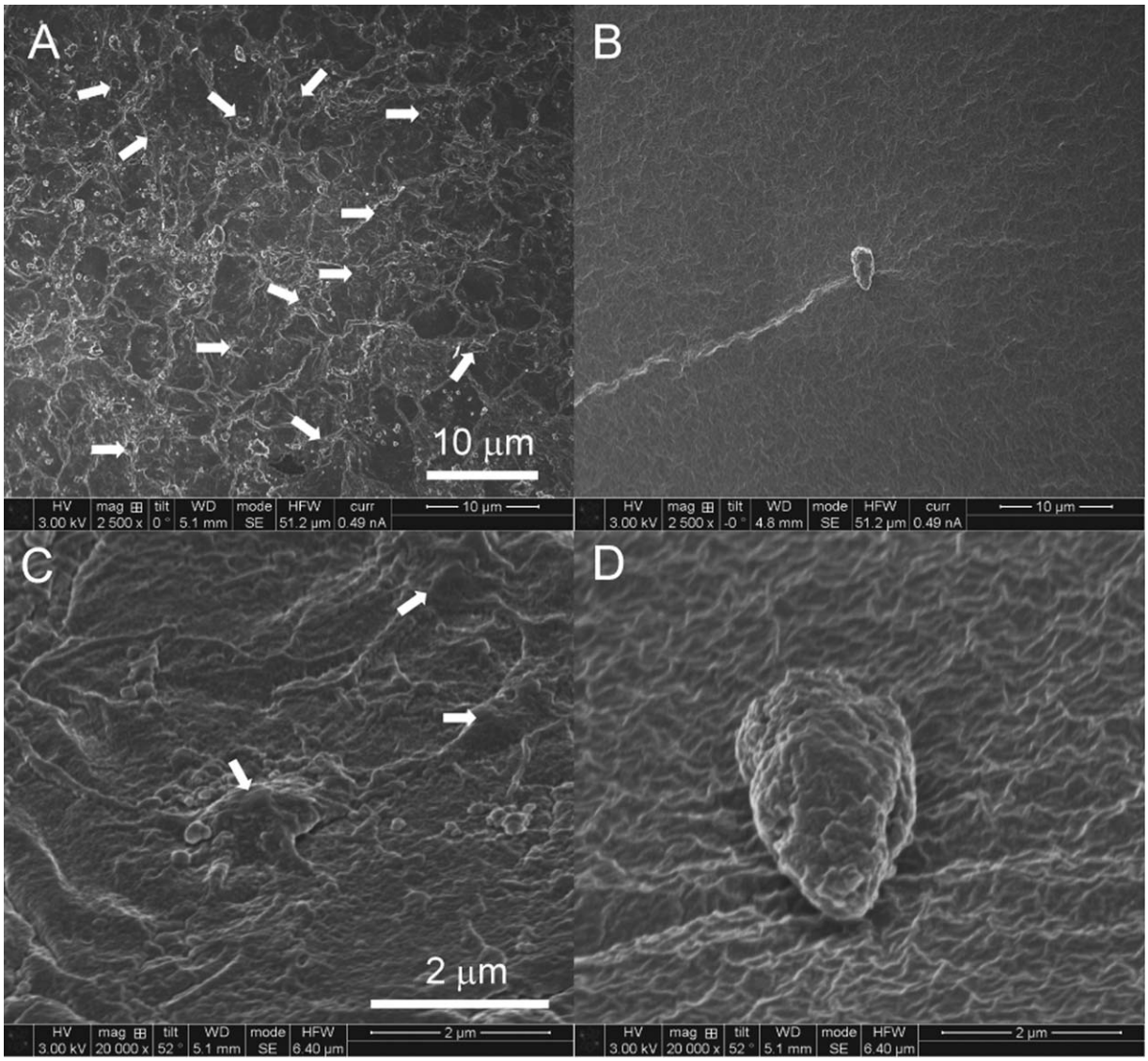

FIGURE 7. Representative SEM images of platelets adhered to unmodified (A, C) and PEG-coated (B, D) microchannel surfaces. Arrows in A and C identify activated platelets. Images A and B at 2500× magnification, C and D at 20,000×.

was seen on PEG-coated channel surfaces, as compared to unmodified PDMS microchannels. For platelet adhesion experiments, a flow rate of 0.7 mL/min was again used to mimic physiological shear stress. The observed lack of platelet adhesion presents further evidence of improved blood compatibility on our PEG-coated channels (Fig. 6). It has been suggested that small platelet aggregates form the initial precursors of a larger-scale clotting response.[51]

SEM images of surface adhered platelets reveal additional differences between the two surfaces. High-magnification images show platelets adhered to uncoated surfaces to be spread out and branching [Fig. 7(C)], as compared to the typical rounded shape seen on PEG samples [Fig. 7(D)]. The branched appearance is indicative of activated platelets, which act to bind additional platelets and release pro-coagulatory factors, continuing the coagulation cascade on a biomaterial surface.[51] Thus, we see that not only does the PEG-coated microchannel adhere far fewer cells, but that they are not bound in a pro-coagulatory state. Because of the use of whole blood in the SEM experiment, we also see evidence of significant protein adsorption and fibrin formation [Fig. 7(A)] in addition to platelet adhesion and activation on the uncoated microchannel surface. This combination of events, resulting in surface-bound clotting, is not apparent on the relatively pristine PEG channel [Fig. 7(B)]. Viewed together, these results demonstrate that our coating method is capable of producing highly hemocompatible microchannel surfaces that remain functional over a period of weeks.

Long-term blood flow and coagulation in complex geometries. Following the initial protein and platelet testing in single microchannel devices, the coating was applied to a more complex microfluidic system consisting of thousands

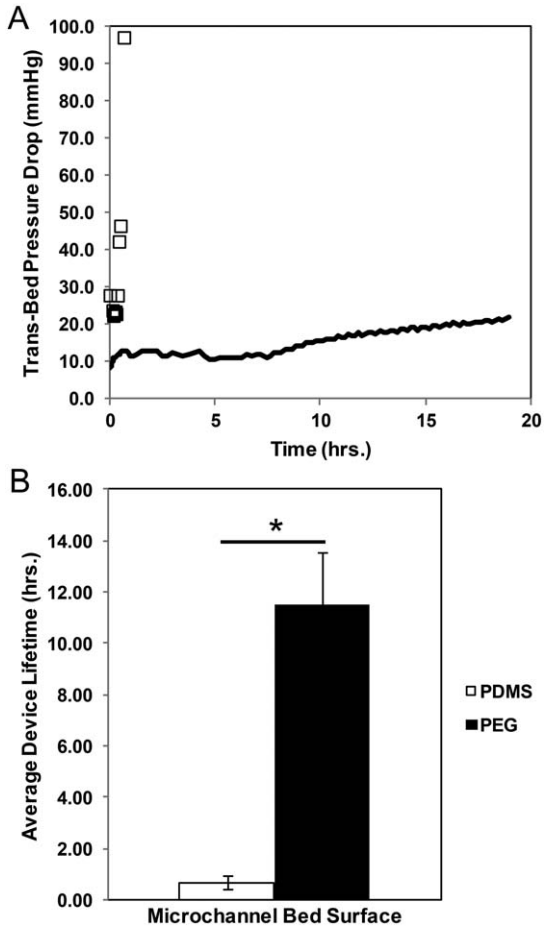

FIGURE 8. (A) Representative plot of microchannel bed pressure drop over time for uncoated PDMS (□) and PEG-coated (−) beds. (B) Average lifetime for uncoated vs. PEG-coated beds. Data are the mean ± std. error ($n = 4$). *$p < 0.05$.

of branching microchannels of varying dimensions. This model allowed us to more rigorously test the coating's ability to resist blood coagulation by using a flow path including dimension changes, potential areas of stasis and turbulent flow, and channel heights on the scale of capillaries and red blood cells (20 μm).

At a fixed blood flow rate, the pressure drop across a microchannel bed correlates directly with the fluidic resistance of the microchannels. In our system of unchanging PDMS channel dimensions and area, increases in resistance (pressure values) were attributed to coagulation and thrombosis within the microchannels.[14]

As expected from protein and platelet adhesion results, PEG-coated microchannel beds displayed high hemocompatibility and a drastically increased lifetime under whole blood flow as compared to uncoated controls (Fig. 8). Despite being exposed to blood flow for 19 h compared to 45 min for the control, a PEG-coated channel bed retained many fewer surface-bound clots after washing [Fig. 9(B), top]. Microscopic images were also taken of the smallest microchannels in areas where no clots were visible to the naked eye [Fig. 9(B), lower insets]. Surface-adhered platelets and red blood cells can be clearly seen in the uncoated channels, while PEG-coated channels remain clear even after 25× longer blood exposure than the uncoated control. Despite the use of heparin to slow the coagulatory response, significant clotting in less than 1 h was seen in uncoated devices. This highlights the need for the PEG coating, especially in applications necessitating periods of longer use. A similar difference in comparative biological response was seen in the platelet adhesion experiment (Fig. 6), which used sodium citrate as a short-term anticoagulant, suggesting that in this model the type of anticoagulant used does not have a significant impact on the relative blood response between uncoated and PEG-coated microchannel surfaces.

In addition to delaying the intrinsic coagulatory response, the hydrophilic PEG coating appeared to decrease the initial pressure drop of the microchannel beds, although the difference was not statistically significant. However, in all experiments, the initial pressure for the PEG-coated bed (avg. ± std. dev. of 15.2 ± 7.2 mmHg) was lower than that of the uncoated control (44.6 ± 29.8 mmHg). It is believed that the lack of significance due to the large variation stems from characteristic differences between the blood samples such as hematocrit.

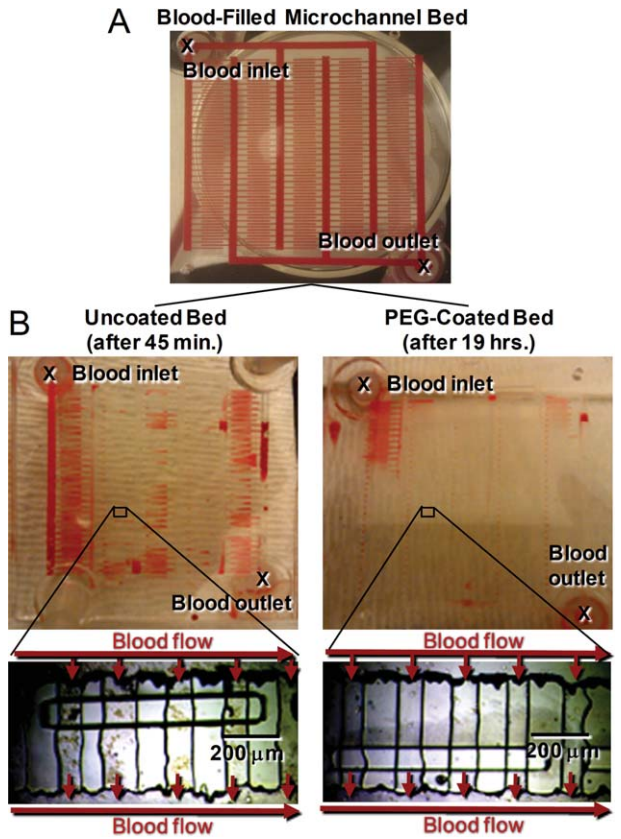

FIGURE 9. (A) Example blood-filled microchannel bed during experiment. (B, top) Representative post-wash images of uncoated and PEG-coated microchannel beds and (B, lower insets) 10× microscopic images of the smallest microchannels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

It is interesting to note that when the PEG-coated microchannel beds were left under blood flow overnight after 7 h, stirring of the blood reservoirs was stopped. It was during this time period that the majority of the pressure increase was seen [Fig. 8(A)]. It is possible that at least some of this pressure increase is due to an increase in relative blood viscosity as opposed to microchannel coagulation, as the cellular component of the blood separates to the bottom of the reservoir over time. Future experiments will incorporate automatic gentle agitation of the blood reservoirs to ensure a constant viscosity.

These results present the strongest evidence of our PEG coating's ability to improve the hemocompatibility of PDMS microchannels, particularly in a complex microfluidic device and over a long period of blood exposure.

Relevance to microfluidic devices. Hemocompatibility is crucial for microdevices in blood-contacting applications, as it delays the intrinsic coagulation response and allows for prolonged, unobstructed flow through the device channels. Coated devices will therefore have increased functional lifetimes and thromboembolism will be less of a risk during *in vivo* device use. Because of the simplicity of the coating application, we believe that this platform is immediately applicable to a wide range of PDMS microfluidic devices. Specifically, our group will be applying the coating to a microfluidic artificial lung,[10] aiming to extend the functional lifetime of the device. Other applications where improved long-term hemocompatibility may be desirable include microfluidic kidneys,[55] synthetic vasculature,[56] and blood separation devices.[57]

The reaction process is rapid, uses common and inexpensive reagents, and occurs at room temperature, allowing for coatings to be applied with minimal additional equipment or training. Also, because the method utilizes a simple silanization reaction, stable surfaces can be produced using any coating molecules with a terminal methoxy-silane group. Thus, microchannels could be easily custom-coated with any number of silane-functional compounds, such as proteins, charged groups, bioactive molecules, or other polymer chemistries. The long term shelf life of the applied coating also allows for devices to be fabricated well in advance of their intended use and stored until needed without sacrificing functionality. Using the same plasma activation step for both PDMS bonding and coating formation may be a drawback to devices which require multiple steps during assembly. However, this strategy is well suited for use with most current microfluidic designs, and with modification may be successfully applied to more complex structures.

CONCLUSIONS

In this work, we have developed a method to apply a silanized PEG coating to intact microchannel structures after assembly. The long-term stability of the coating was characterized for aging periods of up to 28 days, demonstrating an extended shelf life of coated structures. The ability of the channels to resist fibrinogen adsorption under physiologic conditions was investigated as well as platelet adhesion from human blood. Finally, the coating demonstrated a significant hemocompatibility improvement in complex microfluidic devices, increasing the lifetime of coated microchannel networks to more than 11 h under whole blood flow, 16× longer than controls. It is envisioned that this approach can be used as a coating platform for other microfluidic applications to ensure long-term hydrophilicity or hemocompatibility.

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