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Research Article

Enhanced Long-term Antithrombogenicity Instigated by Covalently-Attached Surface Modifier on Biomedical Polymers

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Abstract

During a variety of medical procedures such as renal dialysis, bypass surgery, and lung transplantation patient blood is exposed to the surface of a number of polymeric materials such as polycarbonate (PC), poly (vinyl chloride) (PVC) and polysulfone (PS) for a period up to several days. Such exposure may result in undesirable protein-material interactions that can potentially trigger deleterious biological processes including thrombosis, which may be responsible for other complications such as cognitive disability. In order to address this issue, we have further examined the behavior of an ultrathin antifouling and antithrombogenic coating based on monoethylene glycol silane surface chemistry. Samples of polymeric substrates were exposed to

blood flow at a shear rate of ~20 s^{-1} for time periods of 3 and 6 hours, as well as 3 days. No additional anticoagulant chemistry was applied in the experiments. For all time periods, platelet adhesion, aggregation, and thrombus formation on the coated surfaces was inhibited compared to bare substrates (coated PC performing the best, followed by PVC-, and then PS-coated), strongly supporting the results of previous research conducted over far shorter blood contact times.

Keywords
Blood; antithrombogenicity; hemocompatibility; organosilane adlayer; medical polymers; polycarbonate; poly (vinyl chloride); polysulfone

1. Introduction

Annually, approximately 3000 surgeries are performed per 100,000 head of population, which translates to around ~232 million operations worldwide [1]. As the world population continues to grow in tandem with the expected overall rise in life expectancy and age, the number of surgeries is anticipated to increase significantly, at least according to the current trend [1]. Many such surgeries and other medical procedures require associated medical equipment, instruments and circuitry to come in contact with bodily fluids including blood, which may stimulate biological processes notably those orchestrated by the immune and coagulation systems resulting in highly deleterious outcomes [2-5]. Indeed, postoperative complications such as organ dysfunction are an unfortunate reality. A particularly serious example is the indication that blood–surface interaction during hemodialysis and bypass surgery is linked to incapacitating brain disorder or cognitive disability [6, 7]. The implication here is that clots – formed at the hemo-incompatible surface of biomedical equipment – are transferred to the brain, where they lodge within blood vessels obstructing vital blood supply in the process. This cerebrovascular accident (or "stroke"), in turn, affects brain competence. One crucial consideration with respect to biological fluid–foreign material interaction is the duration of the specific procedure or surgery to be performed. This parameter will have a significant effect since higher contact times are very likely to result in an increased probability of medical complications. Typical durations for various procedures are as follows – apheresis (60-120 minutes) [8], hemodialysis (1-5 hours) [9, 10], hemofiltration (8-12 hours) [11], cardiopulmonary bypass surgery and extracorporeal membrane oxygenation (1-6 hours) [12]. In summary, most surgeries and other medical procedures commonly require 3 or fewer hours, but it is not rare that 6 or more hours may be needed.

In addition to the extracorporeal configurations mentioned above, there also exist issues associated with the ubiquitous *in vivo* employment of catheters (aside from the well-known and -characterized occurrence of infection introduced by such medical equipment). For example, the indwelling central venous catheter (CVC) has extensively been linked to thrombosis and has been reported in up to 33% of catheter uses, as well as up to 21 episodes/1000 catheter-days [13]. Other less common complications that may be related to the treatment of occluded catheters include intracranial

hemorrhage (ICH), major bleeding (MB), and embolism [14-16]. Other cases may result in systemic inflammation and accelerated arteriosclerosis. In view of the various deleterious medical consequences connected to biological fluid–surface interactions occurring daily in a great number of routine medical procedures/surgeries, there has been a long-standing need for the introduction of substrate interfacial modification that is capable of the provision of enhanced biocompatibility.

In the present paper, the hemocompatibility focus is on synthetic polymeric materials that are employed extensively both *in vivo* and extracorporeally. Due to their various intrinsic properties, such as flexibility and chemical stability, different polymers find variable applications in medical procedures [17]. Examples are poly (vinyl chloride) (PVC), which has been employed very widely for the fabrication of blood conduits, blood bags, catheters, and endotracheal tubes, as well as polycarbonate (PC), which is used as a container material for equipment associated with bypass surgery [18, 19]. In order to enhance the biocompatibility of polymeric (and other) materials, an enormous number of surface modifications and treatments have been attempted over a number of years; these are comprehensively reviewed in a recent book by Thompson *et al.* [20]. In addition to physical modification such as plasma treatment, a plethora of coatings have been studied including "bio-inspired" constructs based on single amino acids or longer peptides and peptoids, oligo- and polyethylene oxides/glycols, zwitterionic sulfo- and carboxybetaines and complex hybrids or derivatives of these chemical systems [21-23].

Recently, in our own work, we have successfully derivatized the surfaces of both PC and PVC with an ultra-thin (nanometer-thick), monoethylene glycol-based (MEG-OH) nanogel siloxane adlayer [24]. Regarding PC, the antithrombogenicity of this material was assessed in real-time using a perfusion chamber and fluorescently-labelled whole human blood flown for 5 minutes at a controlled shear rate of 1000 s^{-1}. Remarkably, platelet adhesion, aggregation, and thrombus formation on the MEG-OH coating were greatly inhibited (>97% decrease in surface coverage) compared to the bare substrate [25]. In a similar vein, a decrease in surface coverage by platelet aggregation in the PVC study exceeded 99%, for all the investigated shear rates (300, 900, and 1500 s^{-1}) [26]. Physico-chemical studies of the MEG-OH adlayer on Si/SiO_2 by neutron reflectometry [27] and molecular dynamic simulations [28] have suggested that the antifouling behavior of the adlayer is connected to surface hydration and how water organizes within and atop the film. The intrinsic water domain displays restricted molecular motion, which extends into bulk water to form a physically-distinct interfacial phase. The term "kosmotropic effect" has been used in this context.

In the present paper, we expand the scope of our antithrombogenic investigation and describe experiments wherein blood is exposed to MEG-OH-coated PC, PVC and polysulfone (PS) (Figure 1) over relatively long periods of time (up to 3 days). With regard to platelet integrity, it is known that platelets can indeed be stored at 22.0°C displaying a half-life of around 4 days. Thus, even in the longest experiment we conducted, it is very likely that the majority of the platelets are still alive after 3 days. This work demonstrates that blood incompatibility is not an issue for MEG-OH-coated PC, PVC, and PS when exposed to blood for lengthy time periods.

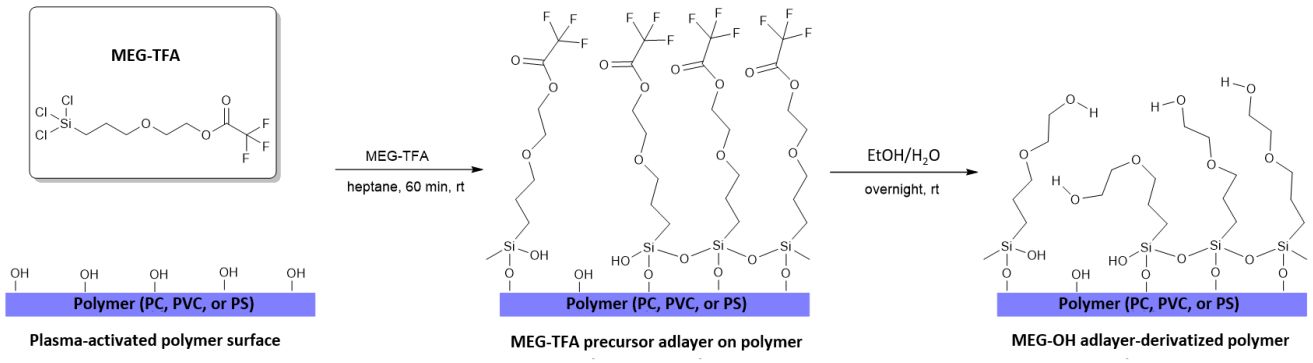

Figure 1 Schematic representation of the straightforward, two-step surface modification of polymer (PC, PVC, or PS) surface with antithrombogenic MEG-OH surface chemistry.

2. Materials and Methods

2.1 Materials

PC, PVC, PS (sheet) were purchased from McMaster-Carr (Elmhurst, USA). Sodium dodecyl sulfate (SDS), ethanol (95%), Dulbecco's phosphate buffered saline (PBS, CaCl2 and MgCl2 free – pH 7.4), and 3, 3'-dihexyloxacarbocyanine iodide ($DiOC_6$), were all purchased from Sigma-Aldrich, Canada and used as received. Heptane was obtained from Caledon Laboratories, Georgetown, Canada. The MEG-TFA precursor molecule for MEG-OH surface modification was synthesized as described previously [29].

2.2 Polymer Substrate Cleaning and Activation

PC, PVC, and PS substrates (0.5 × 0.9 × 0.16 cm in size) were rinsed under tap water and sonicated for 1 minute to remove any major residue. The polymer substrates were then washed in 1% SDS (prepared in tap water), sonicated for 5 minutes in 1% SDS, and then successively rinsed in 1% SDS (×1), tap water (×3), and finally 95% ethanol (copiously). The polymer substrates were then dried under N_2 gas and placed into a plasma cleaner (13.56 MHz, 30W, 150 mTorr) for various durations (depending on the polymer under study). After plasma cleaning, the polymer substrates were placed into a humidity chamber (80% relative humidity, room temperature) for overnight surface hydration.

2.3 Polymer Surface Modification with MEG-OH Adlayer

MEG-TFA surface modifier was diluted with heptane in a glove box kept under inert (N_2) and anhydrous (P_2O_5) atmosphere. Cleaned/activated polymer substrates were then individually placed in pre-silanized glassware that was subsequently filled with a 1 µL/mL solution of MEG-TFA/heptane (at a ratio of 1 mL/1 cm^2 solution volume/substrate surface area) before being placed on a rotary shaker at low speed for 1 hour. After treatment, the samples were removed from the glove box and then successively rinsed with heptane (×3), sonicated for 5 minutes in heptane, and then rinsed once more with this solvent. This rinsing procedure was repeated with 95% ethanol, before drying the samples under a gentle stream of N_2 gas. To convert MEG-TFA adlayers into MEG-OH surfaces, the samples were

individually soaked in 1 mL of a 1/1 (v/v) solution of 95% ethanol and tap water overnight at room temperature. Finally, the MEG-OH-coated surfaces were rinsed in 95% ethanol (×3), dried under a gentle N_2 stream, and then stored in sealed containers awaiting analysis/blood contact experiments.

2.4 X-ray Photoelectron Spectroscopy and Contact Angle Goniometry Adlayer Characterization

X-ray photoelectron spectroscopy (XPS) was performed with a Theta Probe Instrument (ThermoFisher Scientific). Samples were analyzed with monochromated Al Kα X-rays at a take-off angle of 90° relative to the surface. *Avantage* software (ThermoFisher Scientific) was used to perform peak fitting and data analysis with binding energy being calibrated to the main C_{1s} signal at 285 eV. Static contact angles were measured with a CAM101 goniometer (KSV Instruments) and deionized water (18.2 MΩ cm) as the test liquid.

2.5 Blood Contact Experiments

Whole blood was collected in Vacutainers from volunteers with no known blood disorders and labelled with $DIOC_6$ as described previously [25, 26]. The blood from the same volunteer was used for the experiments for equal amounts of control and MEG-OH-coated surfaces to avoid bias. A tubular flow chamber was built in house to expose surfaces to fluorescently-labelled blood and employed in a dark room to avoid any possible photobleaching. PC, PVC, or PS substrates – bare or MEG-OH-coated – were individually inserted into the flow-through chamber and whole blood flown over at a shear rate of ~20 s^{-1} (in a closed-loop system) using a peristaltic pump for 3 hours, 6 hours, as well as 3 days. All chambers were employed separately for the specific time periods, with each experiment being conducted with equivalent bare and MEG-OH samples. Substrates were then removed and gently rinsed in 1 mL PBS for 5 minutes. Next, the substrates were placed in 3% formaldehyde solution (in PBS) for 5 minutes and then removed and rinsed with PBS (×3). Finally, the surfaces were stored in PBS and placed in a cold dark environment prior to microscope measurements.

2.6 Fluorescence Microscopy

Platelet adhesion, aggregation and thrombus formation was visualized with a BX61W1 upright microscope (Olympus, USA), equipped with a Rolera EM-C2 CCD camera (QImaging, Canada) with 8 × 8 µm pixel size. The required exposure time was set to 100 ms without camera gain. The excitation source used was an arc mercury lamp, X-Cite 120 PC fluorescence illumination system (EXFO Photonic Solutions Inc., USA). To filter fluorescent signals, a green 33 fluorescent filter cube set, FITC 3540B (Semrock, USA), which combines a 35 nm wide excitation bandpass filter centered at 482 nm and a 40 nm emission bandpass filter centered at 536 nm, was used. 4× objective lens with a numerical aperture of 0.28 (Olympus, USA) were used with pixel sizes of 2 µm. Videos were collected using both *Micro-Manager* and *ImageJ* software. Platelet aggregate sizes were measured using *ImageJ* software in 2D mode.

3. Results and Discussion

3.1 Polymer Surface Characterization

Prior to studying the interaction of polymer samples with blood, derivatized surfaces were assessed for successful MEG-OH modification using X-ray photoelectron spectroscopy (XPS) (Table 1) [18, 19]. Briefly, it is important to establish that XPS analysis of bare, unmodified polymer substrates displayed intense signals for carbon (C_{1s} at ~285 eV), an element characteristic to PC, PVC, and PS substrates (Table 1). Following plasma activation, the relative intensity of this peak decreased, while that for oxygen (O_{1s} at ~532 eV) increased since the polymer surfaces become activated with -OH groups (Table 1) [30-32]. As expected, silanization of PC, PVC, and PS substrates with oxygen-, silicon-, and fluorine-bearing MEG-TFA surface modifier generated two new XPS peaks – one for silicon (Si_{2p} at ~103 eV) and another for fluorine (F_{1s} at ~688 eV) – as well as an increase in the relative intensity of the oxygen signal (Table 1). With respect to carbon, the XPS signal decreased substantially upon formation of the MEG-TFA adlayer for all the polymers – as anticipated for the now buried substrate from which most of the carbon signal originates (Table 1). Upon conversion of MEG-TFA to MEG-OH adlayer, the fluorine XPS signal disappeared indicating that the labile terminal trifluoroacetyl (TFA) moieties had been completely cleaved upon solvolysis, while the signal for silicon was not affected demonstrating that the solvolytic treatment did not etch the residual MEG-OH coating from the PC, PVC, or PS substrates (Table 1). With respect to PS, the S_{2p} XPS signal (~168 eV) was reduced for MEG-TFA/MEG-OH-coated surfaces (compared to bare PS), which is another indication of adlayer deposition, since the sulfur element is only attributed to PS (Table 1, Figure 2). Surface characterization with XPS concluded that all polymers employed in this study were successfully modified with MEG-OH adlayer.

Table 1 XPS relative atomic percentages for the characteristic elements of PC, PVC, and PS substrates (before and after plasma activation) and MEG-TFA/MEG-OH adlayers, and static contact angle measurements for these surfaces recorded with deionized water (triplicate analysis).

		Relative atomic percentage (%)						**Contact**
		C_{1s}	**Cl_{2p}**	**O_{1s}**	**F_{1s}**	**Si_{2p}**	**S_{2p}**	**angle (°)**
PC surface	Bare	82.8	-	15.4	0.4	0.5	-	94 ± 2
	Plasma-activated	76.3	-	22.8	0.0	0.9	-	45 ± 1
	MEG-TFA adlayer	44.7	-	36.8	5.9	12.6	-	85 ± 1
	MEG-OH adlayer	47.5	-	39.0	0.0	13.5	-	55 ± 2
PVC surface	Bare	69.2	28.1	2.6	0.1	0.0	-	101 ± 4
	Plasma-activated	62.8	27.5	9.7	0.0	0.0	-	62 ± 2
	MEG-TFA adlayer	37.3	1.7	32.2	16.5	12.3	-	91 ± 1
	MEG-OH adlayer	42.5	7.4	35.5	0.4	14.5	-	44 ± 1
PS surface	Bare	79.8	-	13.9	0.5	1.0	3.8	91 ± 2
	Plasma-activated	70.9	-	23.0	0.3	0.8	4.0	22 ± 2

MEG-TFA adlayer	46.0	-	26.9	16.5	9.8	0.4	88 ± 3
MEG-OH adlayer	49.2	-	34.2	2.7	13.4	0.3	55 ± 1

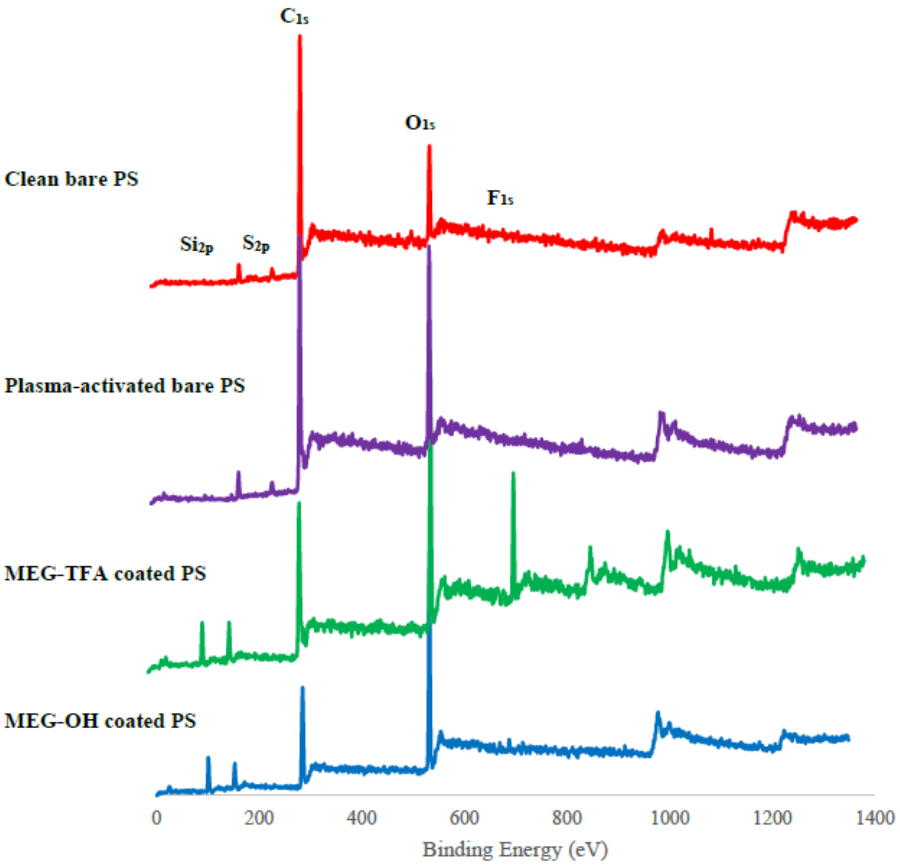

Figure 2 XPS surveys for (top to bottom) clean bare PS (red), plasma-activated bare PS (purple), as well as MEG-TFA (green) and MEG-OH (blue) coated PS. XPS surveys for bare and MEG-OH-coated PC and PVC are found in references 25 and 26, respectively.

To supplement XPS analysis, static contact angle (CA) measurements were also performed with water as the test liquid (Table 1). As received (*i.e.* non-cleaned and non-activated), bare substrates presented a rather hydrophobic surface with CA values ranging from 91 to 101°, depending on the polymer. Upon plasma cleaning and activation, the CA significantly decreased for all polymers, which is consistent with the formation of a more hydrophilic surface presenting hydroxyls and other polar groups. Subsequent silanization with MEG-TFA residues featuring hydrophobic TFA terminal groups increased hydrophobicity for all coated polymers (CA values ranging from 85 to 91°). Finally, the conversion of MEG-TFA into the MEG-OH adlayer with concomitant re-exposure of polar (*i.e.* hydroxyl) moieties resulted in a substantial increase in surface hydrophilicity, as revealed by a decrease in CA (91-101°, depending on the polymer). These CA measurements match well with the changes in wettability associated with the various modifications of surface functionalities and are entirely consistent with the data obtained *via* XPS characterization.

3.2 Fluorescence Microscopy of Polymers Exposed to Blood

Using fluorescence microscopy, we analyzed PC, PVC and PS after exposure to blood at a shear rate of ~20 s^{-1}. This shear rate is rather low; however, low shear rates can be found in the human body (*e.g.* the vena cava, which can have a shear rate as low as 5 s^{-1} [33-35]) as well as within medical equipment, which we calculated to be ~12 s^{-1}. In our previous studies we focused on high shear rates (*e.g.* 300-1500s^{-1}); however, for this investigation we chose a lower shear rate, which is in the applicable range for implants and medical equipment, in order to examine the effect on blood-surface interactions.
Fluorescence spectroscopy analysis of surfaces, bare or MEG-OH-coated, before contact with blood revealed the presence of several background spots that potentially could be erroneously ascribed as originating from blood thrombosis, especially on PC and PVC (Figure 3). In these cases, it is likely that these fluorescence sources originate from either defects in the material or deposited foreign materials, most commonly fungus, could originate from the air during storage or transport and may release fluorescent light when exposed to excitation wavelength extracted by the microscope [36-38]. With respect to defects caused by scratches or dents, they can result in reflected radiation rendering an appearance that can be mistaken for genuine fluorescence. Most scratches had a linear appearance and were avoided during scans. Fluorescent spots are more clearly seen on PVC and PC due to the high transparency of these polymers. On PS, spots were almost non-existent due to the high background signal that overshadows any fluorescence associated with other sources (Figures 3C and 3F). For this material, background fluorescence is associated with conjugated sulfur-oxygen bonds and aromatic rings. The spots, in most cases, do not significantly contribute to a high signal intensity compared to the fluorescence signal later observed due to blood exposure.

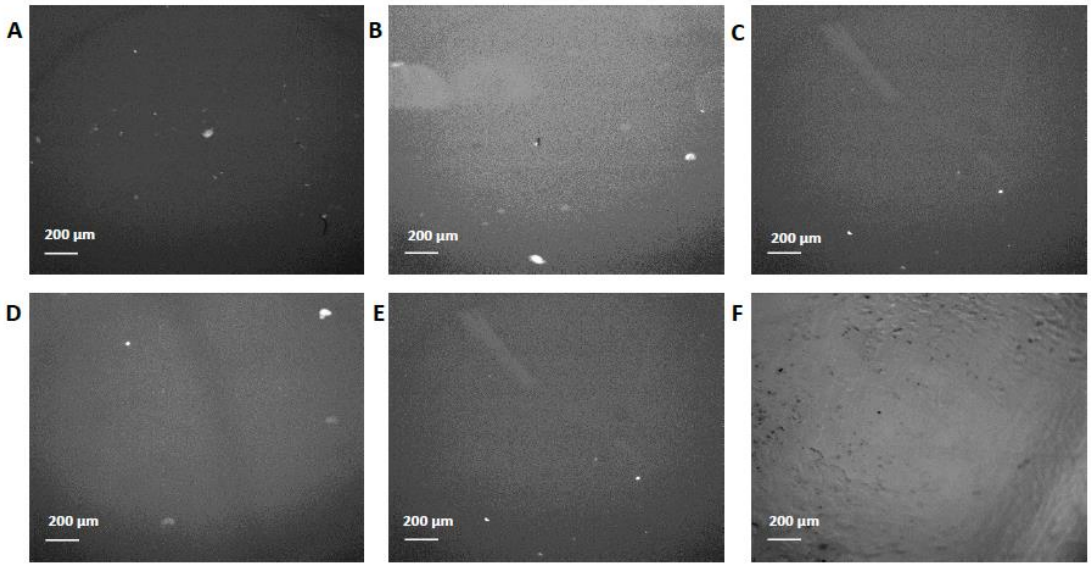

Figure 3 Representative fluorescent images of different surfaces (4× magnification) prior to blood contact. Top (bare surfaces): A) PC, B) PVC, and C) PS; bottom (MEG-OH-coated surfaces): D) PC, E) PVC, and F) PS. Dark areas represent defect- or contamination-free surfaces.

Following 3 hours of exposure to blood, all MEG-OH derivatized surfaces were observed to have significantly lower platelet surface coverage than their corresponding bare counterparts (Figure 4, Table 2). On PC and PVC, as found in our previous studies [25, 26], the reduction was observed to be remarkably high at values of 88% and 90%, respectively (Table 3). When measured with *ImageJ,* the platelet aggregate diameter appears to be below 200 µm and very uncommon on both PC and PVC MEG-OH-coated surfaces (Figures 4D and 4E) compared to much larger aggregates (~400 µm) on bare PC and PVC (Figures 4A and 4B)). On the other hand, experiments on PS, both bare and MEG-OH-coated, yielded no large clots (Figures 4C and 4F) with all clots being approximately only a few micrometers in size. In contrast, the reduction in coverage for PS was much lower at 44%.

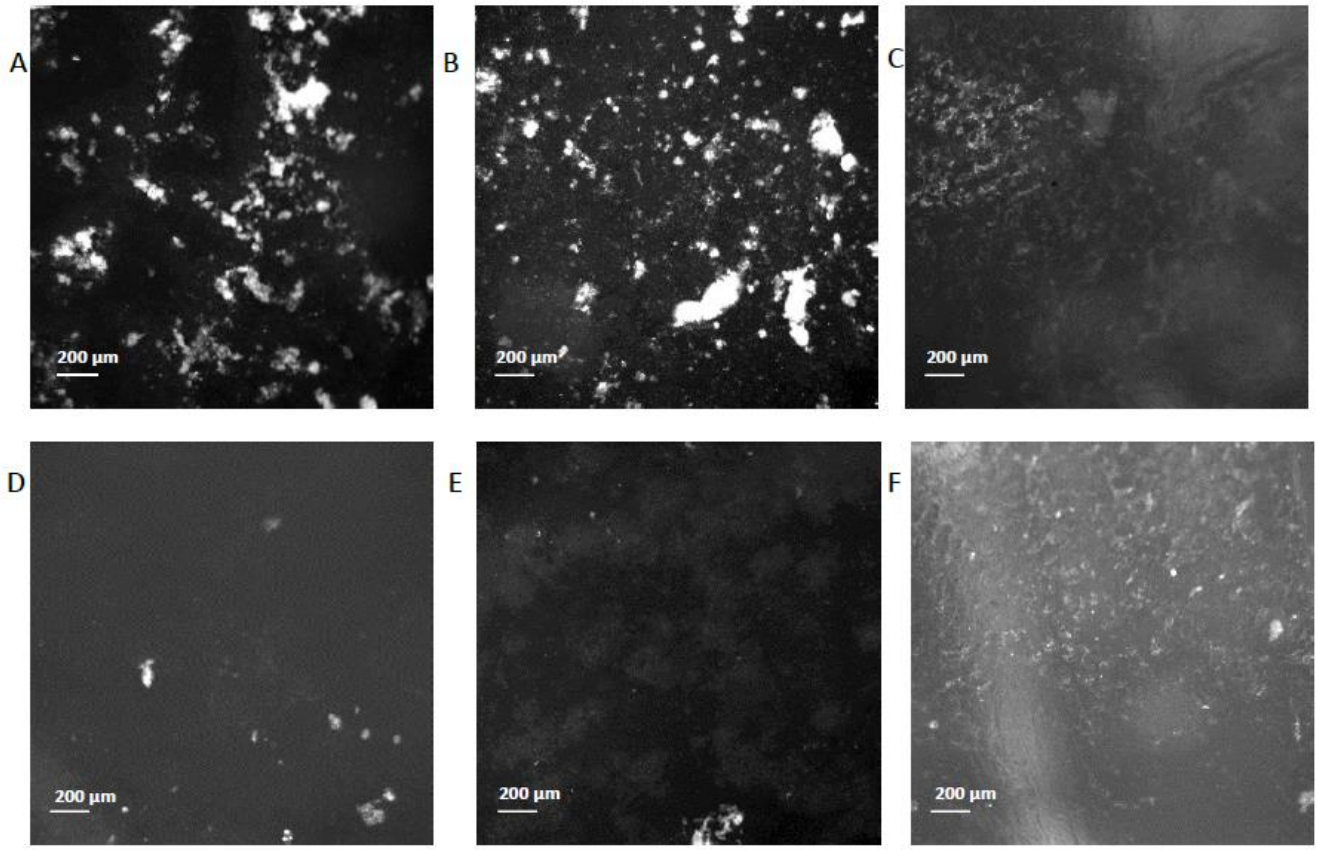

Figure 4 Representative fluorescent images of different surfaces (4× magnification) after 3 hours of blood contact. Top (bare surfaces): A) PC, B) PVC, and C) PS; bottom (MEG-OH-coated surfaces): D) PC, E) PVC, and F) PS. Dark areas represent(platelet-free) polymer surfaces (bare or MEG-OH-derivatized), and the bright areas represent attached fluorescently-labelled platelets.

Table 2 Compilation of surface coverage percentage due to platelet adhesion, aggregation, and thrombus formation on bare and MEG-OH-coated PC, PVC, and PS following blood contact for 3 hours, 6 hours, and 3 days. A minimum of three replicates were performed for each.

		PC surface (% surface coverage)	PVC surface (% surface coverage)	PS surface (% surface coverage)
No blood exposure	Bare	0.4 ± 0.1	0.6 ± 0.1	0.1 ± 0.1
	MEG-OH adlayer	0.8 ± 0.3	1.0 ± 0.6	0.0 ± 0.0
3 hours blood exposure	Bare	10.0 ± 1.0	7.8 ± 0.8	12.0 ± 4.0
	MEG-OH adlayer	1.2 ± 0.2	0.8 ± 0.2	7.0 ± 1.0
6 hours blood exposure	Bare	2.3 ± 0.7	3.6 ± 0.7	2.2 ± 0.5
	MEG-OH adlayer	0.4 ± 0.1	0.2 ± 0.0	0.3 ± 0.2
3 days blood exposure	Bare	68.0 ± 4.0	6.0 ± 1.0	21.0 ± 3.0
	MEG-OH adlayer	6.0 ± 2.0	0.2 ± 0.1	10.0 ± 1.0

Table 3 Percentage reduction by MEG-OH adlayers of surface coverage by platelet aggregates (compared to bare polymer surfaces) when subjected to blood for specific durations.

	PC surface (MEG-OH/Bare %)	PVC surface (MEG-OH/Bare %)	PS surface (MEG-OH/Bare %)
3 hours	88	90	42
6 hours	82	94	86
3 days	89	97	53

After 6 hours of blood exposure, the same trends were observed as seen for 3 hours blood contact with the reduction of platelet aggregates on MEG-OH coated compared to bare PC and PVC surfaces (Table 2, Figure 5) still being at 82% for PC and 94% for PVC (Table 3). In this case, PS had a reduction of 86%, which is significantly lower compared to 3 hours blood exposure. Additionally, compared to 3 hours blood exposure, the clot sizes and platelet aggregate coverage decreased on all coated polymers. The overall observed reduction in surface coverage is likely caused by the removal of large clots during flow or washing procedures. Such entities, in our experience, are more prone to removal by washing and might have been dislodged under flow conditions.

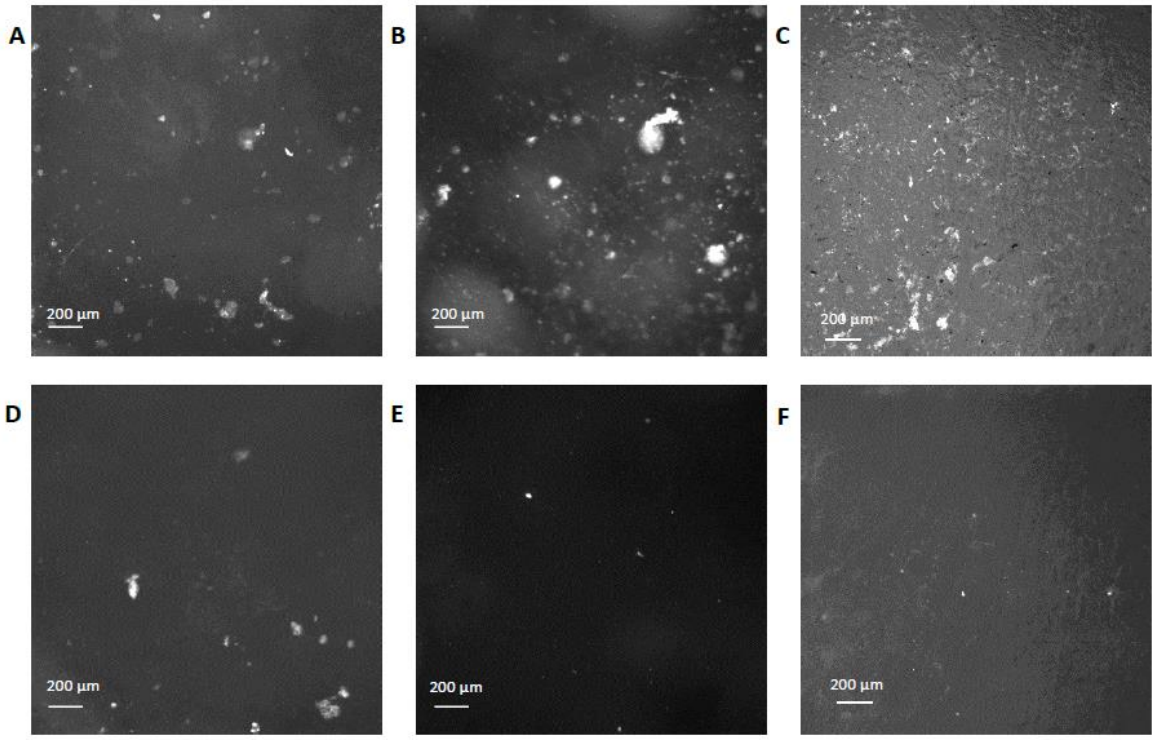

Figure 5 Representative fluorescent images of different surfaces (4× magnification) after 6 hours of blood contact. Top (bare surfaces): A) PC, B) PVC, and C) PS; bottom (MEG-OH-coated surfaces): D) PC, E) PVC, and F) PS. Dark areas represent (platelet-free) polymer surfaces (bare or MEG-OH-derivatized) and the bright areas represent attached fluorescently-labelled platelets.

After 3 days of blood exposure, bare PC and PS substrates exhibited significant platelet aggregation (Figure 6) with values of approximately 68% and 21%, respectively (Table 2). Clot sizes were observed to be as large as ~1 mm on PC and ~0.5 mm on PS; however, for MEG-OH-coated PC and PS, aggregates were much smaller with a size of ~200-300 µm (Figure 6). Remarkably, despite the relatively long period of blood contact, the MEG-OH-coated surfaces show reductions of respectively 89% and 54% for PC and PS, confirming that the surface modification did not lose efficacy over the specified time period. With respect to PVC, this polymer exhibited similar results to 3 hours blood exposure and a 97% reduction in platelet aggregates for MEG-OH-coated compared to bare. It should be noted that these results were achieved despite the relatively harsh conditions of our experiments: (1) in a deliberate fashion, no anticoagulants (besides its collection and storage in heparinized Vacutainers) were added to any of the blood aliquots, (2) blood was stored at room temperature with no special cooling to reduce coagulation, and (3) no coating was applied to any of the conduits employed in the flow-through experiments. Even under these conditions, where a level of coagulation could be anticipated, a major reduction in aggregate deposition was observed on all polymers studied.

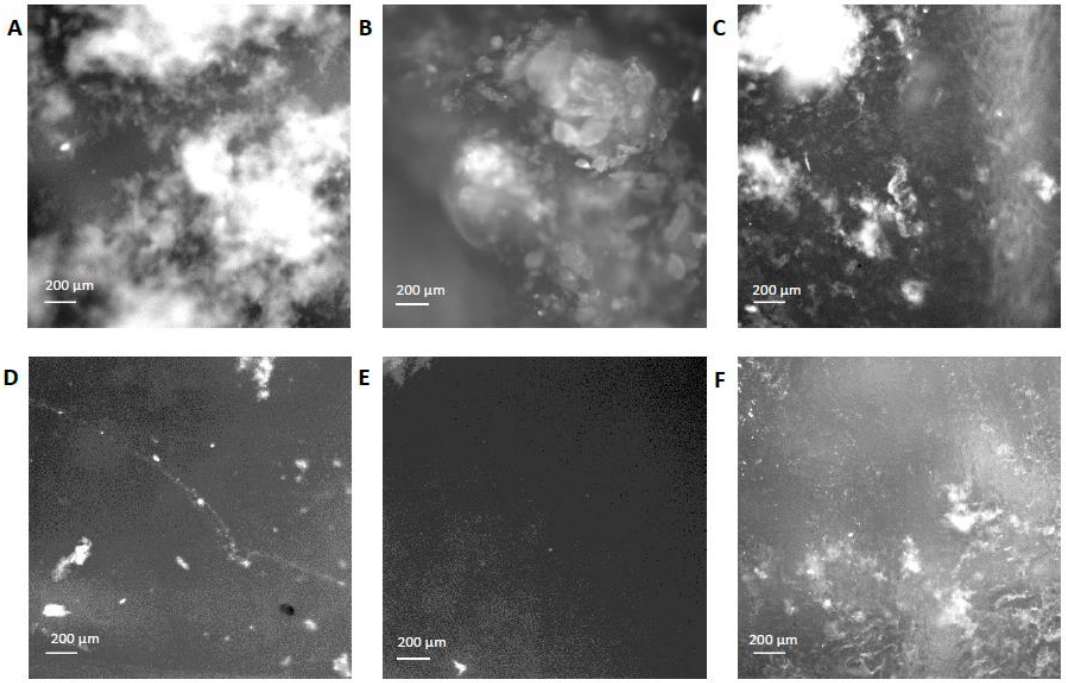

Figure 6 Representative fluorescent images of different surfaces (4× magnification) after 3 days of blood contact. Top (bare surfaces): A) PC, B) PVC, and C) PS; bottom (MEG-OH-coated surfaces): D) PC, E) PVC, and F) PS. Dark areas represent (platelet-free) polymer surfaces (bare or MEG-OH-derivatized) and the bright areas represent attached fluorescently-labelled platelets.

We note that roughness is a possible factor in terms of the instigation of interfacial coagulation for both bare and MEG-OH-coated substrates [26]. PC and PVC have an arithmetical mean roughness (Ra) value of ~0.1 µm, which is standard for manufacturing these polymers. With regards to PS, microscope analysis revealed trough sizes of ~20 µm and a Ra estimate of ~3 µm [39, 40]. Although the roughness may not have been a factor on PC or PVC, we see that it may have been an issue for PS since platelet aggregate coverage was less predictable (Table 2). Fouling on polymers is known to be increased by an elevated level of surface roughness [41, 42]. Thus, the results would be comparable with PVC and PC if PS had a Ra of ~0.1. Moreover, we believe that any coagulation that occurs on MEG-OH-coated polymers was likely the result of the presence of surface defects or areas not coated with MEG-OH. Overall, the best MEG-OH-derivatized polymer system appears to be PVC, PC being a close second. Reduction of platelet surface coverage is observed with PS as well, to a much lesser extent, however.

4. Final Remarks

Surface modification of polycarbonate, poly (vinyl chloride), and polysulfone polymeric materials by MEG-OH silane surface chemistry has been shown to be highly effective with regard to the enhancement of hemocompatibility, even for conditions where surfaces were exposed to blood for extended periods of time (up to several days). MEG-OH adlayer on PC and PVC displayed reductions

ranging between 88-97% in surface coverage by platelets when compared to bare substrates. PS was noticeably less effective (42-86%).

The durations of polymer surface exposure to blood employed in this study are quite reflective of those used in various medical procedures as described earlier. For example, PVC conduits are often used for blood flow over 3-4 hours during patient renal dialysis. An added feature of this work is the potential advantage that aggregates of particularly small dimension, which are evident on coated surfaces, are unlikely to cause complications when released into the bloodstream. In this respect, "micro-clots" are considered to be dissolved by the regulatory fibrinolytic system in the body [43]. Finally, it is useful to discuss the potential of this surface modification strategy in terms of possible commercial exploitation. As specified in this research, a high degree of prevention of platelet aggregation has been achieved over quite long periods of time and, furthermore, the physical chemistry that lies behind this result is reasonably well understood. On the other hand, it is difficult to contemplate the large-scale application of silanization chemistry. The key issue then will be whether alternative chemical or physical protocols can be developed which mimic the result of silanization technology.

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Author Contributions

M.T. and A.R. conceived the experimental strategy; S.S. devised the MEG-OH surface chemistry; K.F. prepared the various surfaces; K.F. and S.S. analyzed XPS and contact angle data; K.F. performed all blood-polymer interaction and fluorescence microscopy work; M.T. wrote the paper in consultation with K.F., which all co-authors edited.

Conflicts of Interest

The authors declare no conflict of interest.

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