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A Nondestructive Surface Zwitterionization of Polydimethylsiloxane for the Improved Human Blood-inert Properties

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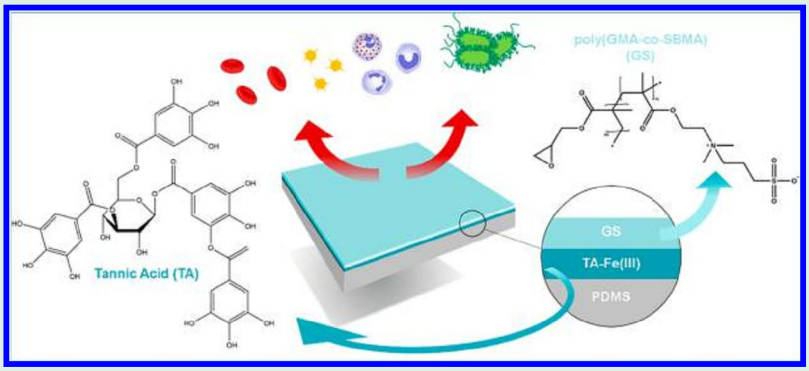

ABSTRACT: Polydimethylsiloxane (PDMS) is extensively used in the field of biomaterials. However, its hydrophobicity still limits its range of applications and makes it prone to biofouling. Various techniques are currently utilized to overcome this limitation, but most of them reduce some of the PDMS prime characteristics, such as its mechanical strength and optical transparency. In this work, we employed an original two-step coating process to bypass harsh treatments on PDMS like UV−ozone or plasma treatment. A pre-coating step of tannic acid-Fe(III) complex was performed prior to the zwitterionization of the PDMS with poly(glycidyl methacrylate-*co*-sulfobetaine methacrylate) or poly(GMA-*co*-SBMA) by a "grafting-to" approach. Successful coating was evidenced by a decrease of the water-contact angle from 118° to 79°. The process was optimized, and the optimized coating condition led to a significant improvement of the PDMS biocompatibility while maintaining its mechanical property and optical transparency. In addition, a 90% reduction of *Escherichia coli* attachment and fibrinogen plasma protein, an 80% reduction of red blood cells and cells from whole-blood attachment, and a 60% reduction of platelets adhesion were measured. We further tested the potential of the zwitterionic PDMS material as a storage vessel for platelet-rich plasma under physiological conditions. Platelet activation was decreased from 10.95% using virgin PDMS to 3.35% with the coated sample in a period of 2 days.

KEYWORDS: *PDMS, tannic acid and Fe(III) complex, zwitterionization, biocompatibility, platelet activation*

INTRODUCTION

When it comes to polymeric materials heavily used in the field of biomaterials, polydimethylsiloxane (PDMS) dominates in usage because of mechanical properties, optical transparency, and general inertness. PDMS has been widely utilized in the biomedical field for fabrication of devices such as catheters, pacemakers, and medical adhesives and tubing and in an array of implants including cochlear, and drainage implants in glaucoma.[1−3] Moreover, its applications have been extended in the manufacturing of microfluidic systems, biological and cellular analysis, wound healing, soft lithography, DNA sequencing, disease diagnostics, drug delivery, and biosensors.[4−13] In addition, the ease of fabrication makes it a more appealing candidate for multitude of applications because it enables PDMS to be fabricated in accordance to the necessary specifications. However, due to its strong hydrophobic nature, it exhibits low wettability; hence, PDMS-based biomedical devices may suffer from absorption of unwanted small molecules such as proteins. Consequently, if these devices are exposed to a blood environment, safety issues arise, and in

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microfluidic systems, its immediate use is impeded because it demands surface modification.[4,14]

Several studies were made to address the limitations hauled by the intrinsic hydrophobicity of PDMS, focusing on surface modification through physical and chemical methods, with the key target to enhance its hydrophilicity and equip it with antifouling properties.[15−20] Modification with physical methods can be done either through chemical alteration on the surface layer via the generation of high-energy species, like radicals, or the formation of sharp interface through deposition of extraneous layer over the existing material.[21] Some conventional physical techniques include plasma treatments, ozone, and ultraviolet (UV) light.[1,21−24] As for chemical methods, two categories are commonly used. The first one is the employment of wet treatment, whereby the substrate is exposed to a given solution to allow direct chemical reaction to occur; the second is through covalent conjugation via surface grafting, which has been widely used for PDMS surface modification.[21] Furthermore, surface grafting can be subdivided into two techniques, namely: "grafting-to" and "grafting-from" approaches. The former involves the grafting of presynthesized polymers onto the surface of the substrate via specific kinds of interactions, while the latter makes use of immobilized initiators on the surface of the substrate to grow the polymer chain from the surface.[1,25,26]

Functional groups utilized for modification process play a vital role because they predict the efficiency of the antifouling property of the substrate. The characteristics that serve as the junction at which these functional groups meet include hydrophilicity and electronic neutrality and is composed of hydrogen acceptor but no donor.[27] One of the widely exploited materials for surface modification for biocompatibility is polyethylene glycol (PEG), but in the long run, the durability and stability of medical devices and implants arise because of the chemical decomposition of PEG via oxidation.[17,28,29] As of today, zwitterionic materials are regarded as the new generation of robust fouling resistant biomaterial systems surpassing PEG. For instance, sulfobetaine methacrylate (SBMA) is one of the widely utilized zwitterionic materials to enhance biocompatibility because of its accessible synthesis at a low cost.[29,30] In a previous study by Chou et al., a zwitterionic copolymer, poly(glycidyl methacrylate-*co*-sulfobetaine methacrylate) (poly(GMA-*co*-SBMA), was designed to form a stealth interface utilizing the "grafting-to" approach.[30] The SBMA segments offer the antifouling property, and its anchoring onto versatile surfaces such as polymer, ceramics, and metal substrates is due to the ring-opening reaction of the epoxide functional group of GMA segments as it interacts with hydroxyl groups generated on the surface after pretreatment with UV and ozone.

In the review article by Zhang and Chiao on the antifouling coatings for PDMS, it was stated that chemical modification of PDMS produces stable surfaces and permanent antifouling properties, yet large-scale production is a concern because successful modifications involve complicated processes.[17] Besides, optical transparency and mechanical properties are also altered when using the strategies available. Indeed, the employment of oxygen plasma and UV/ozone treatment, under extensive exposure time, produces a glass-like layer on the surface of PDMS, ensuing the cracking of this oxidized layer due to mechanical and thermal stresses.[31] In addition, antifouling properties conveyed through physical modification is temporary because PDMS tends to revert to its initial hydrophobic state.[17,32] Despite the efforts made to improve hydrophilicity and the antifouling properties of PDMS, the currently available techniques still require further improvements in terms of process simplicity as well as there is a need for a surface modification strategy that will not sacrifice the innate mechanical and optical properties of PDMS.

In this study, we utilized a simple yet innovative precoating step in an attempt to combat drawbacks, such as the alteration of distinct properties of PDMS offered by the present modification processes. Various studies made use of a one-step assembly of versatile thin films in a simple, rapid, and robust method involving coordination complexes of tannic acid, a natural polyphenol, and Fe(III) ions.[11,33,34] We have adapted the formation of this thin film onto the surface of PDMS as a precoating step, in lieu of current techniques like UV/ozone or plasma treatment, to procure hydroxyl groups. These functional groups enhance the general hydrophilicity of the substrate and serve as reactive sites for the anchoring onto the surface of zwitterionic poly(glycidyl methacrylate-*co*-sulfobetaine methacrylate) copolymer, poly(GMA-*co*-SBMA), that was synthesized by Chou et al. in a previous study. These coated PDMS substrates were then tested for their biocompatibility and effectiveness to inhibit platelet activation in physiological conditions. Biocompatibility was investigated through contact with different biomolecules such as *Escherichia coli*, plasma proteins, erythrocytes, thrombocytes, and human whole blood. Then PDMS was fabricated in a tubular shape with the use of an Eppendorf tube as a mold for platelet activation experiment. Platelet-rich plasma (PRP) was placed in these tubes and stored at physiological conditions to evaluate the change in the degree of platelet activation.

EXPERIMENTAL SECTION

Materials and Reagents. Sylgard 184 PDMS elastomer kit was purchased from Dow Corning. TRIS buffer, ethylenediamine (EDA), bovine serum albumin (BSA), lysozyme, human plasma fibrinogen (fraction I), primary monoclonal antibody, and secondary monoclonal antibody were purchased from Sigma Chemical Co. Tannic acid was purchased from Alfa Aesar. $FeCl_3$ powder was bought from Sigma-Aldrich. Deionized water (DI water) was purified using the Millipore water purification system with a minimum resistivity of 18.0 MΩ.cm.

Fabrication of PDMS. PDMS Dow Corning A was poured into a beaker, followed by the addition of curing agent B onto it in a 10:1 (w/w) ratio, and was mixed using a glass rod. The resulting mixture was sonicated for 30 min or until bubbles were eliminated to obtain a homogeneous mixture. Afterward, it was poured in a tissue culture polystyrene (TCPS) dish, which served as a mold. To fabricate a thinner sheet for mechanical and optical property experiments, a small amount was cast on a glass using a film applicator with an effective thickness of 300 μm. Both were placed in a vacuum oven for curing. Prior to the placement in the vacuum oven, it was ensured that the surface was flat and leveled, such that fabricated PDMS are of the same thickness. Curing was done for 6 h, at 50 °C, and thickness of fabricated PDMS was then measured. Cured PDMS were cut into circles with a 1 cm diameter. For storage, the cut PDMS were immersed in methanol for storage. Before usage, PDMS were washed several times with deionized (DI) water and dried using N_2 gas.

Synthesis of Poly(GMA-*co*-SBMA) Copolymer. The synthesis of poly(GMA-*co*-SBMA) copolymer was based on the paper of Chou et al., and the optimized molar ratio of GMA and SBMA, G20-S80, was adopted.[30] Briefly, GMA and SBMA monomers were dissolved in methanol and deionized water, respectively. The GMA and SBMA solutions were then mixed, and AIBN was added as an initiator, followed by stirring. The stirred mixture was purged with N_2 gas to remove dissolved oxygen. It was then immersed in an oil bath for 6 h at 60 °C to initiate reaction, and the reaction was terminated with an

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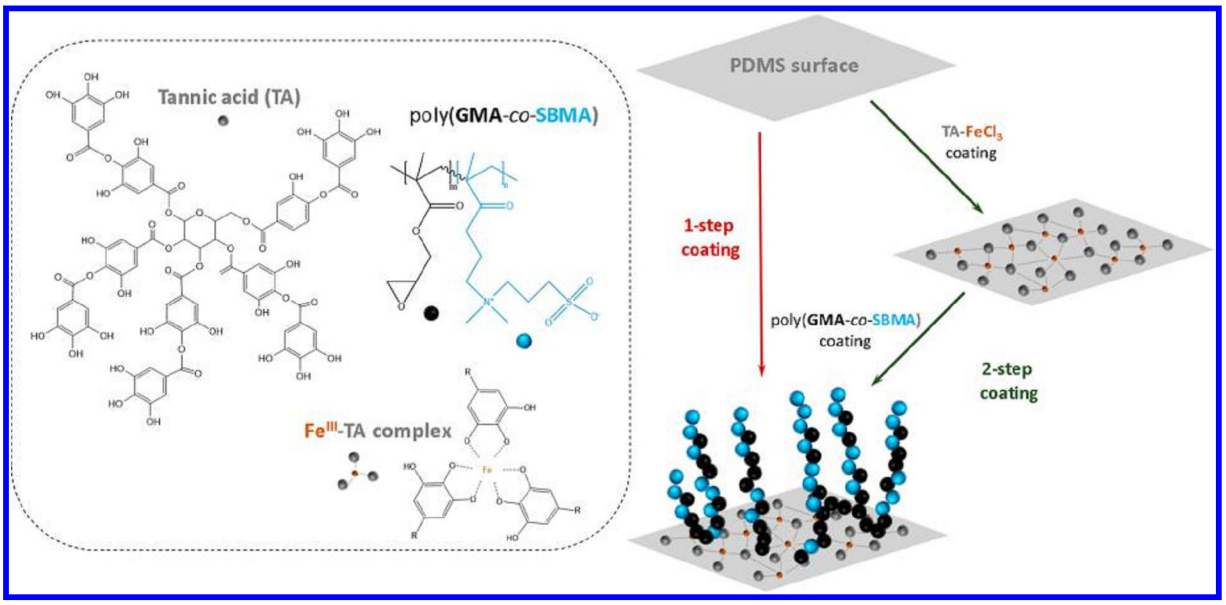
Scheme 1. Simplified Overview of the Grafting Process



ice bath. Purification of the white product obtained was done through washing with methanol three times. Consequently, it was placed in a vacuum oven overnight to evaporate remaining methanol and was freeze-dried to obtain a white crystal powder.

Preparation of Coating Solutions. Tris buffer solution was used as the solvent for the coating solutions. It was prepared by dissolving Tris buffer and NaCl with DI water to a final concentration of 100 mM and 600 mM of Tris buffer and NaCl, respectively. Then the pH of the buffer solution was adjusted with NaOH and HCl to a pH of 8.5. There were three coating solutions utilized in this study [namely, tannic acid (TA), TA with added $FeCl_3$, and poly(GMA-*co*-SBMA) solutions]. To prepare tannic acid solution, TA powder was dissolved with a Tris buffer solution previously prepared. To prepare TA solution with added FeCl3 (TA-$FeCl_3$), prepared TA solution was simply transferred to the bottle containing a pre-weighed $FeCl_3$. TA powder and $FeCl_3$ powder were in 1:0.09 (w/w) ratio. To ensure complete mixing, it was stirred for 15 min at 900 rpm. Poly(GMA-*co*-SBMA) copolymers powder was dissolved with DI water to obtain a poly(GMA-*co*-SBMA) solution. All coating solutions have a concentration of 10 mg/mL. Some samples were coated in the presence of EDA in the zwitterionic coating solution to enhance the cross-linking of the GMA segments. EDA was added in a 1:1 (w/w) ratio with the zwitterionic copolymer.

Grafting of PDMS Surfaces. The substrates were grafted by two methods using either a one-step coating or a two-step coating, which is schematically presented in Scheme 1. Starting with the two-step coating process, substrates were immersed in a coating solution of TA or TA-$FeCl_3$ and were placed inside a shaking oven for 12 h at 37 °C. Afterward, the substrates were washed with DI water three times and were subjected to the second step of the coating, which consisted in immersing the substrates in a bottle containing poly(GMA-*co*-SBMA) solution placed inside a shaking oven for 12 h at 60 °C. All shaking conditions were set at 100 rpm. Finally, the substrates were washed with DI water three times and stored at 4 °C until use. The one-step coating process consisted in directly immersing the substrates in a solution of poly(GMA-*co*-SBMA) solution in similar conditions as in the final step of the two-step coating.

Physicochemical Characterization of Virgin and Grafted PDMS Surfaces. XPS analyses were performed with a PHI Quantera SXM/Auger spectrometer with a monochromatic Al KR X-ray source (1486.6 eV photons), a hemispherical analyzer, and a multichannel detector. Presence of nitrogen and sulfur coming from the zwitterionic copolymer were verified for the coated samples by the taking of high-resolution spectrum for S 2p and N 1s signals. Water contact angle of the coated substrates were determined to assess its wettability. A droplet (4 μL) DI water was carefully allowed to rest on top of the surface of the substrate. An image was then taken using a contact angle equipment (CA-VP, Kyowa Interface Science Co., Ltd., Japan) after it stabilized. Each sample was tested in three different spots. The average of the measured water contact angle values was then determined. To determine the optical transparency of the materials, thin sheets of coated PDMS (~0.18 mm thick) with dimensions of 7 cm × 1 cm were subjected to a UV−vis spectrophotometer (PowerWave XS, BioTek). A spectral scan was performed from 200 to 1000 nm with a 1 nm step. To assess the mechanical strength of the coated substrates, the Young's moduli of the samples were determined using Instron 5544. Thin sheets of coated PDMS (~0.18 mm thick) with dimensions of 7 cm × 1 cm were used for testing. The samples were placed in the instrument by clamping each end separated by a 5 cm distance. Tensile force was then applied to the samples by gradually pulling the ends of the sample at a rate of 10 mm/min until the failure of the samples, which was seen by the breaking of the samples in half. Each condition of the samples has three replicates. The average of the measured Young's moduli was then calculated for each condition.

Evaluation of the Bioinert Property of the Zwitterionic PDMS Materials. A single protein adsorption by enzyme-linked immunosorbent assay (ELISA) was conducted as follows. Prior to testing for protein adsorption, samples were first incubated through its immersion in PBS solution overnight at 37 °C to allow its stabilization under physiological conditions. Samples were then placed in a 24-well TCPS and washed three times with PBS solution preceding the protein adsorption test by indirect ELISA. Preparation of protein solutions and antibodies were first done. Fibrinogen, which is a protein participating in blood clotting, was used as the target protein. To stabilize the target protein and prevent nonspecific interactions, bovine serum albumin (BSA) was used as a blocking protein. Prepared fibrinogen and BSA solutions have concentration of 1 mg/mL, and PBS was used as the solvent. The antibodies, both the first and the second, were also diluted with PBS having a dilution factor of 4000 and 2000, respectively. Initially, the samples were added with 1 mL of target protein solution, fibrinogen, and incubated for 30 min at 37 °C. Fibrinogen solution was then removed, and samples were washed with PBS three times. Consequently, samples were incubated with 1 mL of blocking protein, BSA, for 30 min at 37 °C. The incubation with first and second antibody, which also involved washing and blocking steps, was repeated in the same manner. Moreover, the duration of incubation for both antibodies were also 30

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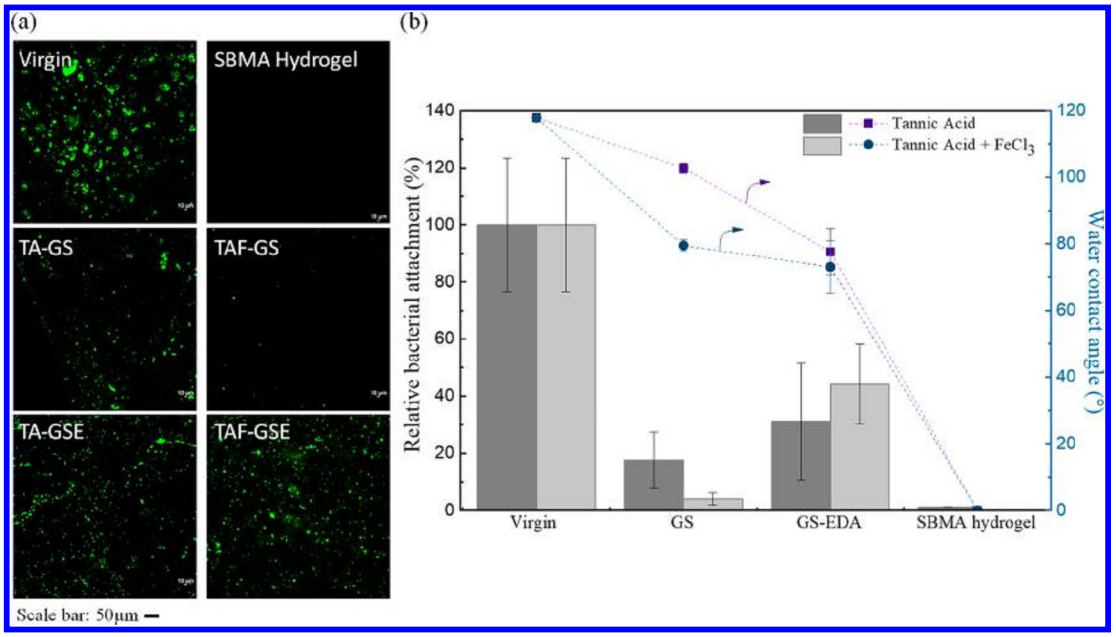

Figure 1. Optimization of the coating conditions. (a) Micrographs of 24 h *Escherichia coli* attachment on PDMS (TA, tannic acid; F, $FeCl_3$; GS, poly(GMA-*co*-SBMA); E, EDA). (b) Quantified data of bacterial attachment on the different surfaces and their corresponding water-contact angles.

min at 37 °C. Lastly, the samples were transferred into a new 24-well TCPS plate such that only protein adsorbed in the samples are detected. To enable visualization through color intensity, 500 μL of TMB peroxidase substrate was added. The termination of the colorization step was done by adding 500 μL of 0.01 M H_2S, which produced a yellow color. The solutions were then transferred to a 96-well UV plate. The wavelength employed was 450 nm, which corresponds to the protein adsorption in each sample. Each condition of the samples has three replicates to determine deviation of results. The same procedure was employed for control samples. The positive and negative controls were SBMA hydrogel and empty 24-well TCPS, respectively.

In addition, blood adhesion tests were performed according to the following protocol. The samples were placed in a 24-well TCPS plate and were immersed in PBS solution overnight at 37 °C to allow its stabilization under physiological conditions. Human blood was obtained from a healthy individual and was separated through centrifugation, with each layer containing different blood cells. Whole blood solution was centrifuged at 1200 rpm for 10 min, which resulted in two layers. The upper layer contained platelet-rich plasma (PRP), which mostly consists of thrombocytes or platelet. As for the bottom layer, the solution is rich in erythrocytes or red blood cells. Before the addition of blood solutions, the samples were washed three times with PBS. To test for thrombocyte adhesion, the samples were immersed in 1 mL of the PRP solution and were incubated at 37 °C for 1 h. Consequently, the samples were washed with PBS for at least 5 times. The fixation of attached blood cells onto the samples was done through addition of 1 mL of 2.5% glutaraldehyde solution. Afterward, samples were stored at 4 °C for 4 h prior to observation with confocal laser scanning microscopy (CLSM) (NIKON CLSM A1R). Images were taken at 200× magnification from 3 different sites, and each condition of the samples has two replicates. Moreover, the erythrocyte adhesion test and whole blood adhesion test was done in the same manner, but samples were immersed in either erythrocyte-rich solution, or whole blood solution, rather than the PRP solution.

To study the behavior of bacterial adhesion on the surface of the coated substrates, *E. coli* modified with a green florescent protein (*E. coli*-GFP) was used. *E. coli* was cultured in a medium containing 5.0 mg/mL peptone, and 3.0 mg/mL beef extract and was incubated at 37 °C and was shaken at 100 rpm for 12 h such that stationary state is achieved with a final bacteria concentration of 107 cells per milliliter. The substrates were rinsed with DI water and then immersed in PBS overnight. Prior to the attachment experiment, substrates were washed with PBS three times. Afterward, 1 mL of bacterial solution was added into each well to allow the immersion of samples, and the plate was incubated for 3 h at 37 °C. Consequently, samples were washed with PBS 3 times to remove any unattached bacteria. Observation of the samples was done using fluorescent microscope (Olympus BX51), and images were taken at a 20× magnification from 5 various sites on each sample.

Platelet-Activation Inhibition Tests. PDMS was fabricated as a tube for the platelet activation experiment. An Eppendorf tube of 2 mL volume was used as a mold. PRP, with a volume of 1.5 mL, was then put into these PDMS tubes and in a 9 mL K3EDTA Vacuette blood vial, which served as the reference for monitoring platelet activation. These PRP-containing tubes were then stored at 37 °C with constant shaking. An aliquot of the samples were collected at the start of the experiment and another after 2 days of storage. The detection of platelet activation was done using flow cytometry by diluting 20 μL of the sample with 1 mL of PBS and adding 20 μL of CD63P marker for activated platelets. The change in the amount of activated platelets from day 2 to the start of the experiment is reported.

RESULTS AND DISCUSSION

Optimization of the Two-Step Grafting Process. The structure of PDMS is composed of repeating units of $-OSi(CH_3)_2-$, endowing it with its characteristic hydrophobicity.[34] This property along with the lack of functional groups that can possibly react with other polymers for antifouling applications impose a challenge in the surface modification of PDMS. To address it, the introduction of reactive groups such as hydroxyl groups enable the modification by increasing its affinity for other polymers to be integrated on its surface.

In this work, the pretreatment of PDMS substrate involved the use of tannic acid to produce a thin coating introducing hydroxyl groups. Tannic acid is a plant-derived polyphenol with the capability to have various interactions with various functional groups. Moreover, polyphenols should have the property of strong solid−liquid interfacial properties exhibited

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by DOPA and dopamine due to their similarity of structure.[35] Wu et al. utilized the self-polymerization capability of tannic acid to coat poly(propylene) (PP) membrane.[36] In the same manner, tannic acid coating on PDMS substrate is though to undergo self-polymerization in the presence of Tris buffer solution, and this is also evident in the study of Barret and colleagues.[35] However, complexes can also be formed with tannic acid through chelation with metal ions.[37,38] Besides, the versatility of this approach was demonstrated in the work of Ejima et al. in coating substrates of different geometries, utilizing Fe(III) ions such that tannic acid coordination complexes are formed.[33] The establishment of a cross-linked film is initiated by the formation of stable octahedral complexes with Fe(III) ions, which serve as their core center, reacting with three galloyl groups from TA; hence, giving rise to the capability of TA molecule to bind with several Fe(III) ions.[39] The coated substrate then possesses hydroxyl groups offered by the tannic acid film, which allows covalent bonds to form with nucleophilic group composed of polymers and other molecules.[40]

To optimize the conditions of the two-step coating on PDMS, we evaluated the effectivity of adding Fe(III) ions to the coating solution of tannic acid. Also, a cross-linker to the zwitterionic copolymer poly(GMA-*co*-SBMA), EDA, was tested for its effectiveness. In Figure 1, we present the hydrophilicity and bacterial resistance property of coated materials to assess whether forming the tannic acid Fe(III) complex helps at improving the biocompatibility of the substrates. Relative to the virgin PDMS, a decrease in contact angle can be seen for all conditions. The addition of Fe(III) ions led to a greater decrease in contact angle than without Fe(III) ions, also adding EDA showed greater increase in hydrophilicity. However, adding EDA did not permit the reduction of bacterial attachment. This phenomenon may be attributed to the interactions of EDA with tannic acid or with the PDMS substrate, producing amine groups, hydrophilic but lacking of biocompatibility. From the observation of the sample with no EDA added, one can see that the addition of Fe(III) ions still helps in giving biocompatibility to the interfaces. These changes signify that tannic acid can modify PDMS to an extent. Thus, the coating conditions presented in Table 1 were used for further tests.

Table 1. Nomenclature of Samples and Their Corresponding Coating Conditions

sample ID	description of the coating condition
virgin	pristine PDMS substrate
1-GS	one-step zwitterionization coating without tannic acid precoating
1-GSE	one-step zwitterionization coating, without tannic acid precoating, with EDA
2-GS	two-step zwitterionization coating with tannic acid/$FeCl_3$ precoating
2-GSE	two-step zwitterionization coating with tannic acid/$FeCl_3$ precoating and with EDA

Mechanical Strength and Optical Transparency of PDMS and Grafted PDMS Surfaces. PDMS has been widely used in the biomedical field, specially to fabricate microfluidic devices due to its low-cost, ease of fabrication, mechanical properties, and optical transparency. For this reason, we evaluated the mechanical properties of PDMS substrates coated using our methods and a more conventional method, UV/ozone treatment. A materials Young's modulus is a good indication of the mechanical strength of a material. Figure 2 shows the Young's modulus of (i) the coated samples,

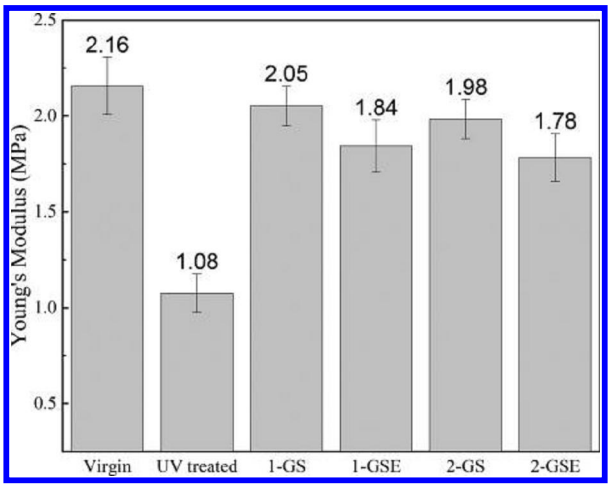

Figure 2. Young's modulus of virgin PDMS, coated PDMS, and UV-treated PDMS.

(ii) an untreated sample, and (iii) a UV-treated PDMS. Only a slight decrease in Young's modulus was observed for all the conditions with respect to that of the virgin sample (2.15 MPa). 2-GS, 1-GS, 2-GSE, and 1-GSE have a Young's modulus (MPa) of: 1.98 ± 0.10, 2.05 ± 0.10, 1.78 ± 0.13, and 1.84 ± 0.14, respectively. However, the UV treated PDMS showed a more significant decrease of Young's modulus as it was measured to be 1.07 ± 0.10 MPa. This decrease can be explained by the emergence of a glassy SiO_2 layer in the surface of PDMS upon UV/ozone treatment. The emergence of this layer makes the bulk material less stiff as seen from its reduced modulus of elasticity. Employing a simple grafting to process to the PDMS substrate did minimal effect on the Young's modulus of the coated samples, overcoming the common problem of the conventional UV-treated PDMS.[17,31]

Furthermore, the optical transparency that PDMS offers is suitable for various biological and chemical studies incorporating well-established fluorescence-based analyses.[41] To examine the optical transparency of the coated substrates, their transmittance values were evaluated (Figure 3). From the graph, transmittance of the modified samples was not lower than that of the virgin, but the sample treated with UV/ozone showed a peak around 480−600 nm, which is in the range of the visible spectrum. This may signify that a slight sample discoloration occurred with the treatment. Another factor that may affect the transmittance of the modified samples is the roughness of the surface. The virgin PDMS surface is a smooth dense surface that may reflect some of the light, thus having the lowest transmittance value. Coated samples will have higher roughness due to the coating layer, and therefore, will make the reflected light lower and transmitted light higher. Overall, the tensile elasticity and optical transparency results simply prove that these properties are not substantially downgraded upon the application of precoating step with the tannic acid and $FeCl_3$ complex on PDMS substrate.

Chemical Evidence of the Presence of Poly(GMA-*co*-SBMA) at the Surface of the Grafted PDMS Samples. To verify the existence of the copolymer coating on the surface of the PDMS, we analyzed the samples by XPS. As seen in Figure

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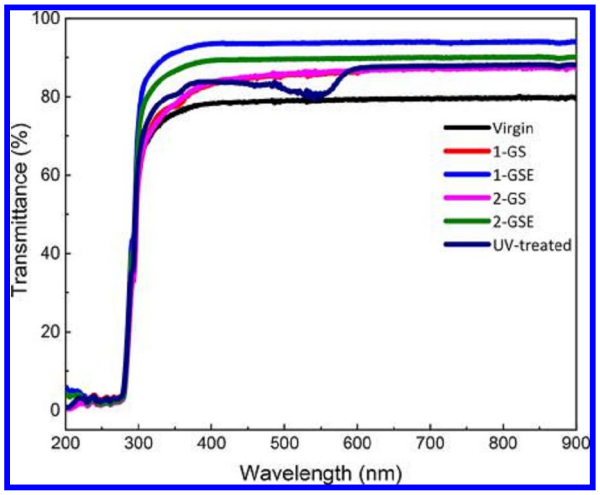

Figure 3. Evaluation of the optical transparency of PDMS and coated PDMS samples.

4, all modified substrates exhibited peaks on both sulfur and nitrogen high-resolution spectra. Regarding the S 2p spectra, 2-GS sample showed the most prominent peak attributed to the SO_3 group in the zwitterionic copolymer. As for N 1s spectra, there are two different nitrogen species present in the coatings, a primary amine with a signal that is located at around 400 eV and a quaternary amine group with a binding energy of 402 eV. The primary amine peak emerges from samples coated via the two-step coating and samples with EDA. The 2-GS sample also contains some primary amine peak that may come from the contribution of the Tris buffer used during the precoating process. Comparing the one-step and two-step coatings, it is seen that a higher intensity for the nitrogen peaks occurred using the two-step coating that may signify higher copolymer deposition on the surface. Samples with EDA, however, have slightly lower peak intensities. This supports that a lower deposition of the copolymer happens when EDA is present because of its reaction with the GMA segments of the copolymer, subsequently reducing the anchoring capabilities of the copolymer.

Effect of Surface Modification on the Biocompatibility of PDMS Surfaces. Blood contacting biomaterials are susceptible to fouling by biomolecules such as bacteria, proteins, and blood cells. Human whole blood is composed of a variety of proteins with their respective biological functions. Proteins are small compared with other components in human blood, which gives them a kinetic advantage. In other words, proteins are the first to attack a foreign material. When a biomaterial does not have sufficient biocompatibility, proteins can adhere to its surface and undergo conformational changes that makes them act as ligands for cell attachment. Among the hundreds of plasma proteins, the most abundant ones are albumins and fibrinogen. Fibrinogen is a key protein that facilitates blood clotting on various biomaterials. Following protein attachment, platelets can also adhere to the biomaterial and activate. Activated platelets can then further interact with various coagulating factors that can promote thrombosis. Other blood cells can also then start fouling on the biomaterial leading to its rejection by our body. In another scenario, biomaterials such as surgical tools may be contaminated with bacteria that may lead to the patient being infected. One of the most common bacteria infecting many is *E. coli*.

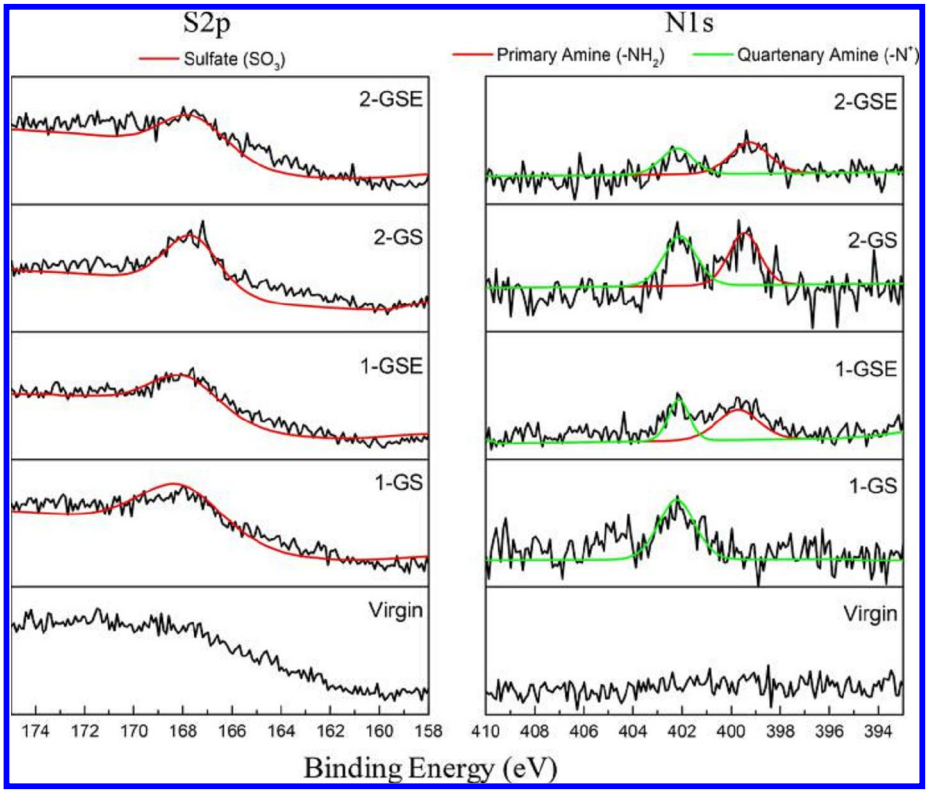

Figure 4. High-resolution S 2p and N 1s XPS spectra.

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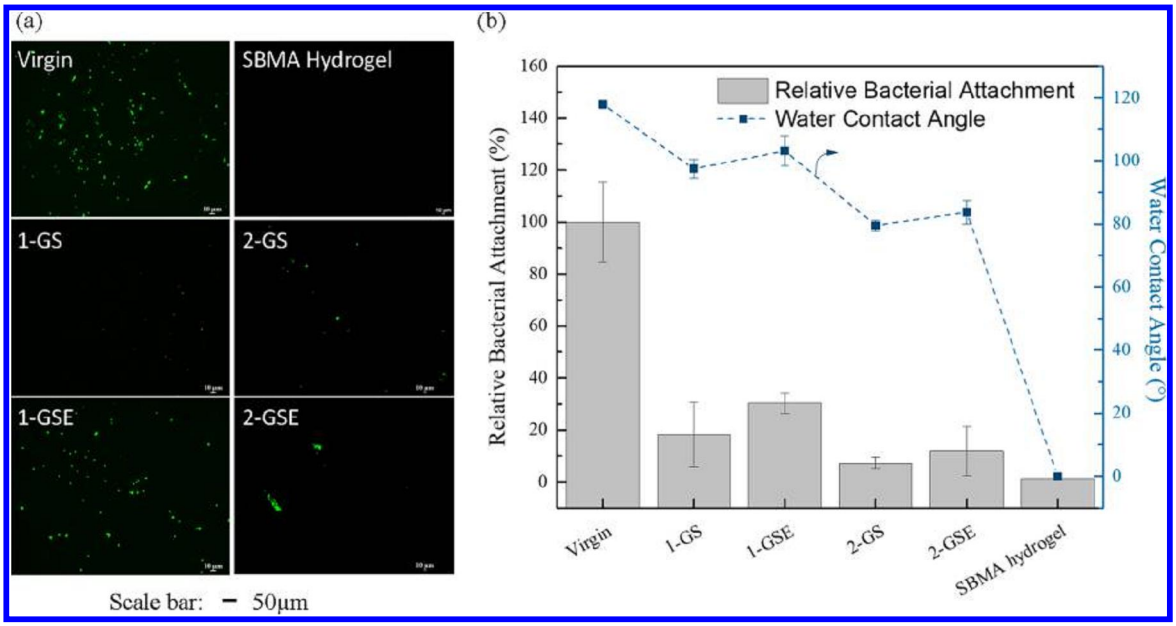

Figure 5. (a) Micrographs of 24 h *E. coli* attachment on PDMS. (b) Quantified data of bacterial attachment onto the different samples and their corresponding water-contact angle.

With all these risks, biomaterials are required to have a very high biocompatibility. In Figure 5, we evaluated the ability of the coated PDMS substrates to resist bacterial adhesion as well as the hydrophilicity of the substrates. The water contact angle was reduced from 117.93° to 79.51° with the two-step coating without adding EDA cross-linker (2-GS). The sample that had the best resistance to the other biomolecules was also 2-GS. Based from the micrographs and the quantified data, a significant bacterial attachment reduction was seen with respect to the uncoated PDMS; it was reduced by 92.67%.

From Figure 6, fibrinogen adsorption was also significantly reduced. With 2-GS, it was decreased to 10.6% relative to the adsorption on TCPS, corresponding to a reduction of 64.8% with respect to the virgin PDMS. Similar to this coated sample as seen in Figure 7, reductions of cells attachment of 86.1%

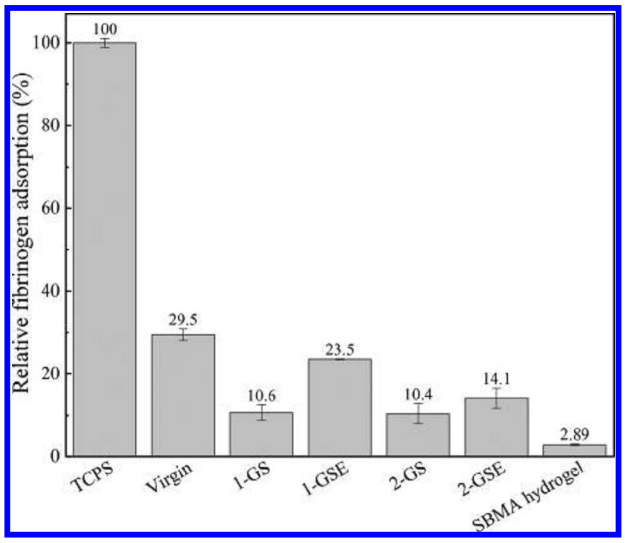

Figure 6. Relative fibrinogen adsorption on coated PDMS samples, where adsorption on TCPS serves as the reference set to 100%.

and 80.3% were measured after the incubating of the material with red blood cells concentrates and whole blood, respectively. These results are on par with other bioinert materials that were studied previously.[30] Platelet attachment, however, had a slightly higher value; a 63.67% reduction was measured. This shows that the two-step zwitterionization approach was successful in enhancing the hydrophilicity and biocompatibility of PDMS. In addition, the two-step approach proved to be better in most cases than the one-step process. These results are attributed to the fact that the addition of the tannic acid/$FeCl_3$ complex during the precoating step increases the amount of available reactive sites for the zwitterionic copolymer to anchor to the surface of PDMS, yielding a higher surface coverage with fewer defects left available for biofouling. Also, as again observed from these results, adding EDA did not help in improving the material's biocompatibility. EDA acts as a cross-linker for the epoxy groups present in the GMA segment of the zwitterionic copolymer; thus, the cross-linking of these segments reduces the anchoring power of the copolymer.

Zwitterionic PDMS as a Potential Storage Material for Platelets: Evaluation of Platelet-Activation Inhibition. Platelets are derived from nucleated precursor cells known as megakaryocytes localized in the bone marrow and flows in the bloodstream as a non-nucleated disk-shaped cell with a diameter of 3−4 um. They play a vital role in hemostasis, and the mechanism of action is designed in such a way that it prevents excessive bleeding caused by ruptured blood vessels by binding into the damaged sites rapidly, followed by thrombosis.[42,43] Major components that play a vital role in platelet activation are platelet membrane glycoproteins.[44] The binding of platelet and fibrinogen can be mediated by the most abundant glycoprotein, GpIIb/IIIa complex, which then leads to platelet aggregation.[45] It is then accelerated upon the release of platelet-derived growth factors and from platelets, which, in turn, is promoted by the glycoprotein CD42b. However, granule glycoproteins CD62p and CD63p can be used as platelet activation markers.[43,44]

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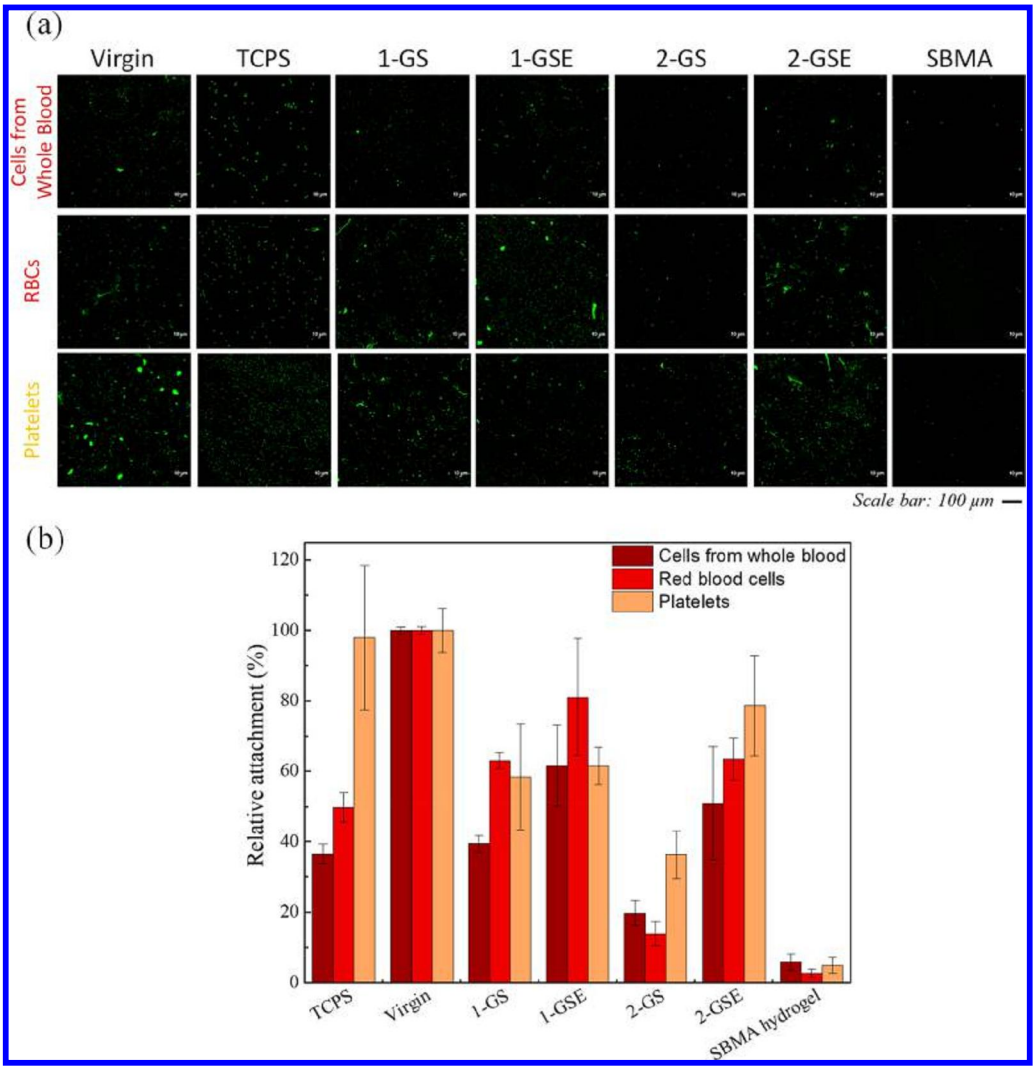

Figure 7. Attachment of different blood cells presented with their (a) micrographs and (b) quantitative analysis.

Furthermore, flow cytometry can be utilized to detect platelet activation as it enables the detection of the activation marker released by individual platelets, with the use of monoclonal antibodies that have already been developed.[43,46]

Exposure of a foreign material to blood can promote platelet adhesion and activation, which can then lead to a series of immuno-response and thrombosis. With sufficient biocompatibility, platelet activation can be suppressed. The sample condition that best performed in terms of biocompatibility, 2-GS, was tested for platelet activation. Flow cytometry permits the evaluation of the degree of platelet activation via measuring the fluorescence intensity of labeled platelet-specific glycoproteins such as CD63P. Figure 8 reports the platelet activity measured upon the storage of PRP into 3 different sample tubes. K3EDTA Vacuette blood vials (9 mL) are used as a reference for investigating platelet activation in this study. The change in platelet activation was determined by getting the difference of the activated platelets monitored upon placement of PRP samples in the tubes from the activated platelet monitored on the second day. Based on the results, the virgin PDMS have increased in amount of the activated platelets almost at the same degree as that of the reference PRP sample amounting to a 10.95% and 11.2% increase, respectively. For 2-GS sample, however, the change in activation was reduced to only 3.35%. A 63.67% reduction of platelet attachment was measured and presented above (Figure 7), suggesting that a

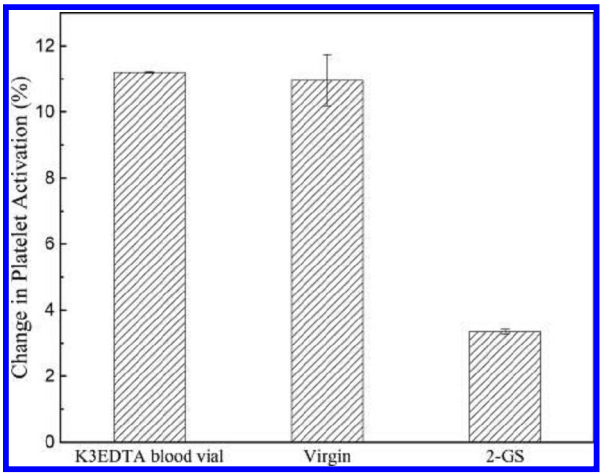

Figure 8. Evaluation of the capability to inhibit platelet activation with a K3EDTA blood vial as a reference.

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significant proportion of platelets still adhered to the surface. However, flow cytometry tests suggest that most of these platelets are not activated and will not trigger any further cell attachment. In other words, the surface-modification process and the zwitterionic material presented in this study are a viable strategy with which to endow PDMS with improved biocompatibility and hemocompatibility, with potential applications as a storage material for blood fractions or, more generally, as a blood-contacting material.

CONCLUSIONS

In this study, tannic acid Fe(III) was used as a precoating material on PDMS to generate multiple hydroxyl groups that can be further utilized for coupling with the ring-opening reaction of the GMA segments of a zwitterionic poly(GMA-*co*-SBMA) copolymer. This method retains the inherent essential properties of PDMS, such as its mechanical properties and optical transparency. With the aid of the precoating step, zwitterionization of the PDMS surface was proved to be successful. Samples coated with EDA, used as a cross-linker for the GMA segments, showed lower biocompatibility. It was due to the competition between the use of EDA as a cross-linking agent and its anchoring to the hydroxyl groups of tannic acid. Comparing PDMS modified after a precoating step versus PDMS directly zwitterionized without a precoating step, samples precoated with the tannic acid were shown to have a better biocompatibility. Protein adsorption was reduced to 89.6%, while the attachment of bacteria, red blood cells, and whole blood was reduced by at least 80% with the precoated samples. Platelet attachment was reduced by 63.67%, and complementary tests showed that the degree of activation of platelets on the coated sample was lower than on the untreated PDMS by a significant margin. Indeed, activated platelets increased by 10.95% in a period of 2 days using uncoated PDMS, while it only increased by 3.35% using our zwitterionic PDMS. Overall, the results of this work have highlighted that PDMS can be modified in a simple manner in view of improving its biocompatibility without compromising its mechanical strength and optical transparency. The poly(GMA-*co*-SBMA)-grafted PDMS materials hold promise for numerous biomedical applications of PDMS, including blood-storage materials, materials for microfluidics-based medical analyses, or blood-typing materials.

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Notes

The authors declare no competing financial interest.

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