In Vitro Blood Compatibility of Modified PDMS Surfaces as Superhydrophobic and Superhydrophilic Materials

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ABSTRACT: The surface of polydimethylsiloxane rubber (PDMS) was irradiated by a CO_2-pulsed laser. The irradiated surfaces were grafted by hydroxyethylmethacrylate phosphatidylcholine (HEMAPC) by using the preirradiation method. The laser-treated surfaces and HEMAPC-grafted PDMS surfaces were characterized by using a variety of techniques including ATR-FTIR spectroscopy, scanning electron microscopy (SEM), and wettability, which was measured by a water-drop contact angle. Different surfaces with different wettability were prepared. These surfaces, including untreated PDMS (hydrophobic), laser-treated PDMS (superhydrophobic), and HEMAPC-grafted surfaces (superhydrophilic), were used for a platelet adhesion study. Results from *in vitro* testing indicated that chemical structures, such as negative-charge polar groups and wettability, are important factors in blood compatibility of these surfaces and the superhydrophilic (the most wettable) and the superhydrophobic (the most unwettable) of modified PDMS surfaces have excellent blood compatibility compared to the unmodified PDMS. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 91: 2042–2047, 2004

Key words: PDMS; surface modification; superhydrophilic; superhydrophobic; blood compatibility

INTRODUCTION

Interactions between the coagulation system and the polymer surfaces are of a highly complex nature and depend on the relative blood compatibility of biomaterials.[1–3] These interactions involve plasmatic enzymes as well as cellular elements and flow conditions.

When a biomaterial is used in direct contact with blood for a short time (e.g., for blood circulation, catheterization, drainage, and temporary blood passage during surgical operation), heparin is generally injected in the blood to prevent it from coagulating on the biomaterial surface. However, the use of such an anticoagulant is not recommended, especially when the patient is apt to hemorrhage by drug administration at high concentration. To develop better biomaterials, in particular for replacement of small vessels, it is important to manipulate platelet–surface interactions to increase the thromboresistance of the foreign surface toward blood. Because platelet adhesion to a biomaterial surface is important, as it results in the formation of a hemostatic plug or thrombus, platelet adhesion and platelet number counting is one of the most popular experimental tools for evaluating the hemocompatible properties of man-made materials.[4,5] Many methods have therefore been proposed to avoid blood coagulation and thrombus formation with no drug administration, among which, surface modification of the blood contacting device is a suitable method.[6]

Several strategies have been proposed to improve the blood compatibility of biomaterials; one strategy involves the synthesis of a highly hydrophilic interface by grafting hydrogel groups to the backbone of a hydrophobic polymer.[7,8] Another approach to thromboresistance includes the introduction of highly hydrophobic groups to the blood-contacting interface by grafting alkyl chains to relatively hydrophilic materials.[9–11]

It is commonly agreed that human blood should be used for *in vitro* tests whenever possible.[12,13] Platelet activation and thrombus formation should be considered to assess accurately thrombogenicity of biomaterials.[14] Silicone rubber has been widely used for medical devices and numerous studies have been conducted on toxicity, stability, and tissue response.[15–17] However, the long-term stability of polydimethylsiloxane rubber (PDMS) in the biological environment has been questioned and the antithrombogenicity of PDMS needs to be largely improved for more biomedical applications.[18,19]

This study was undertaken to reduce platelet adhesion onto the PDMS surface by laser irradiation and also graft polymerization of hydroxyethylmethacrylate phosphatidylcholine (HEMAPC). Hemocompatible properties of silicone-modified surfaces were evaluated by a platelet adhesion study by using platelet-rich plasma

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(PRP) method and counting the platelets by using the lactate dehydrogenase (LDH) method.[20,21]

METHODS

PDMS vulcanization

A medical-grade silicone rubber (M-3090, purchased from Waker Co.) and raw polydimethylsiloxane were milled with 0.5 phr dicumylperoxide (99%) as curing agent. The compound was compression cured at 165°C in a film sheet mold for 5 min. Vulcanized films of 0.5 mm thickness were Soxhlet extracted with toluene/methanol (60/40, v/v) for 24 h to extract impurities and then dried in a vacuum oven at ambient temperature to reach constant weight.

Irradiation procedure

Both sides of the sample surfaces were laser treated and the laser pulses, in ambient conditions, scanned the whole surfaces. No additional chemicals or photosensitizers were used. The laser used was a line-tunable CO_2-pulsed laser (transfer excitation at atmospheric pressure (TEA) CO_2 laser lumonics −103–2), which provides laser beams of wavelengths from 9.1 to 10.6 μm (1098–943 cm^{-1}). The pulse duration was 100 ns. After each exposure, the samples were washed first with acetone : distilled water (50/50, v/v) at 80°C for 48 h. The extracted samples were dried in a vacuum oven at 50°C to achieve constant weight.

Graft copolymerization of HEMAPC

After laser treatment, peroxides were formed on the surface. The treated PDMS films were degassed with a vacuum pump for 6 h under a pressure of 0.2 mm Hg, and HEMAPC monomer aqueous solution, that was degassed by freezing three times with N_2 liquid, was poured into the PDMS film reactor without further exposure to air. Graft polymerization of HEMAPC onto the treated PDMS was allowed to proceed at 55°C for 2 h. The removal of the homopolymers was carried out by Soxhlet extraction by using a mixture of distilled water and acetone (50/50, v/v) at 80°C for 24 h. The samples were dried under standard conditions.

Surface characterization and analysis

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy with KRS-5 prism and with an angle incidence of 45° was used.

Scanning electron microscopy (SEM) was performed on gold-coated samples by using a Polaron sputter coater. A Cambridge S-360 SEM, operating typically at 10 kV, was employed for morphological measurement.

Hydrophilicity was evaluated by measuring the contact angle formed between water drops and the surface of the modified samples by using the contact-angle measuring system G 10 (Kruss). For this purpose, the drops of water were mounted on three different areas of the surface with a microsyringe. Results are the mean values of three measurements on different parts of the film.

Venous blood from healthy human volunteers was collected with a vacuum syringe containing 5% citric acid. The blood was centrifuged at 800 rpm for 10 min at 25°C and the PRP was withdrawn with a polyethylene (PE) pipette and placed in clean vials. The residue of the blood was centrifuged at 3000 rpm for 10 min to obtain platelet-poor plasma (PPP). The platelet count of PRP was determined with Coulter counter (type 4) and adjusted to 150,000 platelets in mm^3. PRP (1 mL) was placed on each of the PDMS films of 1 cm^2 and allowed to stand for 1 h at 37°C. The samples were then vigorously washed with phosphate-buffered saline (PBS) and fixed with 2.5% glutaraldehyde in saline at 20°C overnight. The samples were then dehydrated with ethanol (50–100%) and dried up to the critical point.

The platelets adhering to and spreading on the surfaces were photographed with an SEM.

To determine the number of adhered platelets, 2 mL lysis buffer (0.5% Triton X-100) in PBS was added to the films in a test tube. The lyses were allowed to proceed for at least 1 h at room temperature to ensure complete platelet disruption. The LDH (Horizon Diagnostic, Inc., Ann Arbor, MI), as reagent activity (this agent is intended for *in vitro* quantitative, diagnostic determination of lactic dehydrogenase in human serum or plasma) of lysate was measured by addition of 0.3 mL substrate buffer to the tube.

The change in ultraviolet absorption at 340 nm was measured immediately, using an ultraviolet spectrophotometer (Pharmacia-Biotech R-A-1000-standard).

The initial linear part of the curve was used for calculation of the LDH activity and the LDH calibration curve was obtained by measuring the enzymatic activity of a set of samples with a known concentration of platelets in PBS buffer under the same condition as the films. The experiments were repeated three times by using different PRP. Results are the mean values of three measurements.

RESULTS AND DISCUSSION

Laser-treated samples

Figure 1(a, b) shows the SEM photomicrographs of untreated and laser-treated PDMS surfaces, respectively. These photomicrographs show the continuous and homogeneous porosity of the surface of laser-treated PDMS in comparison with the unmodified

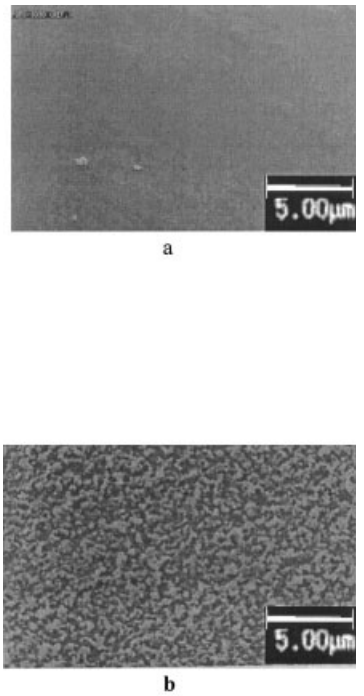

Figure 1 SEM micrographs of (a) surface of virgin PDMS; (b) CO_2-pulsed laser-treated PDMS surface at 9.58 μm wavelength by five laser pulses.

PDMS. The typical dimension of this porous structure is about 400 nm, which is much smaller than the dimension of the platelets (3–5 μm). These dimensions inhibited platelet trapping and further platelet activation.

Surface-graft polymerization

As mentioned in our previous article, the oxidation reaction is a result of the laser irradiation at 9.58 μm wavelength and peroxides were formed onto the PDMS films.[22] Briefly, the radicals produced by laser irradiation immediately reacted with oxygen, peroxy radicals were formed and, after hydrogen abstraction from neighboring PDMS chains, hydroperoxide groups were formed. It should be emphasized that it is critical to exclude oxygen from the silicone prior to the graft polymerization process. If degassing of treated silicone was not complete, the graft polymerization of hydrogel onto the silicone surface did not occur. Silicone contains plenty of dissolved oxygen that can act to inhibit the radical initiation required for graft polymerization.[23] Graft copolymerization of hydrogels onto the laser-treated films took place in the presence of monomer when the systems were heated to a temperature higher than 50°C, whereas no graft polymerization was observed with the untreated silicone under the same conditions. Soxhlet extraction of the grafted PDMS in distilled water was carried out for

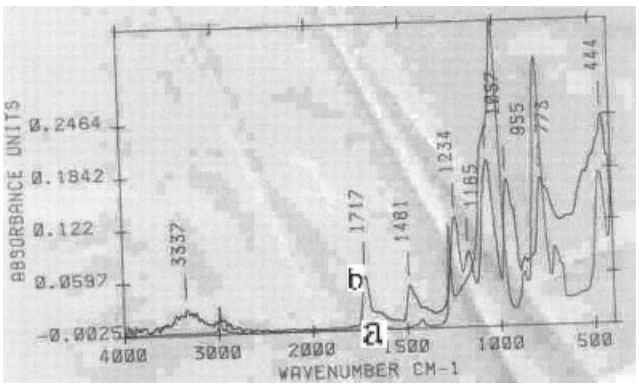

Figure 2 ATR-FTIR spectra of HEMAPC grafted onto the PDMS surfaces by CO_2-pulsed laser: (a) unmodified PDMS; (b) HEMAPC-grafted laser.

72 h to remove the homopolymer from the PDMS surfaces. The samples were dried and the ATR-FTIR spectra were recorded. Figure 2 shows the ATR-FTIR spectra of the HEMAPC grafted onto the PDMS surfaces [Fig. 2(b)] and is compared with the control [Fig. 2(a)]. Comparison of these spectra with that of the unmodified one gives evidence for the presence of grafted of HEMAPC onto the PDMS surfaces. Absorbency bands at 1717, 1481, and 3337 cm^{-1} show HEMAPC functional groups that appear on the PDMS surface. SEM photomicrographs of HEMAPC grafted onto the PDMS surface and cross section of the grafted layer are shown in Figure 3(a, b). As is evident, graft thickness is about 2 μm.

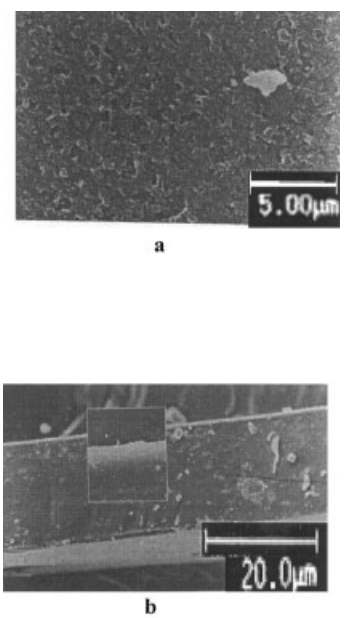

Figure 3 SEM photomicrographs of (a) HEMAPC grafted onto the PDMS surface; (b) cross section of HEMAPC-grafted PDMS.

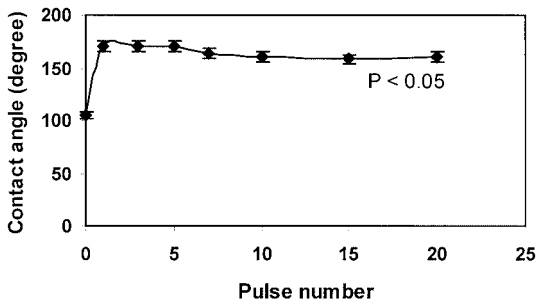

Figure 4 Changes of the water-drop contact angles of the laser-treated PDMS samples with the pulse number (results are mean values of the three measurements ± SE).

Wettability study

PDMS exhibits hydrophobic behavior and poor wettability. The virgin PDMS film has a 105° water-drop contact angle. Water-drop contact angle changes versus pulse number are shown in Figure 4. As can be seen in this figure, the contact angle is increased with the increase in the pulse number up to five pulses (θ = 170°), above which the contact angle is decreased. It means that the surface property of the treated samples has changed and a superhydrophobic surface, compared with the unmodified PDMS, is obtained. As shown by the other studies,[24] it is believed that this unexpected high contact-angle phenomenon in surface-treated polymers is controlled by a large number of different interactions, both chemical and physical, involving modified chains and the environment, surface and subsurface molecules, and interactions among the treatment-introduced functional groups, and finally, the morphology of the polymer. We attributed this superhydrophobicity to the presence of air pockets with hydrophobic property, which have formed due to laser irradiation of the PDMS surface. However, one assumes that it might be the oxidized groups formed on the laser-treated PDMS surface that caused the potential for greater hydrophilicity in comparison with the unmodified PDMS. It seems likely that physical properties of the laser-treated surfaces are predominantly due to surface chemistry and why the PDMS-treated films show higher hydrophobicity in comparison with the unmodified films. In comparison, the single-pulse laser-treated sample, without any graft polymerization, gave a contact angle of 170°. The water-drop contact angle of HEMAPC grafted onto the PDMS surface is about 10°. Results show that there are differences between the wettability of HEMAPC-grafted surfaces and laser-treated PDMS and virgin PDMS. These differences in wettability caused a major effect on blood compatibility of the above-mentioned samples.

Platelet adhesion study

Platelet adhesion experiments were carried out *in vitro* by using the PRP method. SEM was used to study the morphology of the adherent platelets and to compare the platelet shape change responses on the different surfaces. SEM photomicrographs of adhered platelets on the control, laser-treated, and HEMAPC-grafted PDMS surfaces are shown in Figure 5(a–c), respectively. Little platelet spreading is observed onto the laser-treated PDMS in comparison with the unmodified sample. On the untreated materials, the platelets cover the surface completely and form microthrombi. It can be concluded from these studies that the surface of laser-treated PDMS does not induce platelet activation. Platelet spreading on the HEMAPC-grafted PDMS is also less than the virgin PDMS.

The results show that the HEMAPC-grafted PDMS does not induce platelet activation. For better comparison, the number of attached platelets on the HEMAPC-grafted PDMS, control, and laser-treated PDMS without grafting were estimated by measuring LDH activity after detachment of the attached platelets. However, because the method of attached platelet number counting is one of the most popular methods

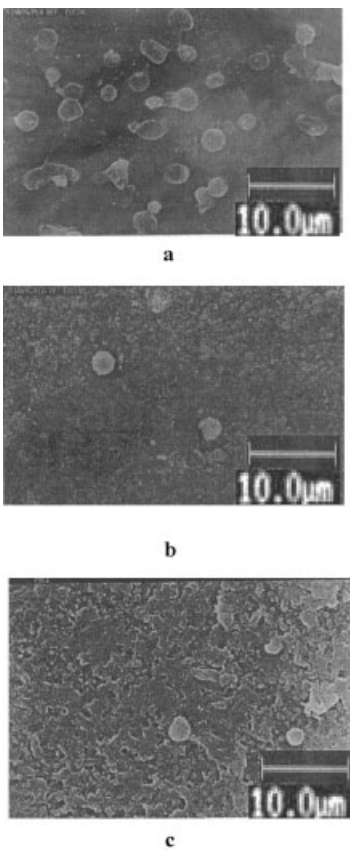

Figure 5 SEM photomicrographs of platelets attached onto the (a) virgin PDMS; (b) laser-treated PDMS; (c) HEMAPC grafted onto the PDMS surface.

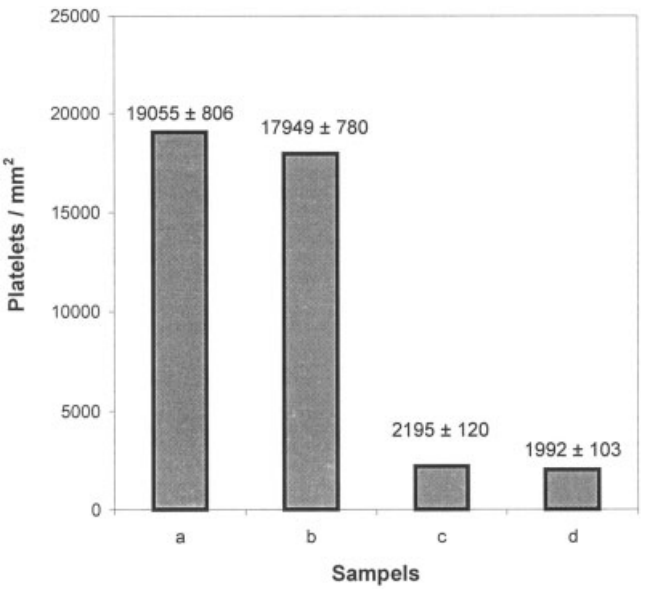

Figure 6 Number of platelets attached onto the different PDMS surfaces measured by LDH method: (a) glass; (b) untreated PDMS; (c) CO_2-pulse laser-treated PDMS (five pulses); (d) HEMAPC-grafted PDMS (results are mean values of three measurements ± SE, $P < 0.05$).

used for evaluating the blood compatibility properties of manmade materials,[25,26] this method was employed as well. The results are shown in Figure 6. The CO_2-pulsed laser-treated PDMS and HEMAPC-grafted PDMS surfaces have better results for blood compatibility compared to the unmodified one. These two surfaces did not induce platelet activation and the numbers of platelets attached are less than the control sample. There are several factors that affect blood compatibility.[27,28] The most important factors are surface roughness and surface wettability (hydrophilic and hydrophobic surface). Ikada et al. reported[29] that there are two possibilities for a polymer surface to have $W_{12,w}$ (work of adhesion between materials 1 and 2 in water) of 0, in other words, to be nonadhesive. One is to create a superhydrophilic surface, in other words, a waterlike surface ($\gamma_{1w} = 0$), and the other is to create a superhydrophobic one ($\gamma_{1w} = 73$) (erg cm^{-2}). Ikada et al. also have reported that it is almost impossible to synthesize a hydrophobic polymer which exhibits γ_{1w} greater than fluoropolymers that possess the lowest γ_1 among the conventional polymers. On the contrary, it may be much easier to modify the polymer surface so as to have a $W_{12,w}$ close to 0. They suggested that the surface, either superhydrophilic ($\gamma_{1w} = 0$, i.e., contact angle of about 0 or superhydrophobic ($\gamma_{1w} = \gamma_w$, i.e., contact angle of about 180°), may possess excellent blood compatibility.

In fact, considering the laser-treated PDMS surfaces, both the roughness (porosity) and the charged chemical nature of the surface (oxygen enriched)[22] serve to alter the properties to nonplatelet activity as compared to the nature of the platelet activity of untreated PDMS. The net effect of the oxygen-enriched nature of the treated PDMS may be to increase negative charge.

Grafting of HEMAPC onto the PDMS surfaces leads to materials with a hydrophilic nature having polar bond groups. However, in each case the polar bond groups are highly hydrated. The HEMAPC-grafted materials contain the phosphotidyl choline zwitterionic head group, which is known to be highly hydrated, and it seems, create a strong bond hydration shield around the molecules, which serves to prevent any molecular recognition process and to avoid recognition from (bonding to) blood proteins and cells.

CONCLUSION

The CO_2-pulsed laser was used to induce the PDMS surface to be graft polymerized with water-soluble HEMAPC monomer through thermal cleavage of the hydroperoxide groups that are generated by the laser. By using this laser tool and graft polymerization technique, superhydrophobic and superhydrophilic surfaces are formed. *In vitro* assays showed that superhydrophobic surface (laser-treated PDMS) and superhydrophilic surface (HEMAPC-grafted PDMS surface) provided surfaces reducing platelet adhesion and its activation in comparison to control surfaces. We conclude that HEMAPC-grafted PDMS and the laser-treated PDMS surfaces show excellent blood compatibility, better than the control, because of the most and the least wettability of these surfaces.

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