Laser Surface Modification of Polymers
A Novel Technique for the Preparation of Blood Compatible Materials (II): In Vitro Assay

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ABSTRACT

Surface modification of polydimethylsiloxane (PDMS) based vulcanizate by CO2-pulsed laser was studied. The modified surfaces were characterized using a variety of techniques including scanning electron microscopy (SEM) combined with energy dispersive X-ray analysis (EDXA), attenuated total reflectance infra-red spectroscopy and the water drop contact angle analysis. EDXA and SEM showed that all of the treated PDMS surfaces contained a higher ratio of O/Si than the base polydimethylsiloxane and the surface morphology of the treated samples were significantly changed. The friction coefficient of the modified silicone drastically decreased. Data from in vitro blood compatibility assessments indicated a significant reduction of platelet adhesion and aggregation for the modified surfaces and those platelets which were adherent remained unspread. The extent of platelet adhesion was correlated to the number of laser pulses.

Key Words: CO2-laser, PDMS, surface modifications, blood compatibility, platelet adhesion

INTRODUCTION

Interaction between the coagulation system and the polymer surface are of a highly complex nature and depend on the relative blood compatibility of a biomaterial [1]. These interactions involve plasmatic enzymes as well as cellular elements and flow conditions. Since platelet adhesion to a biomaterial surface is important as it results in the formation of hemostatic plug or thrombus, platelet number counting is one of the most popular experimental tools for evaluating the haemocompatible properties of manmade materials [2]. 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 [3]. Another approach to thromboresistance includes the

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introduction of highly hydrophobic groups to the blood contacting interface by grafting alkyl chains to a relatively hydrophilic materials [4, 5].

It is also agreed that human blood should be used for in vitro tests whenever possible. The evaluating haemocompatibility in vitro usually involves platelet and coagulation assays [6, 7].

This study was undertaken to reduce platelet adhesion on a polymer surface by laser irradiation, because interactions of these blood components with man-made materials will trigger thrombus formation on the foreign surface when it comes into contact with blood. The rationale for surface modification of polydimethylsiloxane (PDMS) by laser treatment to reduce platelet adhesion is based on the minimal interaction of the super-hydrophobic nature of the PDMS laser-irradiated resulting surfaces with blood components.

As mentioned above these surfaces are widely employed to avoid blood coagulation and thrombus formation [8]. On the other hand, laser induced surface modification of polymers provides a unique and powerful method for the surface modification of polymeric materials without altering their bulk properties [9, 10].

Other advantages of laser sources to initiate chemical reactions have previously been reported [6–9, 10, 11]. Also, surface modification through the use of laser deposition offers several advantages, including ease of treatment of certain surfaces that are difficult to treat with conventional chemical methods. The resulting surfaces are particle-free and sterile, due to the low amount of chemical introduced [10, 11]. The deposition films are thin, tightly adherent and can be deposited on complex geometries. This technique offers possibilities to improve the performance of existing biomaterials and medical devices and for developing new biomaterials [9, 10]. However, irradiating a polymeric material with a continuous laser beam heats the surface and may damage both the surface and the bulk, whilst pulsed lasers allow short exposure times and less thermal damage by optimizing the time intervals between pulses (repetition rate), laser fluency and pulse number [11, 12, 13].

For the long-term application of the silicones used, the stability and the biocompatibility of the polymers are crucial [14].

It has been reported that the polymer surface, either super-hydrophilic or super-hydrophobic which indicate very high and very low surface free energy (γv), respectively, may possess excellent blood compatibility [8].

PDMS has some excellent properties such as high structural resistance towards heat, ozone and chemicals [15]. Medical grade silicone rubber is a widely used biomaterial for different applications including tubing, catheters, vascular grafts, plastic reconstruction, encapsulation of electronic components and voice prostheses [15, 16].

Platelet adhesion to a biomaterial surface is important as it results in the formation of a haemostatic plug or thrombus [4]. There is a general agreement that in vitro tests are most useful in the evaluation of artificial surfaces that are highly reactive with blood [5, 6].

It is well known that the thrombus formation is triggered by an interaction between blood components and the foreign polymer surface [8]. On the other hand the protein adsorption depends greatly upon the surface energy of the substrate [17]. The surface of the treated samples has been characterized and also the surface morphology has been studied.

In the present work we have utilized a CO2-pulsed laser to induce surface modification of PDMS to create a super-hydrophobic polymer to improve blood compatibility while keeping the bulk properties of the substrate intact.

**EXPERIMENTAL**

**PDMS Vulcanization**

Raw PDMS (M 3090 Wacker) was milled with 0.5 phr (per hundred rubber weight) dicumylperoxide (90%) as curing agent at 80 °C. Vulcanized films of 0.3 mm thickness and 3.5x3.5 cm dimensions were Soxhlet extracted with toluene/methanol (60:40 v/v).

**Surface Modification Procedure**

Laser-induced surface modification has previously
been reported [18]. The laser used was a line-tunable pulsed CO₂-laser (TEA CO₂ Laser Lumonics-103-2).

**Surface Characterization and Analysis**

In order to characterize the extracted laser treated samples the following methods were carried out.

- Attenuated total reflectance infra-red (ATR—FTIR) (Bruker—88) with KRS—5 prism and an incident angle of 45° was used.
- Scanning electron microscopy (SEM Cambridge S-360) was performed on gold coated samples using a Polaron sputter coater.
- Energy dispersive X-ray analysis (AN-10000 EDXA) with low electron high tension, EHT, (5—10 kV) was used to measure the O/Si ratio of the modified samples.
- Water drop contact angle (Young angle, θ) was evaluated by measuring the contact angle formed between water drops and the surface of the modified samples. For this purpose, the drops of water were mounted on three different areas of the surface with a microsyringe for 5 min, and then photographs were taken at x50. All contact angles (θ) are the means of three determinations.
- The dynamic mechanical properties of the modified and unmodified samples were studied and compared using a Polymer Laboratories DMTA. For each samples, storage modules (E) and loss tangent (tan δ) versus temperature were recorded.
- Platelets which were adherent to the surface were fixed with 2% (v/v) glutaraldehyde in phosphate buffer saline (PBS) for 1 h and stained with 2.5% eosin (staining agent) for 3 h at room temperature. The stained platelets were observed with a light microscopy (Zeiss).
- Dynamic friction coefficients of PDMS surfaces were measured according to the ASTM D 1894—78 method by using a friction measuring apparatus (Davenport).

**In Vitro Experiments**

Venous blood from healthy human was collected with a vacuum syringe containing 5% citric acid. Platelet rich plasma (PRP) was obtained. The platelet count of PRP was determined with a Coulter counter (type 4) and adjusted to 150,000 platelets per mm². PRP (1 mL) was placed on each of the PDMS films of area 1 cm² and allowed to stand for 1 h at 37 °C. The samples were then vigorously washed with PBS and treated with 2.5% glutaraldehyde in saline at 20 °C over night. The samples were then dehydrated with serial dilution of ethanol (50–100 %) and dried to the critical point. The specimens were examined with 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 lysis was allowed to proceed for at least 1 h at room temperature to ensure complete platelet disruption. The lactase dehydrogenase (LDH), 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 spectrometer.

The initial linear part of 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 film [2].

**RESULTS AND DISCUSSION**

**Surface Characterization**

Table 1 shows the ATR—FTIR absorbance unit of –Si—O– band of the PDMS samples treated with 1, 3, 5, 7, 10, 13 and 15 pulses of CO₂ laser. The intensity of this group was reduced with an increasing number of laser pulses. The reduction in the intensity of the 1011 cm⁻¹ band is an obvious manifestation of the intense fragmentation on the surface of PDMS. Figure 1(a) indicates that the modified PDMS exhibited a strong absorption at 1746 cm⁻¹ which was assigned to hydroperoxide or carbonate groups (–O–COO–) on the surface of laser irradiated PDMS by 3 pulses. This is consistent with the EDXA analysis which shows a
higher percentage of oxygen on the surface of the modified PDMS in comparison with unmodified PDMS. We believe that infra-red laser induced surface modification of PDMS by vibrational excitation of the \(-\text{Si-O-}\) band is through an infra-red multiphoton dissociation (IRMPD) mechanism [19, 20].

According to this mechanism the radicals produced by laser irradiation may be transferred to the \(-\text{CH}\) groups on the skeletal chain of the PDMS and these groups are converted to the hydroperoxide or other oxidized groups (carbonyl groups) in the presence of oxygen. The schematic reactions of the oxidized pathway of the PDMS surface are presented in Scheme I. Among many possible oxidation schemes tested to explain our experimental observation, these reactions, give the most probable mechanism of the \(\text{CO}_2\)-plused laser induced oxidation onto the PDMS surface.

SEM photomicrographs of the treated PDMS surfaces are compared with and unmodified sample as shown in Figures 2 and 3. As it can be seen in Figure 2 the pore size of the modified PDMS with 5 laser pulses is about 750 nm. We found that this parameter highly depends upon the number of laser pulses.
Scheme I. The schematic reactions of the oxidized pathway of the PDMS surface.
pulses delivered to the PDMS films, and the irradiation depth has been determined to be about 3 μm (Figure 4).

The variation of the O/Si intensity ratio determined from EDXA analysis is given in Table 1 as a function of the pulse number.

Contact Angle Change
The variation in hydrophobicity of the treated sample is shown in Table 1. As can be seen, the contact angle is increased with the increasing of pulse number up to 5 pulses above which the contact angle decreases. The surface properties of the treated samples has been changed and a super-hydrophobic surface is obtained.

Friction Coefficient
When the surface of the silicone was laser-treated the hydrophobic surface became very slippery. The observed result is shown in Figure 5. As is apparent, the dynamic friction coefficient of the untreated silicone
is 0.41 but drastically decreases about 0.1 when the surface is laser irradiated even if only one pulse was delivered to the silicone surface.

**Dynamic Mechanical Thermal Analysis (DMTA)**

To study the bulk mechanical properties, laser treated PDMS samples were examined by dynamic mechanical thermal analysis (DMTA). The variation of storage modulus (\(\mathbb{E}\)) and loss tangent (\(\tan \delta\)) versus temperature for treated and untreated samples are shown in Figure 6 (a and b), respectively. It can be seen that the \(\tan \delta\) and \(\mathbb{E}\) for the treated and untreated PDMS samples appeared within the same temperature region. As \(\mathbb{E}\) and \(\tan \delta\) are related to the structure and mechanical properties of the rubber, it can be concluded that the bulk structure and therefore the mechanical properties of the laser-treated samples have remained intact.

**Platelet Adhesion**

Platelet adhesion experiments were carried out in vitro using PRP method [4]. SEM micrographs and
Figure 8. Optical photographs (magnification x 1000) of the adhered stained platelets by eosin on (a) PDMS treated with CO₂-pulsed laser by 10 pulses, (b) untreated PDMS.

photographic images of samples are shown in Figures 7 and 8, respectively. As can be seen, low platelet spreading is observed on the laser treated PDMS in comparison with the unmodified sample which platelets adhered and activated fully on the surface, and platelets covered the surface completely and formed mural microthrombi (Figures 7, 8). The number of platelets adhered on the untreated and treated PDMS films from PRP at different pulses was obtained from the LDH method [2]. Elevated level of LDH are associated with platelet adhesion and aggregations, as shown in Figure 9. For all treated samples the platelet adhesion is reduced in comparison with the untreated one.

However, for the sample which has been treated by 10 laser pulses the reduction in platelet adhesion is significant. We have previously reported that peroxides are initially formed in the surface region of the polymer exposed to the laser irradiation in addition to other carbonyl containing groups which are all oxygen rich [18]. The decrease in platelet adhesion can be attributed both to the higher concentration of above mentioned groups. This also causes a physical change in the water drop contact angle onto the surface of the treated PDMS which has been previously reported [18].

CONCLUSION

PDMS is surface modified by the CO₂-pulsed laser providing it is irradiated by pulses having wavelengths where PDMS has strong absorptions. The CO₂-pulsed laser induced modification on the surface of PDMS at 9.58 μm (1043 cm⁻¹) by vibrational excitation of the –Si–O– bond occurring through infra-red multi-photon dissociation mechanism (IRMPD).
Treated samples showed significant variation in hydrophobicity and this is found to depend upon the number of irradiated laser pulses.

Carbonyl containing groups have been formed onto the surface of laser irradiated PDMS in the presence of oxygen.

The friction coefficient of the surface drastically decreases when the surface is treated with the laser and low platelet spreading and aggregation is observed on the laser treated PDMS surface in comparison with controls.

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REFERENCES