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POLYDIMETHYLSILOXANE-ASSISTED CONTROL OF PLATELET ATTACHMENT FOR RAPID ACTIVATION

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We present the evidence of a direct relationship between surface properties and platelet attachment for rapid activation using polydimethylsiloxane (PDMS). Three different surfaces of one uncoated glass slide and two spin-coated glass slides with two different ratios of PDMS (5:1 and 10:1) were prepared and tested. Prior to the platelet attachment test, nanoscale surface roughness and softness of PDMS, according to the degree of crosslinking, were measured using a compression tester and atomic force microscopy. The surface that encouraged the attachment and activation of platelets was 10:1 PDMS with Young's modulus of 2.58±0.27 MPa and roughness of 0.477nm. These data show that platelet attachment and activation can be triggered and enhanced through the control of nanoscale surface roughness and softness of PDMS, which is promising for biomedical applications using PDMS.

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1. Introduction

Platelets are a type of blood cell with the unique ability to stop the process of bleeding (hemostasis). The mechanism of hemostasis consists of the following sequence of events. Firstly, after platelet attachment on the surface, external stimuli cause platelets to undergo a morphological change, initiating cytoskeleton rearrangement and organelle centralization [1-3]. Secondly, the contents of stored alpha granules are released to the exterior [4]. And finally, arachidonic acid is released, enabling platelets to aggregate for wound repair [5]. After platelet activation, platelet aggregation is known to take a few minutes to be completed.

The platelet activation process can be triggered *in vitro* by physical events such as attachment, adhesion, and centrifugation, as well as other chemical means like collagen [6-8]. The surface conditions play an important role in platelet activation through the encouragement of attachment and adhesion, and many studies have been conducted to illustrate this. Park *et al*. investigated the morphological characterization of surface-induced platelet activation using pre-coated albumin and fibrinogen [9]. Milleret *et al*. reported that alkali treatment of microrough titanium surfaces affected platelet activation [10]. Kammerer *et al*. used topographical, chemical and biomimetic titanium surface modifications *in vitro* to promote platelet activation [11], and

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Zhang *et al*. fabricated a biomolecule-PEG micro-pattern onto a titanium surface in order to observe its effect on platelet adhesion [12].

In the present study, we therefore investigate the dependence of platelet attachment on nanoscale roughness and softness using thin spin-coated polydimethylsiloxane (PDMS) films. Nanoscale roughness of PDMS was measured using a linear scan method of atomic force microscope (AFM), and softness of PDMS was determined by compression testing.

2. Experimental

2.1 Sample preparation

3ml whole-blood was prepared in an anticoagulated vacutainer (K_2 EDTA BD Vacutainer, BD Biosciences, Oxford, UK). EDTA was used to segregate Ca^{2+} ions, in order to block the coagulation mechanism of whole blood without affecting platelet function. Platelet-rich plasma (PRP) was collected using the following protocol. Briefly, the 3ml whole-blood sample was centrifuged with a relative centrifugal force (RCF) of 150 x g for 15 minutes, and the red blood cell (RBC) fraction removed from the vacutainer. Subsequent centrifugation was performed at 800 x g for 8 minutes, in order to obtain 0.5ml of concentrated PRP. The PRP was rinsed twice with phosphate buffered saline (PBS) by centrifugation, and subsequently the pellet was resuspended in 2ml PBS. Finally, a PRP solution with a density of 50,000 cells/μl was prepared by counting the cells using a haemocytometer and further dilution in PBS.

2.2 Fabrication of PDMS-coated glass slides and testing of platelet attachment

Two different surfaces were prepared by coating glass slides with two different ratios of PDMS, which is widely used in biomedical applications. An uncoated glass slide was used as a control. Two different ratios of PDMS (Sylgard 184 silicone elastomer kit; Dow Corning, Midland, MI, USA) were prepared by mixing the silicone elastomer base and the curing agent at a ratio of 5:1 and 10:1, respectively. Glass slides (1 in. x 3 in.) were cleaned with ethanol and subsequently spin-coated at 1,000 rpm, and cured on a hot plate at 80^{o}C for 10 minutes. In order to investigate the effect of surface roughness and softness on platelet attachment and activation, the prepared platelets were placed on the two different PDMS surfaces, as well as the uncoated control surface, and observed under a microscope for 7 minutes.

2.3 Compressive strength testing

The mechanical properties of the two PDMS different ratios were determined using an unconfined compression test. PDMS samples with ratios of 5:1 and 10:1 were prepared in cylindrical structures (1cm x 1cm) for three repeat compression tests. The PDMS samples were placed on a compression testing system (Instron Model 5542, Norwood, MA, USA), and the test was conducted at a constant strain rate of 1mm/min. The 10 N load cell was used to monitor the applied load. The Young's modulus of each PDMS sample was calculated based on the obtained compressive stress-strain curve.

2.4 Atomic force microscopy

AFM (Multimode-8, Bruker, Santa Barbara, CA, USA) imaging was carried out to determine the three-dimensional surface roughness of the two different ratios of PDMS. The scan size for AFM was chosen as 1.0μm at a resonance frequency of 1.0 Hz (Tapping mode).

3. Results and Discussion

The stiffness of the PDMS was investigated using compressive strength tests. In Figure 1(a), two compressive stress-strain curves were measured for two different PDMS samples with a 5:1 and a 10:1 ratio. Based on the curves, elastic moduli of two PDMS samples were measured as 3.53±0.48 MPa and 2.58±0.27 MPa for the 5:1 and 10:1, respectively, according to the elastomer base and curing agent ratio, as shown in Figure 1(b). These results show that increasing the PDMS,

and effectively decreasing the concentration of curing agent, causes less cross-linking of the PDMS. Therefore, the PDMS sample with a 10:1 ratio was softer compared with the PDMS sample with a 5:1 ratio.

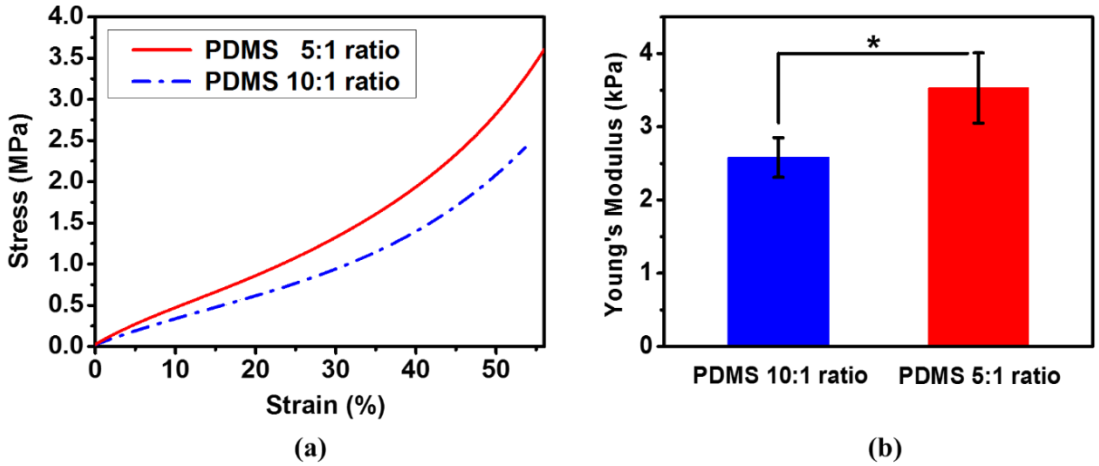

*Fig. 1. Compressive strength test. (a) Stress-strain curves and (b) Young's modulus of the fabricated PDMS with 5:1 and 10:1 ratios. (*p<0.001).*

Fig. 2 shows the obtained AFM surface images of the two different ratios of PDMS. The roughness (Ra) of each PDMS sample with a 5:1 and a 10:1 ratio was 0.435nm and 0.477nm respectively. As the concentration of curing agent was reduced, less cross-linking of PDMS generated a rougher surface.

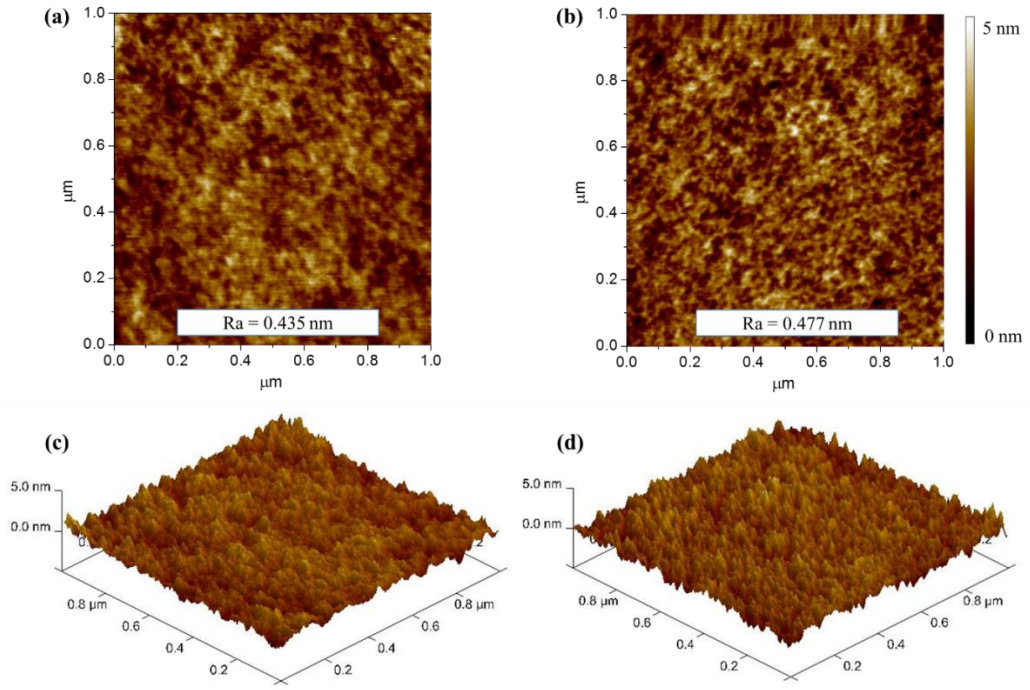

Fig. 2. AFM surface images of two different ratios of PDMS. (a) & (c) PDMS with a 5:1 ratio; (b) & (d) PDMS with a 10:1 ratio.

Platelet attachment tests were performed under a microscope on glass slides spin-coated

with two different ratios of PDMS, 5:1 and 10:1. An uncoated glass slide was used as a control. Figure 3 shows platelet attachment and activation as a function of time, from 0 minute to 6 minutes, on the three different surface conditions. The surface to which the most number of platelets were attached was the 10:1 ratio of PDMS, while the surface to which the least number of platelets were attached was the uncoated glass slide. When correlated with surface roughness and softness, platelets seem to prefer rougher and softer surface conditions for attachment.

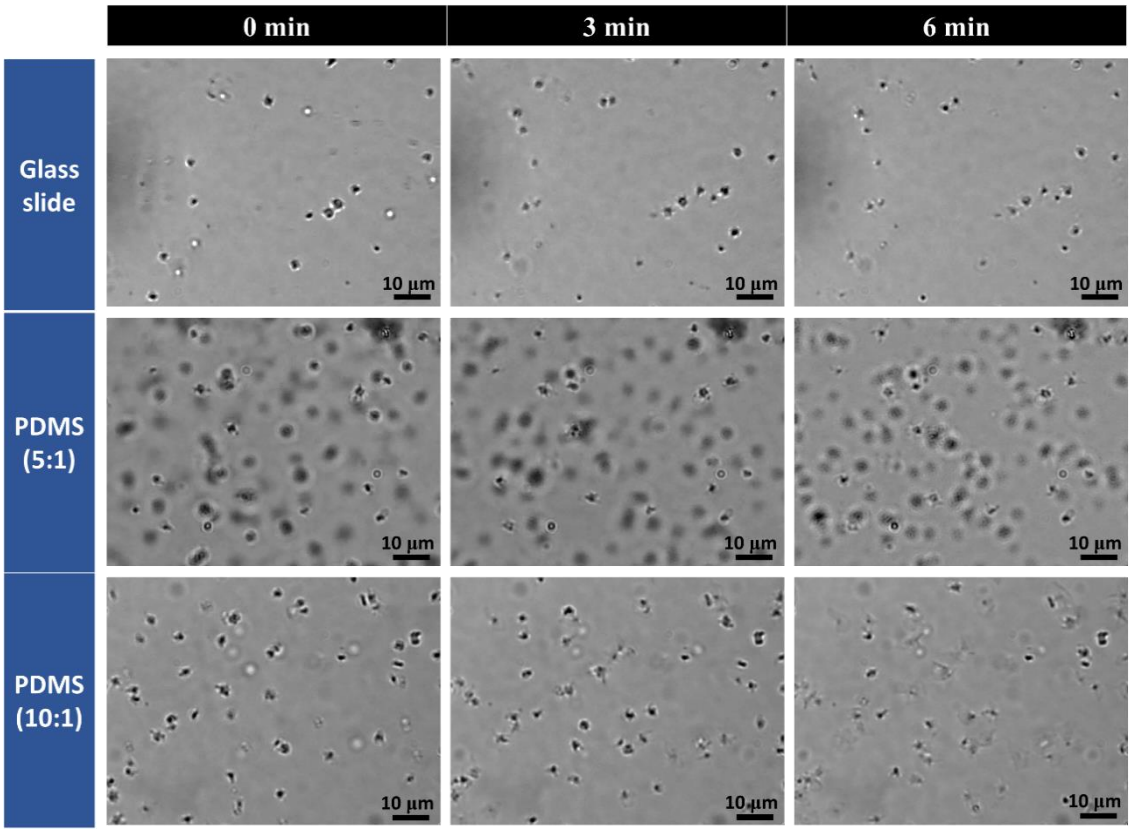

Fig. 3. Microscopic images of platelet activation on three different surfaces as a function of time (from 0 to 6 minutes).

Fig. 4 shows comparative analysis using the number of platelets attached on three different surfaces (uncoated, 5:1 PDMS-coated, and 10:1 PDMS-coated glass slides) as a function of time for 0, 3, and 6 minutes. At 0 minute, the mean number of platelets attached on the surface of 10:1 PDMS coated glass slide was 2.7 times greater than that of uncoated glass slide. After 6 minutes, the difference in platelet attachment between the uncoated and 10:1 PDMS-coated glass slide increased by 3.1 times. These results show that softer surface condition could enhance platelet attachment. Since slide glass with a ratio of 10:1 PDMS provided platelets with rougher and softer surface conditions, the number of platelets attached was greater by about 2 times compared to slide glass with a ratio of 5:1 PDMS.

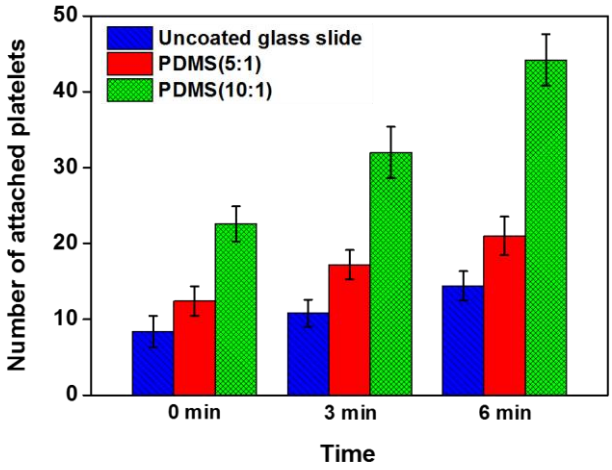

Fig. 4. Comparison of platelets attachment within 50μm × 50μm area on the surfaces of uncoated, 5:1 PDMS-coated, and 10:1 PDMS-coated glass slides as a function of time (0, 3, and 6 minutes).

Interestingly, after attachment, platelets were found to keep rotating for settling down only on the uncoated glass slide. Since adhesion on the uncoated glass slide was comparatively weaker than on the PDMS surface, it seems that the platelets were not attached sufficiently enough to the surface for activation to occur. Notably, the most rapid platelet attachment and activation, in terms of morphological change in Figure 3, was observed on the surface of 10:1 PDMS. Therefore, we confirm that platelet attachment and activation can be triggered using PDMS with rough and soft surface conditions.

4. Conclusion

The effect of surface roughness and softness on platelet attachment and activation were observed on three different surface conditions using a compression tester and AFM. The surface to which the most number of platelets became attached, and subsequently activated, was the 10:1 PDMS. This indicates that platelet attachment and activation can be triggered and enhanced on rough and soft surface conditions. Based on these results, nanoscale surface roughness and softness are primarily responsible for platelet attachment and activation. This information will be extremely valuable for a variety of biomedical applications using PDMS, such as hemostasis and tissue regeneration.

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