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SURFACE-ENGINEERED PLATELET ACTIVATION FOR IMPLANTABLE APPLICATIONS OF PARYLENE-COATED POLYDIMETHYLSILOXANE

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This study analyzed the characteristics of platelet attachment and activation depending on different condition of surfaces (PDMS and parylene on PDMS) for implantable biomaterials. The attached platelets were observed more on the PDMS substrate with the softer surface property, as compared to the parylene-on-the-PDMS substrate. However, due to the effect of the roughness of substrates, the platelets were rapidly activated on the parylene-on-PDMS substrate. Therefore, both softness and roughness of implantable biomaterials need to be regarded as important parameters in surface-engineered platelet applications.

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Keyword: Platelet; Attachment; Activation; Roughness; Softness

1. Introduction

In regenerative medicine, platelets are well known as effective contributors to the prevention of bleeding by the release of inherent growth factors [1, 2]. The platelet-responsive mechanism on the exact damaged spot is based on its attachment and activation that stem from chemical and physical interactions [3-7]. Those interactions strongly correlate with biomaterials and surface conditions; therefore, the release of growth factors can be controlled.

Accordingly, in-depth studies on the release control of growth factors from platelet have been conducted with a variety of biomaterials and surface conditions. For example. Weisenberg *et al.* reported hemocompatibility of materials used in microelectromechanical systems [8]. Furthermore, Chen *et al.* investigated the effect of surface microtopography of poly(dimethylsiloxane) on protein adsorption, platelet, and cell adhesion [9]. In addition, Koh *et al.* studied the effect of topography of polymer surfaces on platelet adhesion [10]. Another study by Alfarsi *et al.* showed that titanium surface could enhance platelet activation [11]. Adamson *et al.* presented ligand capture and activation of human platelets at monolayer modified gold surfaces [12]. Recently, our group introduced polydimethylsiloxane-assisted control of platelet attachment for rapid activation [13].

In the present study, we introduce characteristics of attachment and activation of platelet according to two different surface conditions: polydimethylsiloxane (PDMS) and parylene on PDMS. Its behaviors depending on two different types of surface conditions used as major biomaterials were observed based on both morphological change and the release of growth factors with time. The observation was clearly analyzed using mechanical properties, such as roughness and contact angle.

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2. Experimental methods

2.1 Materials

The effects of surface conditions with two different materials (PDMS and parylene on PDMS) on platelet behavior were investigated. The PDMS-based surface condition was prepared by mixing the silicone elastomer base and curing agent at the ratio of 10:1. The mixed PDMS solution was placed on a slide glass (25 mm x 75 mm) and then subsequently spin-coated at 6,000 rpm up to 10 μm. The PDMS-coated slide glass was cured on a hot plate at 80 °C for 10 minutes. The parylene-on-PDMS samples were prepared by coating an additional layer (1 μm) of parylene-C on the 10 μm PDMS-coated slide glass. To enhance the adhesion between PDMS and parylene, the PDMS-coated slide glass was treated with plasma discharge (CUTE-MP, Femto Science, Inc., Gyeonggi, South Korea) at 400 Watts for 300 seconds.

3 ml of whole blood samples were prepared in a vacutainer anticoagulated with K2 ethylenediaminetetraacetic acid (EDTA) (BD Vacutainer, BD biosciences, Oxford, UK). EDTA was used to segregate Ca^{2+} ions to prevent the coagulation mechanism of whole blood without affecting the platelet functions. The whole blood samples were centrifuged with a relative centrifugal force (RCF) of 150 g for 15 minutes and the red blood cells (RBCs) were removed from the vacutainer. Subsequent centrifugation was performed at 800 g for 8 minutes to obtain 0.5 ml of concentrated platelet-rich plasma (PRP). The obtained PRP was rinsed twice with phosphate buffered saline (PBS) by centrifugation. The number of platelets was counted using a haemocytometer (Haemacytometers, Superior Marienfeeld, Lauda-Königshofen, Germany). Finally, the PRP solution with the density of 2.0×10^6 cells/ml was prepared.

2.2 Experimental setup on the different surfaces

In order to investigate the behaviors of the platelets depending on the different surface conditions, 10-μl PRP solutions were dropped in the Ø 3-mm PDMS reservoirs placed on each surface of PDMS and parylene on PDMS, respectively. Furthermore, the behaviors of the platelets were observed under a microscope for 1, 5, and 10 minutes, respectively. The platelet samples were then fixed to prevent further changes in the morphology of the platelets. Cell fixation was conducted by immersing the samples in the 200 μl solution of 2% paraformaldehyde and 2% glutaraldehyde for 2 hours at room temperature. After removing the solution, the samples were rinsed twice with PBS.

2.3 Visualization for attachment and activation of platelets

The morphology changes of the platelets on the different surfaces were analyzed using a scanning electron microscope equipped with a field emission gun (FE-SEM; SUPRA 40VP, Carl Zeiss, Germany). For FE-SEM analysis, the fixed platelets were dehydrated with a graded ethanol (30%, 50%, 70%, 90%, and 100% (v/v)) for 10 minutes, orderly, and then coated with platinum.

The activation of the platelets on different surface conditions were fluorescently visualized using the primary antibody P-selectin (CD62) (sc-6941, Santa Cruz Biotechnology, CA, USA). P-selectin is a protein that is inherent in the α-granules of the platelets. It is released when the platelets are activated to enhance the coagulation and tissue regeneration mechanisms. The aforementioned fixed platelets were stained with the primary antibody P-selectin for 2 hours. After washing the samples twice with PBS, the samples were treated with the secondary antibody, donkey anti-goat IgG-FITC (sc-62021, Santa Cruz Biotechnology CA, USA). The samples were rinsed twice with PBS and then dried at room temperature. The magnitude of platelet activation was then analyzed using a fluorescent microscope (BX51TF, Olympus corporation, Tokyo, Japan).

2.4 Analyses of mechanical properties

Surface roughness of two different substrates (PDMS and parylene on PDMS) was measured using atomic force microscopy (AFM) (Multimode-8, Bruker, Santa Barbara, CA, USA). The scan areas were 2 μm x 2 μm at a resonance frequency of 0.996Hz (Tapping mode). Also atomic force microscopy (AFM) (XE-100, Park Systems, South Korea) was used to determine Young's moduli of substrates (PDMS and parylene on PDMS). The scan areas were 10 μm x 10 μm with a resonance frequency of 1.0Hz (contact mode, force-distance spectroscopy). The contact mode of a cantilever was applied with a spring constant of 42 N/m, a tip radius of 10 nm, and a constant force of 10 nN. The measurements were repeated six times.

3. Results and discussion

The adhesive behaviors of the platelets to two different surfaces were observed using FE-SEM images at a magnification of ×1,000 (see Fig. 1). Overall, the amounts of the adhered platelets on the substrates proportionally increased with time. At 1, 5, and 10 minutes, the platelets were attached more on the PDMS substrate, while the parylene-on-PDMS substrate showed less number of platelets attached on the substrate. The reason underlying this difference might be that the PDMS substrate provided the softer surface property suitable for platelet attachment. Force-displacement data obtained from AFM measurement, as shown in Fig. 2(a) and (b), support this hypothesis. The values of Young's moduli of PDMS and parylene-on-PDMS substrates were calculated as 0.273±0.001 and 25.8±1.2 MPa from the force-displacement curves, respectively, resulting in the harder surface of the parylene-on-PDMS substrate than that of the PDMS substrate by 95 times.

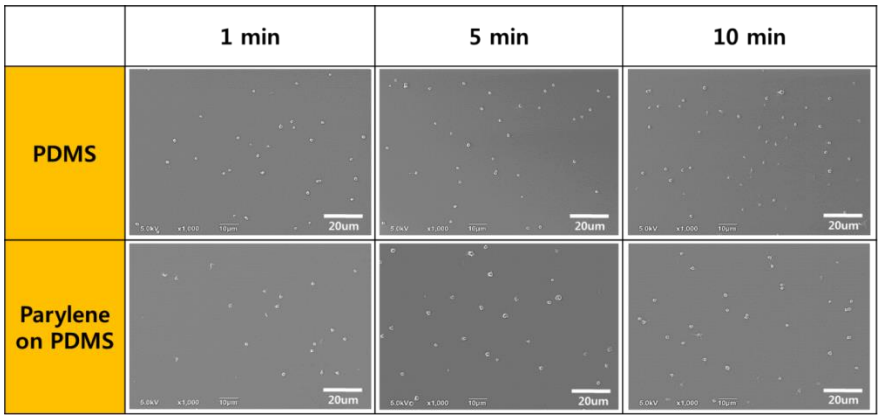
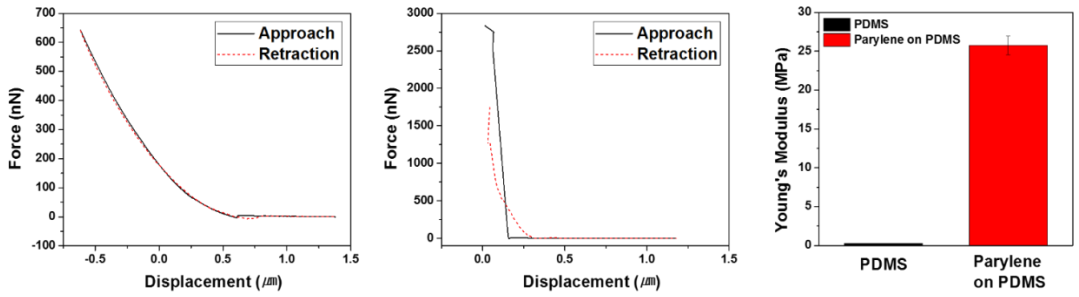


Fig. 1. High-resolution scanning electron microscopic images of platelet attachments according to the PDMS and parylene-on-PDMS substrates with time (1, 5, and 10 minutes).



(a) (b) (c)

Fig. 2. Force-displacement curves of (a) PDMS, (b) parylene on PDMS recorded from approach to retraction of a cantilever, and (c) Young's moduli obtained at retraction from force-displacement curves.

Fig. 3 shows the number of activated platelets stained with P-selectin as a function of time. Based on the fluorescent images in Fig. 3(a), the activation of platelets on the substrates showed the increasing trend with time. Initially, the activated platelets were observed more on the parylene-on-PDMS substrate when compared to the PDMS substrate. After 10 minutes of observation, the platelets on the parylene-on-PDMS substrate still showed the greater number of activation than those on the PDMS substrate. In contrast with the attachment results in Figure 1, the activated number of platelets were greater on the parylene-on-PDMS substrate, as shown in Fig. 3(b).

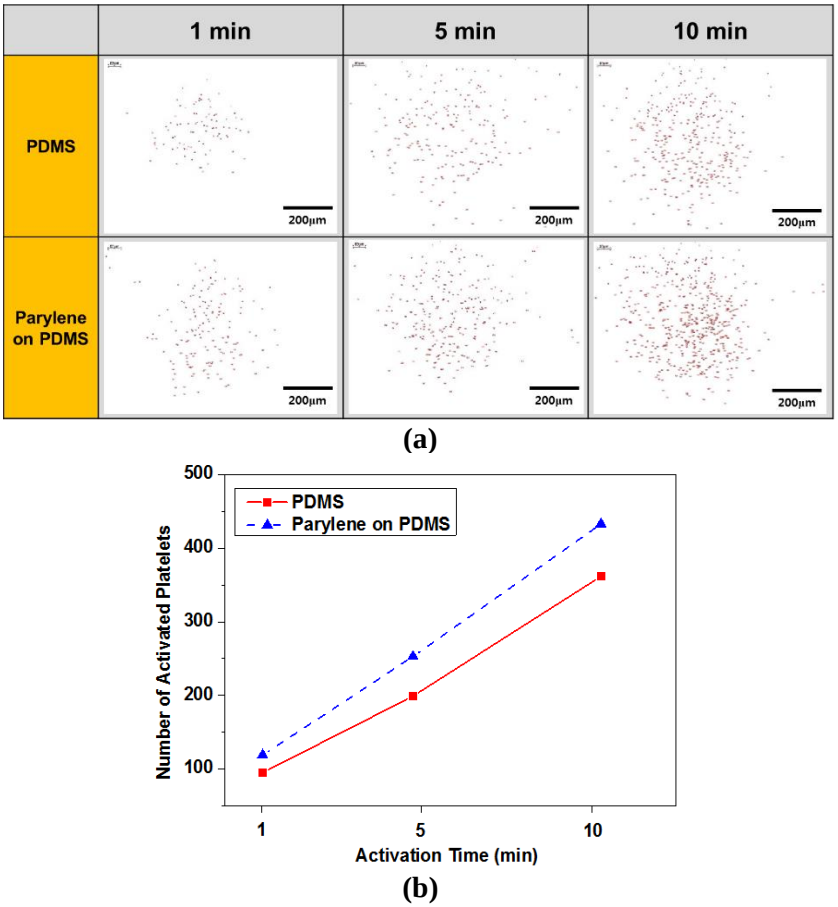


Fig. 3. (a) Fluorescent images of the activated platelets stained with P-selectin and (b) the graph showing the number of activated platelets on the PDMS and parylene-on-PDMS substrates with time (1, 5, and 10 minutes), respectively.

In order to explain this trend, the AFM-based roughness measurements were conducted for two different surfaces (see Fig. 4). The roughness of the parylene-on-PDMS substrate was 3.243±0.778 nm, while that of the PDMS substrate was 0.220±0.031 nm. These results suggest that the platelets seemed to be activated greater on the rougher surface condition. Therefore, although the softer surface showed more amount of platelet attachment, roughness was more essential to enhance the platelet activation after attachment.

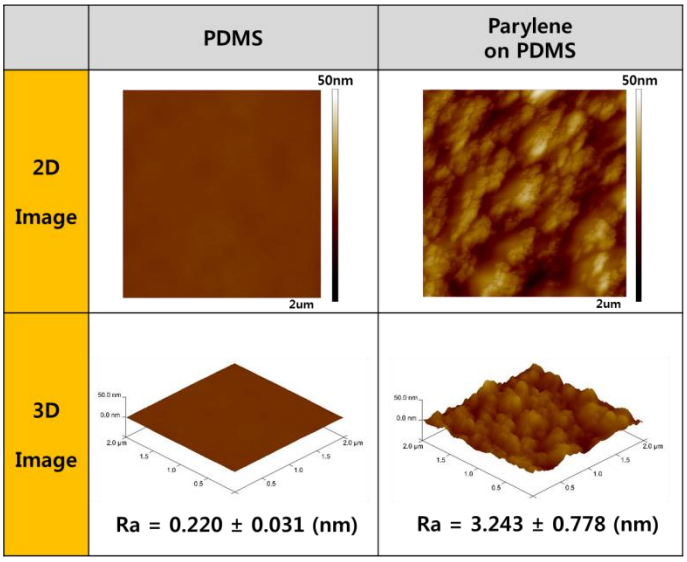


Fig. 4. Roughness of the PDMS and parylene-on-PDMS substrates measured with an atomic force microscope (AFM).

Fig. 5 shows the morphologic change of a single platelet after attachment on the PDMS and parylene-on-PDMS substrates with time (1, 5, and 10 minutes). A continuous series of morphologic changes were observed when platelets were attached on the substrate. First, at 1 minute after attachment, the platelets on the PDMS substrate maintained a spherical shape, while those on the parylene-on-PDMS substrate began to form a whisker-like shape. Second, at 5 minutes after attachment, all platelets formed the extension of filopodia. Only the filopodia protruded on the parylene-on-PDMS substrate were retracted, resulting in forming the platy structure. Based on the high-resolution FE-SEM images, it was observed that the height of the central body of platelets was lowered as the morphology of platelets was changed into the platy structure. At 10 minutes after attachment, the platelets on the parylene-on-PDMS substrate filled the spaces between the retracted filopodia, forming lamellae-like extensions of platelet membrane. However, the platelets on the PDMS substrate were still experiencing the whisker-like shape, without further morphologic changes.

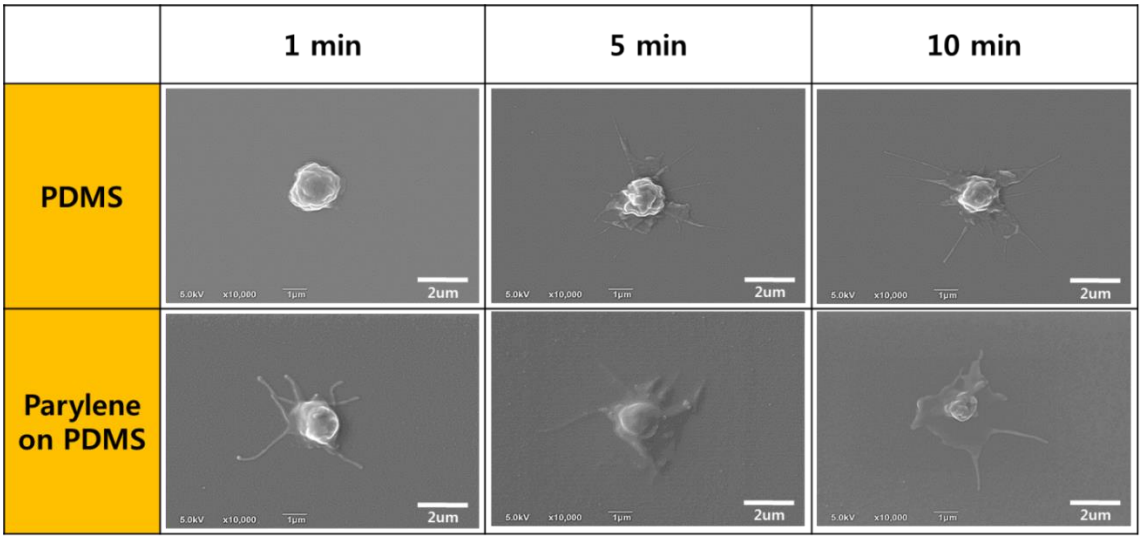


Fig. 5. Time-dependent morphologic changes of a single platelet in the activation process according to the PDMS and parylene-on-PDMS substrates.

To conclude, even though the softness of the surface is dominant to platelet attachment at an initial stage, the roughness of the surface showed a preponderance of the platelet activation process. Rapid activation process of the platelets after attachment could be determined with roughness of the surface. Therefore, controlling both the softness and roughness of the surface could be invaluable for the applications of regenerative medicine and tissue engineering.

4. Conclusion

The present study investigated, characterized, and analyzed surface-engineered platelet attachment and activation according to the PDMS and parylene-on-PDMS substrates using softness and roughness. While the soft surface promoted the platelet attachment in the initial stage, the roughness of the surfaces has considerably contributed to the fast process of platelet activation. Therefore, the results of this surface-engineered study could provide useful information for a variety of implantable biomedical applications.

Acknowledgements

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