A HEMOCOMPATIBLE ARRAY OF CYLINDRICAL NANOSHELLS WITH A REDUCED EFFECTIVE BLOOD CONTACT AREA

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ABSTRACT

Inhibition of the blood coagulation that arises from the natural defense system of humans is of crucial concern for implanted blood-handling devices, such as a heart valve and stent. Unless the coagulation is effectively suppressed, patients with an artificial heart valve may be burdened with the inconvenience of taking an anticoagulant for the rest of their lives. The anti-blood-coagulation effect is demonstrated on the surface of an array of cylindrical nanoshells. The resultant blood contact area is 99.2% less than that of the referenced flat surface, indicating a high level of water repellency. The significant reduction in the effective contact area leads to the notable anti-blood-coagulation effect because the clotting process relies on the surface reaction.

KEYWORDS: superhydrophobicity, water repellency, platelet adhesion, hemocompatibility, anti-blood-coagulation

INTRODUCTION

Any material implanted in the human body is considered to be an invader whenever it makes contact with blood. The natural defense system of humans initiates the clotting reaction as a way of isolating the foreign surface. For biomedical devices, the issue of hemocompatibility has been studied intensively[1] to prevent implanted devices from having negative effects, such as thrombosis.[2]

Because the blood coagulation is a surface reaction, any reduction in the effective contact area between the blood and the implanted surface tends to suppress blood clotting; hence, as shown in Fig. 1, a superhydrophobic (SHP) surface with geometrical roughness is attractive for anticoagulation.[2] This paper reports on a dramatically improved anticoagulation on an SHP surface of an array of cylindrical nanoshells.

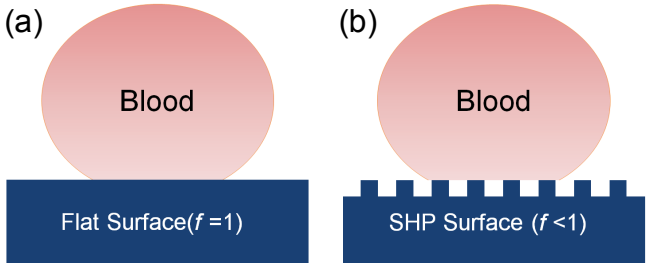

Figure 1: Schematics of the blood-contact area fraction, f, for (a) a flat surface and (b) an SHP rough surface

EXPERIMENTAL

Fig. 2 illustrates the process flow of the cylindrical nanoshell array. An array of oxide pillars was made by means of lithographic patterning before the deposition of poly-crystalline silicon (poly-Si). The poly-Si was then etched by means of reactive ion etching until the top surface of the oxide pillars was revealed. Afterwards the poly-Si sidewalls were left along the oxide pillars. The subsequent removal of the oxide pillars with a buffered oxide etchant completed the fabrication of the cylindrical shell of the poly-Si.

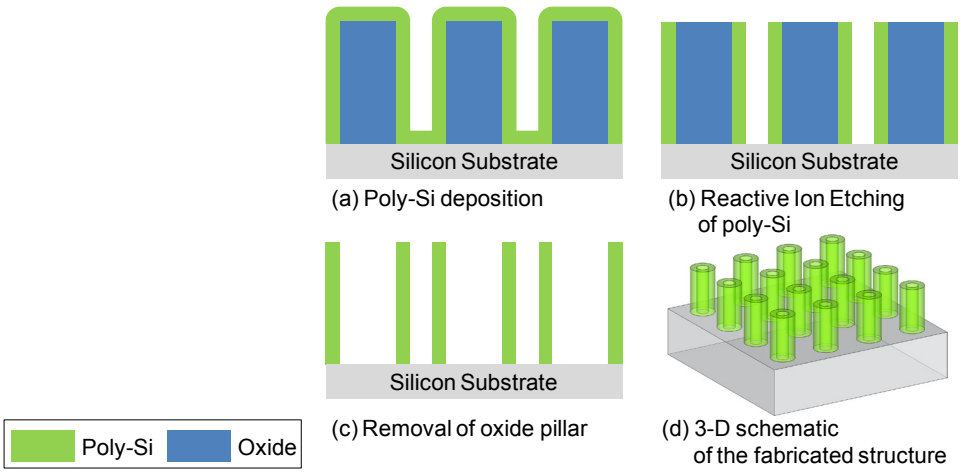

Figure 2: (a-c) Process flow of the SHP cylindrical nanoshell array and (d) a 3-D schematic of the fabricated structure

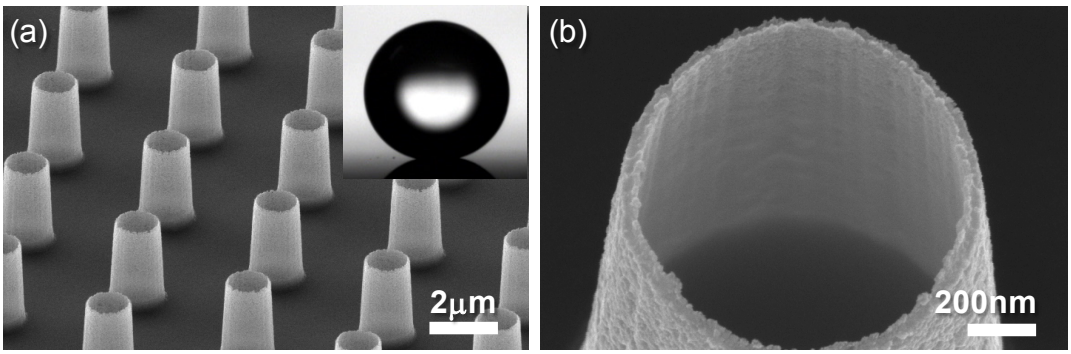

Figure 3: Scanning electron microscope (SEM) images of the cylindrical nanoshell array: (a) tilted view of the fabricated nanoshell array with an inset showing a water contact angle of 165.5°; (b) close-up view of a unit cell of the cylindrical nanoshell array, indicating a cylindrical shell with a thickness of less than 50 nm

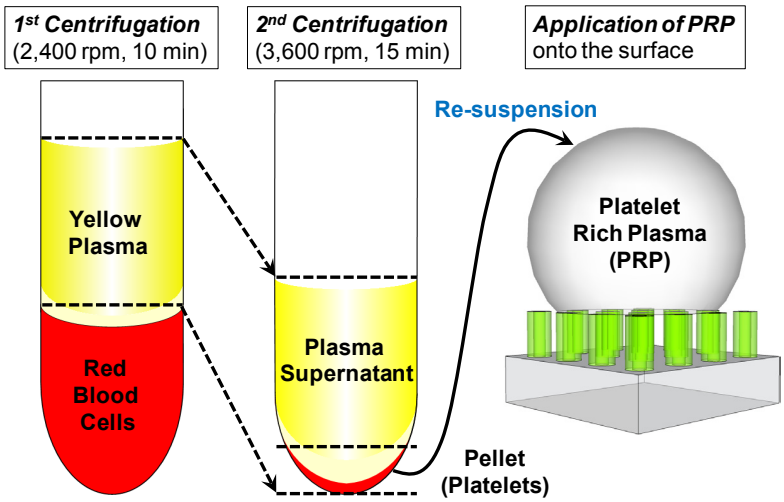

Figure 4: A schematic of the experimental steps of extracting platelet-rich plasma. First, the citrated blood is centrifuged at 2,400 rpm for 10 min and the supernatant is separated and transferred to a new tube. The second centrifugation is conducted at 3,600 rpm for 15 min. A pellet of platelets is thus prepared, and the platelet-rich plasma is finally produced by means of the resuspension of the pellet in a phosphate buffer solution.

The surface of the cylindrical shells protrudes to a height of 2 μm and a thickness of less than 50 nm. The blood-contact area on this surface is 99.2% less than that of the referenced flat surface. Fig. 3 shows scanning electron microscope (SEM) images of the fabricated cylindrical nanoshell array; the inset shows a water contact angle of 165.5°, which ensures an SHP surface.

To investigate the anticoagulation effect of the SHP cylindrical nanoshell array, we performed an experiment that is similar to that of a previous work.[2] We primarily examined the results of blood coagulation (namely the agglomeration and adsorption of platelets on the surface) because clotting is a routine set of chemical reactions, such as the initial adsorption of proteins and the agglomeration of platelets on the proteins, which eventually form a clot.

Fig. 4 shows experimental flows. The citrated blood was centrifuged twice to extract platelet-rich plasma: the first time at 2,400 rpm for 10 min and the second time at 3,600 rpm for 15 min.[3] We then used a hemocytometer to measure the concentration of the platelets in the centrifuged plasma, which was diluted to 1.5×10^5 platelets/mm^3 with a phosphate buffer solution (PBS). The diluted plasma was applied to both the cylindrical nanoshell surface and the flat surface. Both samples were subsequently incubated at 37°C for 1 hour to promote the agglomeration and adsorption of the platelets. After being washed with PBS, the samples were then soaked for 8 hours in 2.5% glutaraldehyde and PBS to fix the platelets onto each surface. Finally, the samples were dehydrated with ethanol.

RESULTS AND DISCUSSION

Fig. 5 shows SEM images taken after the platinum coating. The two images were taken with an equally magnified view (×1,000). The platelets, which have a diameter of 12 μm to 15 μm, clearly adhered to the flat surface (Fig. 5(a)). Platelets in blood are typically 2 μm to 3μm; however, when activated, they begin to aggregate and form a larger clot. As predicted, the platelets easily adhered to the referenced flat surface and were subsequently activated.

In contrast, the results on the SHP surface of the cylindrical nanoshell array (Fig. 5(b)) differed significantly from those on the flat surface. Only two platelets were observed on the 1 cm^2 area of the perfectly ordered cylindrical nanoshell array. Note also that the two platelets were in the 2 μm to 3 μm range, which is comparable to their original size in the blood. The size measurements confirm that the platelets on the surface of the cylindrical nanoshell array were not

activated. This notable anticoagulation effect on the surface of the cylindrical nanoshell array can be attributed to the fact that the blood contact area is 99.2% less than that of the referenced flat surface. Thus, the high level of water repellency effectively prevents any clotting.

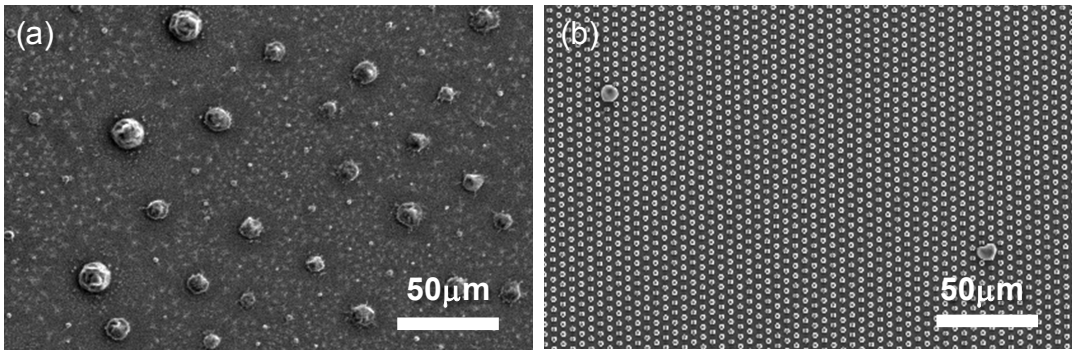

Figure 5: SEM images of the agglomerated platelets after fixation onto (a) a flat poly-Si surface and (b) the SHP surface of the fabricated poly-Si nanoshell array. Only the platelets on the flat poly-Si surface were activated. The platelets on the flat surface are bigger than those on the SHP surface because the activated platelets on the flat surface begin to aggregate and form a larger clot.

CONCLUSION

The perfectly ordered and protruded cylindrical nanoshell array has a high level of water repellency. The blood contact area of its surface is 99.2% less than that of the referenced flat surface. To investigate the effect of the blood contact area on hemocompatibility, we applied a platelet-rich plasma extracted from human blood onto the protruded surface and the flat surface. The results confirm that a much smaller number of platelets adhere to the surface of the fabricated cylindrical nanoshell array than to the flat surface. Moreover, the activation process that increases the size of the platelets was totally inhibited on the surface of the cylindrical nanoshell array. Thus, the proposed surface structure can be used on implantable biomedical devices, such as stents and heart valves. The modification of the surface and the resultant reduction in the effective blood contact area prevent the body's natural defense system from coagulating the blood.

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REFERENCES

[1] C. Werner, M. F. Maitz, and C. Sperling, Current strategies towards hemocompatible coatings, Journal of Materials Chemistry, 17(32), pp. 3376-3384, (2007).

[2] A. J. Schrauth, and N. P. Suh, Axiomatic design of non-wetting hemocompatible surfaces, Proceedings of 4th International Conference on Axiomatic Design, Firenze, Italy, (2006).

[3] G. Weibrich, W. K. G. Kleis, G. Hafner, and W. E. Hitzler, Comparison of platelet, luekocyte, and growth factor levels in point-of-care platelet-enriched plasma, prepared using a modified Curasan kit, with preparations received from a local blood bank, Clinical Oral Implant Research, 14(3), pp. 357-362, (2003).

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