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Control of Cell Adhesion on a Superhydrophobic Surface by Polydopamine Coating

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Because the uncontrolled adhesion of cells onto synthetic surfaces causes the malfunctioning of biomedical devices, the control of cell adhesion on artificial surfaces is of importance, especially in the biomedical field.[1,2] Thus, various approaches have been developed to control cell adhesion on surfaces;[3,4] among them, cell-resistant polymer coatings[5] have extensively been utilized. Poly(ethylene glycol)[6,7] and zwitterionic polymers[8,9] are representative examples of polymers that display cell-resistant properties, and they have successfully been introduced onto a wide range of surfaces by appropriate immobilization techniques.[5] With the growing interest in control over cell adhesion onto surfaces, another approach to achieving this goal has recently been developed, which is based on superhydrophobic surfaces.

As hydrophobic coating of nanostructured surfaces has been identified as a key method for introducing the self-cleaning- and superhydrophobic-surface properties of lotus leaves,[10-12] both the construction[13-15] and applications[16-18] of biomimetic superhydrophobic surfaces have extensively been investigated. As a result, several promising properties, including the repellence of cells, have been reported.[19-23] For instance, Lei *et al.* fabricated superhydrophobic surfaces by using aligned carbon nanotubes and reported that platelet adhesion was considerably reduced.[23] Stratakis *et al.* investigated cell adhesion on nanostructured superhydrophobic surfaces as well, and concluded that the mammalian cell adhesion could be controlled by varying the roughness and wettability of the surface.[20]

The application of superhydrophobic surface has further advanced to the selective attachment of cells.[24] Patterning of cells on superhydrophobic surfaces was, for instance, realized by site-specific UV/plasma treatment, giving rise to the hydrophilic/superhydrophobic patterning.[25-27] Line-, circle-, and square-shaped hydrophilic patterns were fabricated on the surface, with the cell adherence only onto the hydrophilic region, resulting in spatio-selective cell adhesion.

Although superhydrophobic surfaces have been applied to the preparation of cell patterns, the applied methods display several drawbacks with respect to practical use. They require external instruments for modifying the superhydrophobic surfaces. In addition, the methods are transient; the hydrophilicity decreases over time, eventually reverting to the original (hydrophobic) state.[28] Therefore, a permanent method for modifying superhydrophobic surfaces is required. Indeed, recently, a facile and robust approach to modifying superhydrophobic surfaces was developed based on polydopamine coating.[29,30] During polydopamine coating, dopamine is used to modify the surface under alkaline conditions, resulting in polydopamine-coated substrates. The advantage of this method is that it can be applied to any material surfaces, including superhydrophobic surfaces.[29] Moreover, it is known that polydopamine-coated surfaces show excellent cell-adhesive properties.[31,32] We, therefore, reasoned that polydopamine-coated superhydrophobic surfaces could provide efficient platforms for controlling cell adhesion.

For the preparation of a superhydrophobic surface, anodized aluminum oxide was used as a nanostructured surface onto which hydrophobic fluorosilane was deposited.[30] After preparation, the surface was selectively coated with polydopamine to induce cell-adhesive properties. Selective polydopamine coating was performed by half-masking the surface using a polydimethylsiloxane (PDMS) slab, which was carefully removed after 18-h coating (Figure 1). Modification of the superhydrophobic surface by polydopamine was characterized by water-contact angle measurements. The significant change in the angle from $157.5^{o} \pm 3.0$ to $36.3^{o} \pm 1.4$ indicated that the surface was successfully modified by a layer of polydopamine (Figure 2). The surface was further analyzed by X-ray photoelectron spectroscopy (XPS). The unmodified superhydrophobic surface showed an intense CF_2 peak at 291 eV, originating from the hydrophobic fluorosilane layers. After polydopamine coating, a decrease in intensity of the peak at 291 eV was observed, and the peak at 284 eV, corresponding to the C 1s of the polydopamine

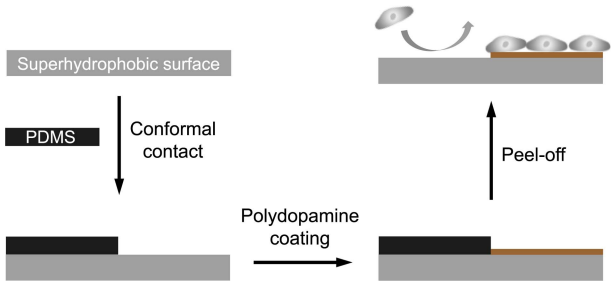


Figure 1. A schematic description for the controlled cell adhesion on a superhydrophobic surface.

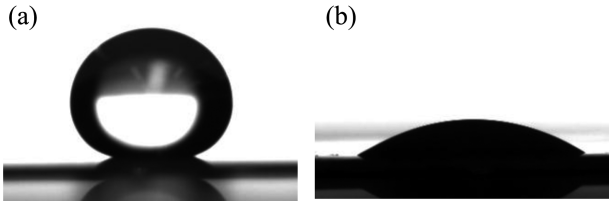


Figure 2. Water contact angle images of (a) unmodified and (b) polydopamine-coated superhydrophobic surfaces.

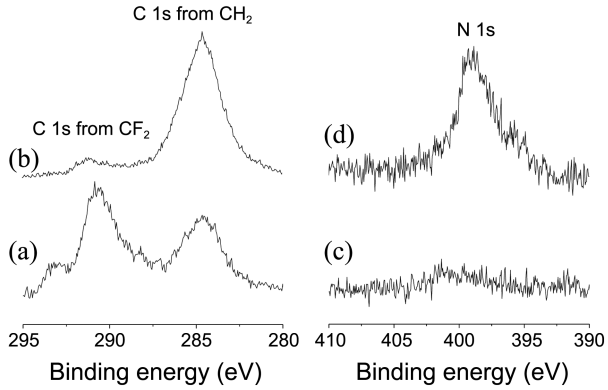


Figure 3. XPS spectra of (a and c) unmodified and (b and d) polydopamine-coated superhydrophobic surfaces.

layers, considerably increased. In addition, a new peak, corresponding to N 1s of polydopamine was observed after coating. Taken together, these results clearly confirmed the successful modification of the superhydrophobic surface by polydopamine (Figure 3).

Cell adhesion was then investigated on the selectively modified superhydrophobic surface using NIH3T3 fibroblasts by seeding a cell suspension (2.5×10^5 cells/mL, 6 mL) onto the modified surface and validating adhesion on the surface after 24 h. Prior to analysis, the cells were stained with 4',6-diamidino-2-phenylindole (DAPI) and characterized by fluorescence microscopy. Figure 4 shows the selective attachment of the cells only onto the polydopamine-coated region of the superhydrophobic surface. As expected, the cells did not attach onto the superhydrophobic region, whereas the polydopamine-coated region efficiently recruited fibroblasts. The preference of cell's attachment to hydrophilic surfaces is due to differences in surface energy; it is known that the substrates that exhibit low surface energy inhibit cell adhesion.[33] Thus, superhydrophobic surfaces, which display extremely low surface energy, reduce cell adhesion, whereas polydopamine coatings on solid substrates are known to be excellent platforms for cell adhesion by virtue of their high surface energy. As a result, polydopamine coating on the superhydrophobic background enabled us to control cell adhesion.

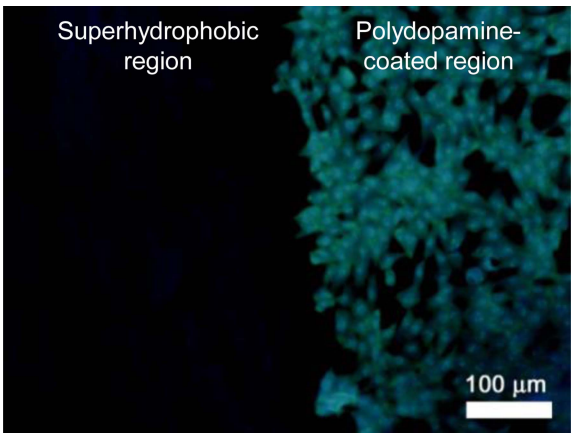


Figure 4. Representative fluorescence image of the cells that selectively adhered to the polydopamine-coated superhydrophobic region.

In summary, the controlled cell adhesion on a superhydrophobic surface was demonstrated by simple surface coating. More specifically, the superhydrophobic surface was utilized as a cell-repellent background, allowing control over cell adhesion by selective polydopamine coating. We believe that this method can be used as an efficient tool for achieving precision control over cell adhesion.

Experimental

Materials. Pure aluminum sheets (99.999%, 0.25 mm in thickness) were purchased from Goodfellow (Cambridge, UK). Perchloric acid ($HClO_4$, 70%, Junsei), ethanol (99.8%, Merck), methanol (HPLC grade, Merck), acetone (extrapure, Daejung), dopamine hydrochloride (98%, Aldrich), trizma base (99%, Sigma), trizma HCl (99%, Sigma), (tridecafluoro-1,1,2,2-tetrahydrooctyl)trichlorosilane (Gelest, Inc.), phosphate buffered saline (PBS, Sigma), phosphoric acid (H_3PO_4, 85%, Junsei), DAPI (Vector Lab.), and chromium(VI) oxide (CrO_3, 99.9%, Sigma-Aldrich) were used as received.

Selective Polydopamine Coating of the Superhydrophobic Surface. The superhydrophobic surface was fabricated according to the previous report.[30] Polydopamine coating was selectively performed by a partial exposure of the superhydrophobic surface in the dopamine solution (10 mM), and the methanol and Tris buffer solution (pH 8.5) (CH_3OH: buffer = 2:3 in volume ratio) was used as a co-solvent of dopamine. The partial exposure of the superhydrophobic surface was achieved by half-masking the surface with a PDMS slab. The coated substrates were rinsed with deionized water, and dried under a stream of argon.

Cell Adhesion Test. NIH-3T3 cells were seeded onto the substrates and incubated at 37 °C with 5% CO_2. After 1 d, nonadherent cells were removed by aspirating the medium and carefully washed twice with PBS. Adherent cells were fixed in 4% paraformaldehyde for 10 min, and the nuclei of cells were stained with DAPI. Cell attachment onto the substrates was characterized by fluorescence microscopy (IX 71 fluorescence microscope, Olympus, Japan).

Characterizations. The XPS study was performed with a VG-ScientificESCALAB250 spectrometer (U.K.) with a monochromatized Al Kα line as an X-ray source. Emitted photoelectrons were detected by a multi-channel detector at a takeoff angle of 90° relative to the surface. During the measurements, the base pressure was 10^{-9}-10^{-10} Torr. Static

water contact angle measurements were performed using a Phoenix 300 goniometer (Surface Electro Optics Co., Ltd., Korea).

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