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Bio-Inspired Silicification on Patterned Surfaces Generated by Microcontact Printing and Layer-by-Layer Self-Assembly

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Fabrication of inorganic micropatterns with low cost and large area uniformity has potential applications in biomedicine[1] and microelectronics.[2] As an alternative to conventional fabrication methods such as lithography and electrodeposition, bio-inspired, chemical methods involving controlled deposition of inorganic materials on organic interfaces have been suggested.[3] Although micropatterns of various inorganic materials[4] such as TiO_2, ZrO_2, $SrTiO_3$, SnO_2, ZnO, Ta_2O_5, Fe_3O_4, and Cu have been generated by chemoselective deposition of inorganic species on patterns of self-assembled monolayers (SAMs) through specific chemical reactions, these depositions were usually performed under acidic conditions (near pH 2) and/or relatively high temperatures (50–120 °C). In contrast, the physicochemical control of inorganic materials in biological systems is achieved intrinsically under physiological conditions (at near neutral pH and room temperature). In the aim of developing methods for generating inorganic micropatterns under mild and biofriendly conditions, biomimetic approaches that utilize micropatterns of enzymes or biomimetic polymers as catalytic templates have been investigated.[5,6] For example, the micropatterns of catalytic templates for silica deposition were fabricated by sophisticated techniques of holographic two-photon-induced photopolymerization[5a] and photolithography,[5b,c] and the micropatterns of silica nanoparticles were generated.[5d,e] The thickness of the patterned silica/template hybrid films was also controlled by varying the thickness of the template films via surface-initiated, atom transfer radical polymerization.[6] Albeit successful, the methods still lacked the biocompatibility that was required for interfacing biomimetic mineralization with biological entities, such as living cells. Compared with the radical polymerization, layer-by-layer (LbL) processes are considered to be more biofriendly[7] in addition to the fact that the processes have been frequently used to form micropatterns of polymers and metals with simple controllability of thickness under mild conditions.[8] In this work, we demonstrate the biomimetic formation of silica micropatterns as a first step toward the realization of living-cell–silica interfaces, where all the processes were performed in aqueous solutions under mild conditions, by taking advantage of the LbL technique.

Poly(diallyl dimethyl ammonium chloride) (PDADMA, M_w: 100000–200000) was selected as a catalytic template for biomimetic silicification, because the previous studies indicated that polyamine-containing, cationic peptides, named silaffins, played important roles in biological silica formation of diatoms,[9] and quaternary amine-containing synthetic polymers have proven chemically catalytic for biomimetic silica formation.[10] In addition, PDADMA has been used for the formation of multilayered silica/PDADMA films.[11] PDADMA and sodium polystyrene sulfonate (PSS, M_w: 70000) were alternatively deposited onto the surface that had been patterned with hexadecanethiol and triethylene glycol mono-11-mercaptoundecyl ether by microcontact printing (see the Supporting Information).[8] The patterns in this study were circular (diameter: 30 μm; separated by 10 μm). The patterned substrate was alternately immersed in aqueous NaCl (0.5 M) solutions of PDADMA (5 mg mL^{-1}) and PSS (5 mg mL^{-1}) for 5 min each, and the thickness of the films was varied by the number of the immersion steps. The LbL process was finished with PDADMA for achieving catalytic interactions with silicic acid derivatives at the interface. After thoroughly washing the substrate with deionized water, the PDADMA/PSS-patterned substrate was placed for 30 min in the 50 mM silicic acid solution that had been made by adding 0.1 mM HCl solution of tetramethyl orthosilicate (100 mM) to 100 mM phosphate buffer (pH 5.5) with 1:1 (v/v) ratio.

After biomimetic silicification, the XPS spectrum showed Si peaks at 102.20 (Si2p) and 153.47 eV (Si2s; see the Supporting Information). In addition, a P2p peak was also observed at 132.50 eV with a weak intensity, indicating the in-

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volvement of phosphate anions in the silicification process. The IR spectrum also confirmed the successful silicification on the patterned surface (Figure 1). New peaks were observed at 1216, 969, and 800 cm^{-1}, which were assigned as Si-O-Si asymmetric stretching, Si-O^- stretching, and Si-O-Si symmetric stretching, respectively.[12]

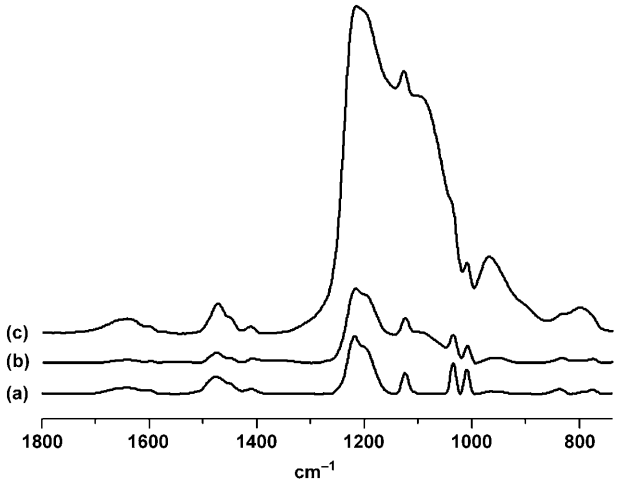

Figure 1. IR spectra of the patterned PDADMA/PSS (11/10) multilayer: a) before silicification, b) after silicification without phosphates, and c) after silicification with phosphates.

Silicification occurred spatio- and chemoselectively, and silica patterns were generated only on the circular patterns of PDADMA/PSS (11/10) films (Figure 2a,b). The AFM micrograph of the patterned PDADMA/PSS (11/10) film showed that the thickness was about 60 nm with root-mean-square (rms) roughness of 29.9 nm (Figure 2c). After silicification, the thickness of the circular patterns increased to about 200 nm (rms: 91.3 nm; Figure 2d). The thickness increase indicated that the silicification was not limited to the top-most layer of PDADMA, but occurred in the entire layer of PDADMA/PSS films. As a comparison, we and others previously reported the formation of ultrathin silica films (< 10 nm) when biomimetic silicification was catalyzed by top-most functional groups.[5b,13] The presence of silica in the inner layers of the films was confirmed further by energy-dispersive X-ray (EDX) spectroscopy. The EDX line profiles for the cross-section of the dissected films indicated the presence of silica in the whole range of the films, and the elemental analyses showed that the compositions of silica at the top layer were similar or nearly identical to those at the inner layer (see the Supporting Information).

Another characteristic of the formed silica was the presence of nanospherical structures, which resulted in the increased roughness. The use of poly(2-dimethylaminoethyl methacrylate) films as a catalytic template generally yielded quite smooth silica films,[6,12c] while solution-based silicification produced nanoparticles in most cases.[9,14] We believe that the observed nanospherical structures were caused by the noncovalent nature of the LbL process. Sumper et al. reported that silica nanoparticles were generated in solution by predetermined polymeric templates that were formed by electrostatic interactions.[9,14,15] In other words, electrostatically associated polymeric chains were thought to induce the formation of silica nanoparticles. The LbL deposition was performed under mild conditions, and the deposited polymers interacted electrostatically with one another. The surface density of the polymers would not be as dense as that of the polymers grafted by surface-initiated polymerization,[6,10a,12c] and the polymers themselves would be relatively free locally. Therefore, the conformational change of the polymeric chains yielded more silica deposition (increase in the thickness) as well as formation of silica nanoparticles that had been observed with free polymers in solution. This hypothesis was supported by the required presence of phosphates for biomimetic silicification in our systems. Phosphate or other polyvalent anions were found to act as a flocculating agent required to trigger biomimetic silica formation.[15] Control experiments without phosphates showed imperfect deposition of silica (Figure 1b), implying that the polymers in the LbL layer were less dense than the polymers grafted by surface-initiated polymerization and free to form complexes with phosphates; the latter did not require phosphates for biomimetic silica formation,[10a] because the polymers themselves were densely packed without the aid of phosphates.

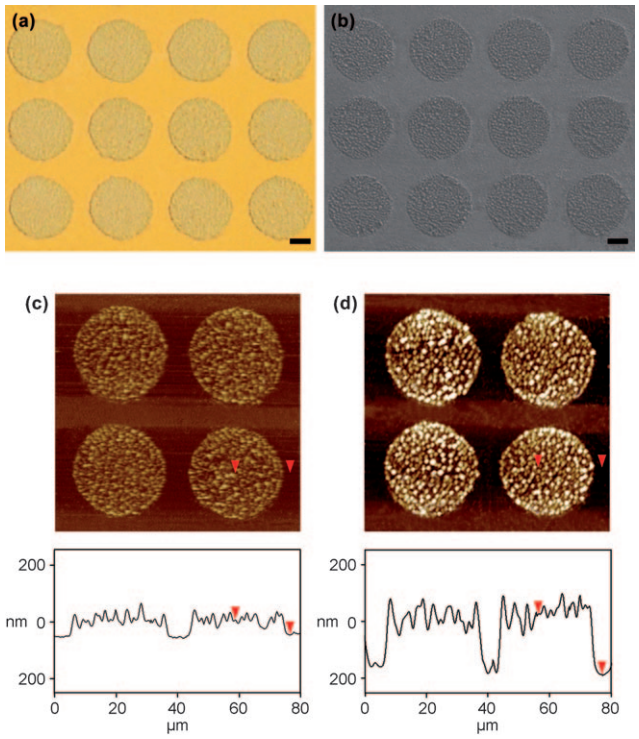

Figure 2. a) Optical micrograph of the patterned PDADMA/PSS (11/10) multilayer. b) SEM micrograph of the patterned silica–PDADMA/PSS (11/10) multilayer. Scale bars for (a) and (b): 10 µm. AFM micrographs of c) the patterned PDADMA/PSS (11/10) multilayer and d) the patterned silica-PDADMA/PSS (11/10) multilayer. Their sectional views are also shown.

The thickness of patterned silica films was controlled by the number of multilayers. We prepared three different samples of PDADMA/PSS mutilayers (6/5, 11/10, and 16/15), and performed biomimetic silicification. The cross-sectional SEM micrographs showed that the thickness of the silica layers increased as that of the polymeric multilayers increased (Figure 3). Of interest, the thickness change of the

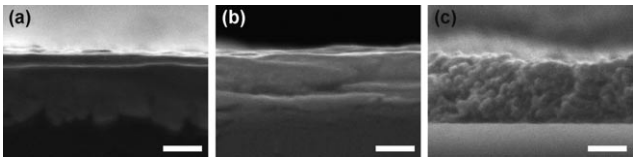

Figure 3. Cross-sectional SEM micrographs of the silica–PDADMA/PSS films formed from the PDADMA/PSS (6/5) (a), (11/10) (b), and (16/15) (c) multilayers. Scale bars: 100 nm.

multilayers also affected the nanoscopic morphology of the formed silica. The nanospherical morphology, predominant in the structure from the PDADMA/PSS (16/15) mutilayer (Figure 3c), became unobservable in the structures from the (6/5) and (11/10) multilayers (Figure 3a,b). We think that thinner films might have less spatial flexibility for the processes—self-aggregation and polycondensation of silica precursors—that would lead to the formation of silica nanoparticles.

In summary, we have demonstrated a simple but versatile method for generating silica patterns under mild conditions. The method has several advantages: micro- and nanofabrication techniques allow the formation of any arbitrary silica shape because the biomimetic silicification occurs only at the PDADMA/PSS-presenting areas; the thickness of the silica films (in other words, the aspect ratio of the silica structures) is controlled simply by the thickness of the PDADMA/PSS films, which is varied by the number of LbL depositions; the method is compatible with biological entities and biomaterials because all the processes proceed in aqueous solutions under mild conditions; the method is not limited to silica and could be extended to other inorganic materials, such as titanium oxides and other metal oxides.[16]

Experimental Section

Materials: PDMS prepolymer (Sylgard 184, Dow Corning Corp.), (tridecafluoro-1,1,2,2-tetrahydrooctyl)trichlorosilane (United Chemical Technologies, Inc.), gold-coated silicon wafers (with a titanium adhesion layer of 5 nm and thermally evaporated gold layer of 100 nm, K-MAC, Korea), triethylene glycol mono-11-mercaptoundecyl ether (95%, Aldrich), 1-hexadecanethiol (99%, Aldrich), poly(diallyl dimethyl ammonium chloride) (PDADMA, average M_w: 100000–200000, 20 wt% in H_2O, Aldrich), sodium polystyrene sulfonate (PSS, average M_w: ca. 70000, powder, Aldrich), hydrochloric acid (HCl, 35%, Junsei), tetramethyl orthosilicate (TMOS, 99%, Aldrich), and absolute ethanol (99.8%, Merck) were used as received. Ultrapure water (18.3 MΩ cm) from the Human Ultrapure System (Human Corp., Korea) was used.

Microcontact printing: A PDMS prepolymer was cast against a microfabricated photoresist master (30 μm circular wells separated by 10 μm). Curing the prepolymer (at 60 °C for 12 h) and peeling it away from the master provided a negative replica of the two-dimensional pattern of the photoresist. The negative replica of PDMS, composed of protruding cylindrical features on the surface, was used as a stamp. Before casting the PDMS prepolymer, the master was pretreated with (tridecafluoro-1,1,2,2-tetrahydrooctyl)trichlorosilane for 1 h under vacuum at room temperature to functionalize the surface with the fluorocarbon. The stamp was inked with a drop of an ethanolic solution (10 mM) of hexadecanethiol, which was contact-printed onto a gold substrate that had been cleaned with piranha solution. After printing, the gold substrate was immersed for 1 min in an ethanolic solution (10 mM) of triethylene glycol mono-11-mercaptoundecyl ether.

Layer-by-layer (LbL) self-assembly: An aqueous NaCl solution (0.5 M) was used for preparing the PDADMA and PSS solutions. The final concentration of the solutions was 5 mg mL^{-1}. The patterned gold substrate was alternately immersed in 2 mL of the PDADMA solution and 2 mL of the PSS solution for 5 min for each step. The thickness of the polymeric pattern was arbitrarily adjusted by controlling the number of layer-by-layer depositions. The LbL process was always finished with PDADMA for good interactions between the biomimetic polymer and silicic acid derivatives.

Biomimetic silicification: After thoroughly washing the substrate with deionized water and drying under a stream of argon, a PDADMA/PSS-patterned substrate was placed in 2 mL of 50 mM silicic acid solution, which had been independently prepared by stirring an HCl solution (0.1 mM) of TMOS (100 mM) at room temperature for 20 min and adding the resulting solution to aqueous sodium phosphate buffer (100 mM, pH 5.5) with 1:1 (v/v) ratio. After 30 min, the substrate was taken, washed with deionized water and ethanol, and dried under a stream of argon.

Characterizations: Scanning electron microscopy (SEM) images were obtained using an FEI XL FEG/SFEG microscope (FEI Co., Netherlands) with an accelerating voltage of 10 eV. All samples were sputter-coated with platinum. IR spectra were recorded on a Thermo Nicolet Nexus FTIR spectrometer in a SAGA mode. The X-ray photoelectron spectroscopy (XPS) study was performed with a VG-Scientific ESCALAB 250 spectrometer (United Kingdom) with a monochromatized $Al_{K\alpha}$ X-ray source (1486.6 eV). Atomic force microscopy (AFM) imaging was performed in a tapping mode on a Nanoscope IIIa multimode scanning probe microscope (Veeco, United States) with a tapping mode etched silicon probe (TESP). The thickness of monolayer and polymeric films was measured with a Gaertner L116s ellipsometer (Gaertner Scientific Corporation, IL) equipped with a He–Ne laser (632.8 nm) at a 70° angle of incidence. A refractive index of 1.46 was used for all the films.

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Keywords: biomimetic synthesis · layer-by-layer deposition · micropatterns · silica · silicification

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