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Notes

Bioinspired Fabrication of Silica Thin Films on Histidine-Terminated Self-Assembled Monolayers

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There exists a great deal of interest in silica thin films, which potentially allow us to develop various applications including heterogeneous catalysis,[1] wettability control,[2] and microelectronics[3] because of their unique physicochemical properties, such as transparency, mechanical hardness, chemical stability, and low dielectric constant. Recently, the scope of substrates, on which silica thin films are deposited, has been expanded to biological entities for exploiting the emerging fields of drug delivery,[4] cell culture,[5] biosensors,[6] and cell encapsulation.[7-9] Although chemical deposition and thermal treatment have been considered as a simple way to fabricate silica thin films, toxic chemicals and extreme temperature are required for silica-forming processes.[10]

In contrast to the chemical and thermal methods, biosilicification occurring in diatoms and glass sponges has its own characteristic strategies to concentrate a small amount of silicic acid from sea water and construct the siliceous exoskeletons under mild conditions (*e.g.*, neutral pH, room temperature, aqueous media).[11,12] Despite the similarity in materials, it is known that silica-forming mechanisms in the two organisms are completely different. The biosilicification in diatoms is achieved by silaffins, highly phosphorylated silica-forming peptides extracted from the cell wall of diatoms, where ε-amino group in lysine residues are post-translationally modified to ε-*N*-dimethyllysine or ε-*N*,*N*,*N*-trimethyl-δ-hydroxylysine or a long-chain polyamine comprising of a few *N*-methylpropylamine units.[11] The self-assembled structure of the cationic polypeptides in silaffins is believed to act as a catalytic template for the *in vivo* polycondensation of silicic acid derivatives. Inspired by silaffins, cationic synthetic polymers and polypeptides have been used as catalytic templates for fabricating silica thin films. For example, silica thin films were formed on a gold substrate by using poly((2-dimethylamino)ethyl methacrylate) (PDMAEMA) grown by surface-initiated polymerization.[13,14] The positively charged PDMAEMA could attract the negatively charged silicic acids, followed by the polycondensation to silica. In a similar fashion, polyelectrolyte multilayers of poly(diallyldimethylammonium chloride) (PDADMA), formed by the layer-by-layer method, were used as a catalytic template for generating micro-patterned silica films.[15]

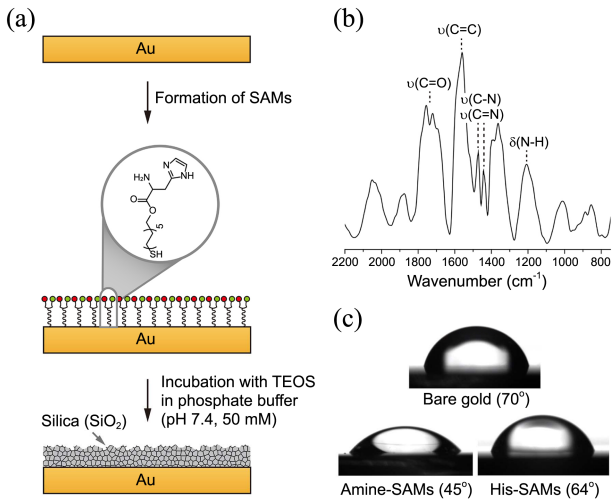

Figure 1. (a) Experimental procedure for the formation of silica thin films on histidine-terminated SAMs. (b) FT-IR spectrum of His-SAMs. (c) Water contact angle images of Amine-, His-SAMs, and bare gold.

On the other hand, silicateins, silica-forming proteins extracted from the spicules of various kinds of glass sponges, catalyze the polycondensation of silicic acids by mainly using two specific amino acids: histidine and serine that are in close proximity for hydrogen bonding.[12] Because of the hydrogen bonding, the nucleophilic attack of oxygen in serine to silicon alkoxide is believed to occur effectively. In contrast to silaffins, the silicatein-catalyzed formation of silica thin films was carried out with silicon alkoxide at pH 7.4. For example, Perry *et al.* reported the formation of uniform silica thin films by immobilizing recombinant silicateins on gold substrates.[16]

To date, most of silica thin films have been formed with

macromolecules on solid substrates as a catalytic template, but studies on single-molecular catalytic templates, mimicking biosilicification, have rarely been attempted for the generation of silica thin films. Herein, we developed a simple method for fabricating silica thin films on gold substrates by using a rationally designed molecule as a catalytic template. We synthesized a catalytic molecule, histidine-conjugated thiol (11-mercaptoundecyl 2-amino-3-(1*H*-imidazol-2-yl)propanoate; denoted as His-SH). The thiol group acted as a surface-anchoring group, leading to the formation of self-assembled monolayers (SAMs) on gold. We thought that the histidine moiety in His-SH would be catalytically active for bioinspired silicification, because it contained both amine and imidazole functional groups; the former mimicked silaffins leading to strong interactions with silicic acid derivatives, and the latter did silicateins and would act as a general base, which catalyzed the condensation reaction.

The experimental procedure for the formation of silica thin films was depicted in Figure 1(a). Briefly, we prepared His-SAMs on gold by immersing a gold substrate in an ethanolic solution of His-SH, and the resulting gold substrate was incubated with tetraethyl orthosilicate (TEOS) in an aqueous phosphate buffer solution (pH 7.4, 50 mM). The formation of His-SAMs was confirmed by Fourier-transform infrared (FT-IR) spectroscopy (Figure 1(b)). The peaks at 1214, 1442, 1476, 1561, and 1730 cm^{-1} corresponded to δ(N-H), ν(C-N), ν(C=N), ν(C=C), and ν(C=O), respectively (δ: in-plane bending vibration; ν: stretching vibration).[17] The thickness of His-SAMs was measured to be 12.45 Å by ellipsometry. The formation of His-SAMs was also supported by water contact angle measurement. The water contact angle of His-SAMs was 65°, which was higher than that of Amine-SAMs (45°). The exposed imidazole ring on the top of the surface was expected to render the surface more hydrophobic.

We screened the concentrations of TEOS to compare the catalytic activity of His-SAMs with that of Amine-SAMs. With more than 10 mM of TEOS, the characteristic bands of silica at 1000 and 1250 cm^{-1} (Si-O-Si stretching)[18] were found for both SAMs after 2-day incubation (data not shown).

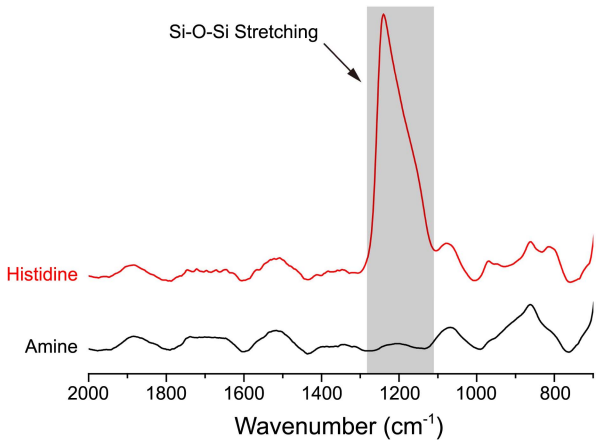

Figure 2. FT-IR spectra of Amine- and His-SAMs after incubation with TEOS (5 mM) for 2 days.

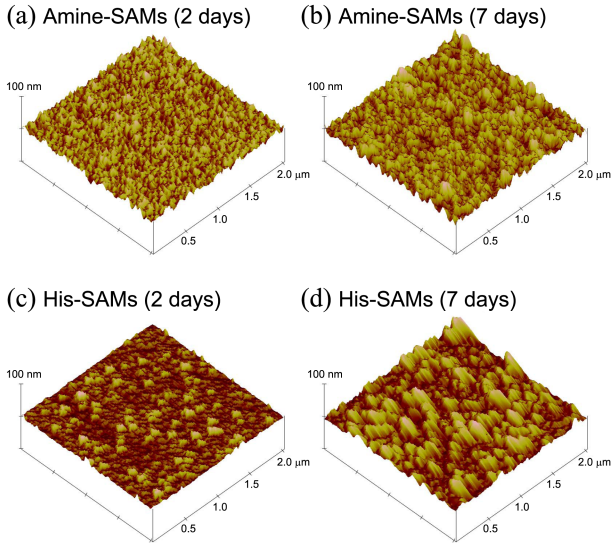

Figure 3. AFM images of Amine- and His-SAMs after (a and c) 2-day and (b and d) 7-day incubation.

In contrast, with 5 mM of TEOS were found the characteristic peaks of silica only for His-SAMs (Figure 2, red line), not for Amine-SAMs (Figure 2, black line). When the concentration of TEOS decreased further to 2.5 mM, no characteristic peaks of silica were observed for both SAMs.

It is noteworthy that His-SAMs catalyzed the silicification at relatively low concentration (5 mM) of TEOS, which is stable in an aqueous solution at neutral pH. We thought that the two functional moieties (amine and imidazole) in histidine were synergistically involved in the bioinspired silicification. Because the pK_a values of amine and imidazole are ~10 and ~6, repectively, the pH value of 7.4 is intermediate between pK_a of amine and that of imidazole, which would lead to the partial protonation of the amine group and the partial deprotonation of the imidazole group. Therefore, the protonated amine would attract negatively charged silicic acids and the deprotonated imidazole react with TEOS as a Lewis base, leading to acceleration of silicification. The morphological differences became more distinct, when Amine- and His-SAMs were incubated further for 7 days. Amine-SAMs displayed little changes in the surface morphology compared with the atomic force microscopy (AFM) image after 2-day incubation (Figure 3(a), (b)). However, the significant changes in the surface morphology were observed for His-SAMs (Figure 3(c), (d)). The result also confirmed the higher catalytic activity of His-SAMs in the silicification than Amine-SAMs.

In conclusion, we showed that the single molecule (His-SH) could be used for fabricating silica thin films on gold at pH 7.4 with a relatively low concentration of TEOS (5 mM). Our synthetic approach could be applied to the interdisciplinary fields, specifically related to living cells, such as nanobiotechnology, biomedical applications, and cell-based nano/micro devices, because these applications require the cytocompatible process to integrate low cytotoxic materials with cell surfaces.[19-22] In addition, the silicification carried

out in the synergistic fashion could be another tool for investigating silicification mechanisms in nature.

Experimental

Synthesis of the Histidine-conjugated Thiol (His-SH). His-SH was synthesized based on the previous report.[23] A solution of triphenylmethanethiol (6.6 g, 24 mmol) in 30 mL of ethanol-benzene (1:1) was added to a solution of 11-bromoundecanol (5 g, 20 mmol) in 30 mL of ethanol-benzene (1:1) with stirring at room temperature. After the reaction mixture was cooled to 0 ºC, 10% aqueous sodium hydroxide solution was added, and the resultant was stirred at room temperature for 16 h. The solvents were concentrated *in vacuo*, and the saturated aqueous sodium bicarbonate solution was added to the reaction flask. The mixture was extracted with dichloromethane. The combined extracts were washed with brine and sodium sulfate for dryness. The mixture was purified by column chromatography (ethyl acetate:hexane = 1:5) to afford 11-triphenylmethylthio-1-undecanol. N_a-Boc-L-Histidine (1.38 mmol), *N*,*N*′-dicyclohexylcarbodiimide (1.38 mmol) and 4-dimethylaminopyridine (1.38 mmol) were added sequentially to 20 mL of dichloromethane at 0 ºC. After 10 min, 11-triphenylmethylthio-1-undecanol was added, and the reaction mixture was stirred for 16 h at room temperature. The mixture was filtered through a short plug of Celite with deionized water, and the residue was purified by flash column chromatography (dichloromethane:methanol = 19:1) to afford the Boc-protected His-SH. To the 15-mL dichloromethane solution of the Boc-protected His-SH (1 mmol) at room temperature were added 20 mmol of trifluoroacetic acid and 1.1 mmol of triisopropylsilane. The reaction mixture was stirred for 6 h and then quenched by deionized water. The residue was concentrated *in vacuo* and purified by flash column chromatography (dichloromethane:methanol = 9:1) to give His-SH.

Formation of Silica Thin Films on Gold. The gold substrates were prepared by the thermal evaporation of gold (thickness: 100 nm) with a titanium adhesion layer (thickness: 5 nm) on a four-inch silicon wafer. They were cut into small pieces (1 × 1 cm^2) and cleaned with piranha solution (a mixture of 7:3 (v/v) 98% sulfuric acid and 35% hydrogen peroxide). The His-SAMs were formed by immersing gold substrates in a 1-mM ethanolic solution of His-SH overnight at room temperature. After the formation of SAMs, the resulting gold substrates were rinsed with ethanol and water several times and then dried under a stream of argon. The amine-terminated SAMs (Amine-SAMs) were also prepared by following the same procedures with 11-amino-1-undecanethiol based on previous report.[24] To form silica thin films, the substrates were incubated with tetraethyl orthosilicate (TEOS) at various concentrations in an aqueous phosphate buffer solution (pH 7.4, 50 mM). The resulting SAMs and silica thin films were characterized with a FT-IR spectrophotometer equipped with smart-apertured grazing-angle accessory and AFM.

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