Alternate Soaking Technique for Micropatterning Alginate Hydrogels on Wettability-patterned Substrates

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Abstract: Techniques for patterning hydrogels are important for fabrication of cell culture, analytical, and actuator devices at the micro- and nanometer length scales. In this study, we fabricated alginate hydrogels cross-linked by divalent cations on wettability-patterned substrates by alternate soaking of precursor solutions of sodium alginate and divalent cations. The wettability-patterned substrates were fabricated on hydrophilic glass plates modified with hydrophobic self-assembled monolayers of hexamethyldisilazane followed by exposure to an ultraviolet/ozone atmosphere through a metal mask. The film thickness of alginate gels with a width and length of 0.1 and 4 mm were tuned stepwise from 30 nm to 200 nm by adjusting the precursor conditions, including the pH, type of divalent metal ions, and sodium alginate concentration, and the alternate soaking conditions, including the dipping/withdrawal speed and number of alternate soaking cycles. This technique can be applied to other functional gels and will contribute to fabrication of hydrogel devices at the micro- and nanometer scales in the future.

Key words: alginate hydrogel, wettability-patterned substrate, alternate soaking, divalent metal

1 INTRODUCTION

Polymer hydrogels have attracted much attention for application to artificial muscles¹⁻³, the stationary phase in chromatography⁴⁻⁰, and gelling agents for foods⁷⁻⁹ because of their unique properties, such as high swelling/shrinking and low diffusion coefficient of water. Recent developments in photoradical polymerization and printing techniques enable patterning of hydrogels, which is expected to lead to microcell cultures for control of the shape and orientation of cells¹⁰⁻¹³, micro total analysis systems combined with gels for chromatography and sensors¹⁴⁻¹⁶, and microactuator devices for micromachines¹⁷, ¹⁸. Patterned hydrogels are normally fabricated by photoradical polymerization through photomasks owing to the difficulty in patterning hydrogels by conventional photolithography using etching and lift-off.

Patterning techniques that use wettability-patterned substrates consisting of patterned self-assembled monolayers have been developed to pattern polymeric or other functional materials at less than the micrometer length scale. Patterning techniques for self-assembled monolayers can be divided into top-down techniques, including photolithography and soft lithography, and bottom-up techniques, such as thermodynamic and dissipative self-assembly. To form polymer networks on wettability-patterned substrates, one strategy is direct polymerization of self-assembled monolayers¹⁹⁻²¹ and another is gelation in the solution adsorbed on the patterned surface. Here, we focus on the latter strategy because an easy fabrication process and high swelling degree of the gels are expected. Typically, wettability-patterned substrates consist of hydrophilic substrates surrounded by hydrophobic self-assembled monolayers (or the opposite combination) for selective adsorption of functional material solutions on the surfaces. Casting²²⁻²⁴, solvent evaporation²⁵, and dip-coating²⁶ techniques have been reported for fabrication of films on wettability-patterned substrates. However, these patterning techniques often encounter tradeoff problems between the film thickness and the uniformity of the film on the patterned regions.

As one solution to the above problem, we have reported a new soft liquid phase adsorption technique for fabrication of organic and inorganic films on wettability-patterned substrates using methanol/hexane or toluene/water emul-
Recently, we also reported alternate soaking techniques for fabrication of calcium phosphate films on wettability-patterned substrates\(^2\) or in silicone elastomer molds\(^3\), which can be used to fabricate patterned films consisting of insoluble materials that cannot be used in soft liquid phase adsorption techniques. In this study, we fabricated alginate hydrogels cross-linked by Ca cations on wettability-patterned substrates by alternate soaking with precursor solutions of sodium alginate and divalent ions.

2 EXPERIMENTAL PROCEDURES

2.1 Materials

Acetone, chloroform, sodium alginate (the viscosity of a 10 g L\(^{-1}\) aqueous solution was 500–600 mP\(\cdot\)s at 20\(\degree\)C), MgCl\(_2\cdot6\)H\(_2\)O, CaCl\(_2\cdot6\)H\(_2\)O, methanol, ammonia water, and hydrochloric acid were purchased from Wako Pure Chemical Industries, Ltd. Hexamethyldisilazane was purchased from Aldrich. The glass plates were obtained from Matsunami Glass Ind., Ltd.

2.2 Preparation

Preparation procedure is shown in Fig. 1. The glass plates were ultrasonically cleaned in acetone and chloroform for 20 min each and then exposed to an ultraviolet/ozone atmosphere generated by an ultraviolet/ozone cleaner (PL16-110, Sen Lights Corp., Japan) for 15 min. The glass plates were immersed in neat hexamethyldisilazane for 6 h, rinsed with pure water, and heated at 110\(\degree\)C for 1 h to immobilize hexamethyldisilazane on the glass plates. Metal masks with four slits on the long axis with a length of 4 mm and a width of 0.1 mm (Okenshoji Co., Ltd., Japan) were placed on the hydrophobic glass plates and then exposed to an ultraviolet/ozone atmosphere for 15 min to selectively remove hexamethyldisilazane monolayers, resulting in formation of wettability-patterned substrates. The wettability-patterned substrates were immersed in 0.1 M CaCl\(_2\), MgCl\(_2\), or SrCl\(_2\) in methanol, withdrawn, and kept in an air atmosphere for 5 min to form alkali earth metal halides films on the hydrophilic surface of the wettability-patterned substrates. These patterned alkali earth metal halides films were dipped in sodium alginate aqueous solution for 30 s, withdrawn, and then kept in an air atmosphere for 10 min to evaporate the excess water on the alginate gels. These dipping and withdrawal procedures were repeated one to three times. The pH of the sodium alginate solution was adjusted with ammonia and hydrochloric acid aqueous solutions.

2.3 Measurements

The optical microscopic observations were performed with a BX-60 optical microscope (Olympus, Japan). The thicknesses of the alginate hydrogels were determined with

![Fabrication scheme](image-url)
3 RESULTS

In a previous study, we selectively formed CaCl₂ films on the hydrophilic surfaces (contact angle of water less than 10°) of substrates surrounded by a hydrophobic monolayer of hexamethyldisilazane (contact angle of water 80–90°) by immersing the wettability-patterned substrates in CaCl₂ in methanol followed by withdrawing at an adjusted speed. Ca²⁺ was reacted with PO₄³⁻ by immersing the samples in aqueous Na₂HPO₄, resulting in formation of calcium phosphate films on the wettability-patterned substrates. We expect that alginate gels can be formed on wettability-patterned substrates because alginate ions can be cross-linked with cross-linker of Ca²⁺ to form “egg-box” structures.

3.1 Variation of the pH

Adjusting the pH is important because the solubilities of sodium alginate and CaCl₂ in water change are dependent on the pH. The pKₐ values of sodium alginate and CaCl₂ are about 1.5–3.5 and 8–9, and alginic acid and Ca(OH)₂ form at low and high pH, respectively. These values indicate a decrease in the solubilities of both alginate and calcium ions in water, and we performed the experiments in the pH range 4–9. The pH was adjusted by adding hydrochloric acid or ammonia water to avoid using buffer solutions because the type of monovalent ions and concentration affect formation of alginate gels, which will be discussed later.

Figure 2a–c show optical microscope images of the alginate gels on the wettability-patterned substrates fabricated at pH values of 4.5, 7.2, and 8.8, respectively, with a dipping/withdrawal speed of 200 mm min⁻¹. The number of alternate soaking cycles, concentration of CaCl₂ in methanol, and concentration of sodium alginate in water were fixed at 1, 0.1 mol L⁻¹, and 0.5 wt%, respectively. At pH = 4.5, the alginate gel formed over the complete regions of the wettability-patterned substrate. The high viscosity of alginate ions owing to formation of aggregates at pH values near the pKₐ prevents selective adsorption of the solution on the wettability-patterned substrate. At near-neutral pH, such as 7.2 and 8.8, the alginate gels selectively formed on the hydrophilic surface of the wettability-patterned substrates. These alginate gels did not dissolve with the pure water rinsing treatment, indicating formation of Ca²⁺-crossed-linked alginate networks.

3.2 Effect of the dipping/withdrawal speed

Given that solvent evaporation can be ignored, the amount of the solution adsorbed on the wettability-patterned substrates can be determined by the balance between the interfacial tensions of the solvents and substrate surfaces, and the gravity force. The withdrawal speed and angle increase the effect of the gravity force for dip-coating techniques. In the case where solvent evaporation cannot be ignored, evaporation increases the concentration of the solution with elapsed time, which changes the viscosity of the adsorbed solution and the interfacial tension. The changes of the viscosity and interfacial tension affect not only the amount of the adsorbed solution and the selectivity of adsorption on the wettability-patterned substrates, but also the film morphology owing to the drying history. The experimental parameters, such as the boiling points of the solvents, temperature, vapor pressure owing to the patterned size, and humidity, should be precisely controlled to obtain good reproducibility. We set the dipping angle at 90° with respect to the water surface and the dipping/withdrawal speed at more than 50 mm min⁻¹ to suppress evaporation during the withdrawal process.

Figure 2d–f show optical microscope images of the alginate gels fabricated at dipping/withdrawal speeds of 50,
100, and 200 mm min\(^{-1}\), respectively. The number of alternate soaking cycles, concentration of CaCl\(_2\) in methanol, and concentration and pH of sodium alginate in water were fixed at 1, 0.1 mol L\(^{-1}\), and 0.5 wt% and 8.8, respectively. For a dipping/withdrawal speed of 50 mm min\(^{-1}\), alginate gels formed on the complete regions of the wettability-patterned substrates because of the excess amount of the adsorbed solutions. At dipping/withdrawal speeds of 100 and 200 mm min\(^{-1}\), alginate gels selectively formed on the hydrophilic surfaces of the wettability-patterned substrates. We did not obtain good reproducibility for formation of the alginate gels on the wettability-patterned substrates for dipping/withdrawal speeds of more than 400 mm min\(^{-1}\).

### 3.3 Control of the amount of the alginate gel

Next, the concentration of sodium alginate in water and the number of alternate soaking cycles were varied to control the amount of the alginate gel on the wettability-patterned substrate. Figure 3a shows optical microscope images of the alginate gels on the wettability-patterned substrates for various concentrations of sodium alginate and number of alternate soaking cycles. The concentration

![Fig. 3](image_url)

Fig. 3 (a) Optical microscope images and (b) thickness plotted with concentration on alginate gels fabricated at pH = 8.8 and a dipping/withdrawal speed of 100 mm min\(^{-1}\) with variation in the concentration of sodium alginate and number of alternate soaking cycles on wettability-patterned substrates.
of CaCl₂ in methanol, pH, and dipping/withdrawal speed were fixed at 0.1 mol L⁻¹, 8.8, and 100 mm min⁻¹, respectively. After each alternate soaking cycle, the image contrast of the alginate gel increases with increasing concentration of sodium alginate. The width of the alginate gel remains almost constant with increasing sodium alginate concentration. Figure 3b shows the thicknesses of the same alginate gels on wettability-patterned substrates. After one alternate soaking cycle, increasing the sodium alginate concentration from 0.5 to 1.5 wt% increases the alginate gel thickness from 30 to 80 nm with a saturated tendency. For each sodium alginate concentration, increasing the number of the alternate soaking cycles increases the thickness of the alginate gel with a saturated tendency. The width of the alginate gel remains almost constant. Compared with layer-by-layer techniques, the film thickness greatly increases by several tens of nanometers because undissolved materials precipitate on the substrates in alternate soaking techniques.

The formation mechanism for the first and two or more layers have to be considered separately. The first layer of the alginate gel forms by cross-linking between Ca²⁺ from the CaCl₂ film and sodium alginate from solution. For formation of the second layer, Ca²⁺ diffuses from the first layer of the alginate gel and reacts with the alginate ions in solution because CaCl₂ adsorbs on the surface of the first alginate gel layer and penetrates into the alginate gel. The amount of Ca²⁺ in the alginate gel on the surface is lower than that in the first CaCl₂ film, resulting in a decrease in the thickness of the second and subsequent layers of the alginate gel. If this process is dominant for determining the film thickness, the increase of the film thickness would be constant for the second and subsequent layers. We are currently investigating the thickness of alginate gels fabricated by more than four alternate cycles. As another factor, sodium alginate in the adsorbed solution reacts with Ca²⁺ and slowly diffuses from the first inner gel. The immersion time before withdrawal has to be considered for reaction with all Ca²⁺ in the gel. The gel strength at the gel interface should be different from that in the bulk gel because the alginate network in the upper gel would penetrate into the lower gel.

3.4 Effect of the type of metal ions

Sodium alginate can cross-link with divalent or higher valence metal ions in addition to Ca²⁺. The concentration of monovalent metal ions changes the gelation concentration of divalent metal ions and the physical properties of the formed gel. Next, we varied of the type of divalent ions for cross-linking sodium alginate without adding monovalent metal ions.

Figure 4 shows the thickness of alginate gels cross-linked with Mg²⁺, Ca²⁺, and Sr²⁺ on wettability-patterned substrates for one alternate soaking cycle and a dipping/withdrawal speed of 100 mm min⁻¹ plotted against the sodium alginate concentration. The film thickness increases in the order Mg²⁺, Ca²⁺, and Sr²⁺ for each sodium alginate concentration. The gelation concentration of divalent metal ions decreases in the same order because of the size mismatch between the metal ions and the egg-box structures of the alginate ions. In our experiments, divalent metal ions with a small gelation concentration increase the thickness of the alginate gel. This can be explained by considering the small crosslink densities of the alginate gels, which leads to increases in the swelling degree of the gels and total amount of the alginate ions that form the gels.

4 CONCLUSION

We have investigated the alternate soaking technique for fabrication of alginate gels on wettability-patterned substrates. The film thickness of alginate gels with a length of 4 mm and a width of 0.1 mm can be stepwise tuned from 30 to 200 nm by adjusting the precursor conditions, such as the pH, type of divalent metal ion, and sodium alginate concentration, and the alternate soaking conditions, such as the dipping/withdrawal speed and number of alternate soaking cycles. The molecular weight of sodium alginate also has to be considered. A large molecular weight would increase the viscosity of the alginate solution and swelling degree of the alginate gel, leading to the need to readjust the deposition conditions.

The in-plane patterned size of wettability-patterned substrates can be decreased from the micrometer to the nanometer length scale using conventional photolithography, soft lithography, and electron beam lithography, leading to
the potential of fabricating nanosize gels in a tunable pattern. It should be noted that for nanopatterning of alginate gels, it is necessary to overcome the difficulties in the absorbed amount of the precursor solution owing to the decrease in the surface area of the wettability-patterned substrate, and formation of uniform gel films owing to the increase in the vapor pressure of the adsorbed solution. This concept of gelation of two precursors on wettability-patterned substrates can be applied to other gel materials, such as high swelling degree aqua gels\(^6\), high strength clay gels\(^6, 47\), and gels consisting of tetrahedron-line macro-monomers\(^8\), which will contribute to device fabrication for bioanalysis and actuators at the micrometer and nanometer scales.

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