

Self-Folding of Three-Dimensional Hydrogel Microstructures

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This letter describes the fabrication of three-dimensional particulate-like hydrogel microstructures using a combination of soft lithography and volume expansion induced self-folding. Bilayer structures are produced by solvent casting and photocuring of liquid resins. They curl into three-dimensional (3D) structures upon contacting with water due to differential swelling of the two layers. The curvature can be controlled by adjusting the polymer composition of the primary swelling layer. A simple semiempirical mathematical model is used to predict this self-folding behavior. By designing the two-dimensional (2D) shapes of the bilayers, this technique can lead to complicated 3D microstructures.

Introduction

Micron-sized colloidal particles, or microparticles, have long been used for a wide variety of applications. While spherical and irregular-shaped microparticles can be synthesized by many methods,¹ microparticles with well-defined nonspherical geometries have not been produced until recent years. These “highly engineered” particulate microstructures have attracted growing interest for some applications such as drug delivery and photonic/electronic microsystems.^{2–4}

Several methods have been used for the fabrication of nonspherical particulate microstructures. For example, polystyrene microspheres were assembled into clusters with well-controlled structures.² Plate-like silicon dioxide and poly(methyl methacrylate) microparticles with drug reservoirs were fabricated by photolithography-based microfabrication technology.³ Through self-folding by surface energy minimization of molten solder, microfabricated silicon plates were assembled into various polyhedra.⁴ We have also developed simple soft lithographic techniques to prepare plate-like microparticles made of thermoplastic polymers.⁵ In this study, we extend this method to the fabrication of 3D hydrogel microstructures from 2D precursors.

Experimental Section

Materials. The poly(dimethylsiloxane) (PDMS, T2) was purchased from Dow-Corning. The chitosan, poly(ethylene glycol methacrylate) (PEGMA) with average Mn of 526, poly(ethylene glycol dimethacrylate) (PEGDMA) with Mn of 330, methacrylic acid (MAA), ethylene glycol methacrylate (EGDMA), cold water soluble poly(vinyl alcohol) (PVA), and 50 wt % glutaraldehyde aqueous solution were purchased from Sigma-Aldrich. Irgacure 651 was donated by Ciba Specialty Chemicals (Tarrytown, NY). An aqueous solution of 3 wt % acetic acid was used as solvent to make a 3 wt % chitosan solution. UV-

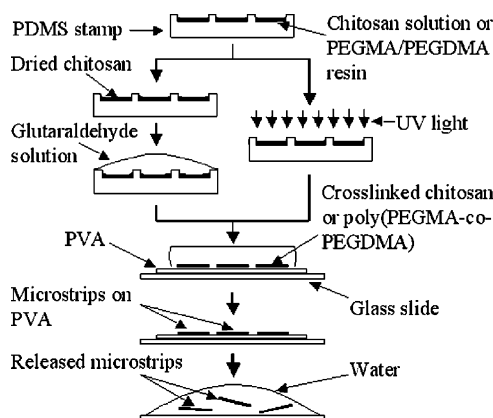


Figure 1. Fabrication schematic of microstrips.

curable resins with PEGMA/PEGDMA weight ratios of 1:1, 3:1, and 5:1 were prepared. In all three samples, Irgacure 651 was used as the photoinitiator with a concentration of 3 wt %.

PDMS Mold Preparation. PDMS molds were made by casting a PDMS prepolymer and a curing agent at 10:1 weight ratio onto a complementary relief structure prepared by the photolithographic method. Three molds were used in this study. The first consisted of an array of rectangular microwells, each 95 μm long, 20 μm wide, and 6.9 μm deep. The second consisted of an array of cross-shaped microwells with two perpendicularly crossed rectangular microwells, each 40 μm wide, 200 μm long, and 6.9 μm deep. Finally, the third consisted of an array of 6.9 μm deep microwells composed of a 100 μm square with three 100 μm long and 20 μm wide rectangular trenches on each side.

Preparation of Single-Layer Chitosan Microstrips. As shown in Figure 1, the chitosan solution was brushed onto the PDMS mold with an applicator. The solution was trapped in the discrete microwells due to discontinuous dewetting. Water in the solution evaporated rapidly, leaving a solid chitosan film. 50 wt % glutaraldehyde aqueous solution was added on the PDMS mold for 10 min to cross-link the chitosan. The sample

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was then rinsed with deionized water and dried in air. To take out the chitosan microstrips in the microwells, the PDMS mold was placed on a sticky substrate with the side having the microstrips facing down. The sticky substrate was prepared by briefly exposing a PVA film on a glass slide to water vapor generated from a hot water bath and was used immediately. A solid weight was set on the PDMS mold (300 g/cm^2) for 5 min. The mold was then removed with the microstrips stuck to the PVA/glass slide. Water was added to release the microstrips by dissolving the underlying PVA film. Micrographs of the microparticles were captured using an Olympus BH-2 optical microscope, a Nikon TMS inverted phase contrast optical microscope, and a Fujifilm Finepix 2600 digital camera.

Preparation of Single-Layer Poly(PEGMA-co-PEGDMA) Microstrips. As shown in Figure 1, the fabrication of poly(PEGMA-co-PEGDMA) microstrips started with filling the PEGMA/PEGDMA resin into the microwells using the same brushing method described above. Then resin was polymerized via exposure to UV light for 2 min in a chamber flushed with nitrogen gas. The wavelength and intensity of the UV light was 365 nm and 30 mW/cm^2 , respectively. The poly(PEGMA-co-PEGDMA) microstrips were transferred onto the PVA sacrificial layer and released in water using the same procedure as above.

Preparation of Self-Folded Chitosan/Poly(PEGMA-co-PEGDMA) Bilayers. Fabrication of curled bilayer micropads combined the two procedures for preparing chitosan and poly(PEGMA-co-PEGDMA) microstrips. The chitosan solution was first brushed into microwells and dried, followed by cross-linking by glutaraldehyde. The poly(PEGMA-co-PEGDMA) resin was then filled into the microwells and cross-linked by exposure to UV light. Finally, the bilayers were transferred onto the PVA surface and released in water. The released bilayers curled immediately and spontaneously.

Preparation of Self-Folded Poly(methacrylic acid) (PMAA)/PolyEGDMA Bilayers. The PMAA resin contained 1 wt % Irgacure 651 as photoinitiator, 50 wt % glycerin as solvent, and EGDMA as cross-linker with an EGDMA to PMAA mole ratio of 2:100. It was brushed into the microwells and cross-linked by exposure to UV light (4 mW/cm^2 , 10 min, under nitrogen environment), followed by filling the microwells with EGDMA containing 1 wt % Irgacure 651 and also exposed to the same UV light intensity. The bilayers were transferred onto the PVA surface and released in water. The released bilayers curled immediately and spontaneously.

Measurement of Microstrip Thickness. An optical profiler (WYKO NT3300, Veeco Instruments, Woodbury, NY) was used to characterize the PDMS mold and film thickness in the microwells. Depths of the microwells were measured before the chitosan solution was applied, after cross-linked chitosan layer was formed, and after the poly(PEGMA-co-PEGDMA) layer was formed. Ten microwells at different areas in the mold were measured for each measurement. The depths were used to calculate the mean and standard deviation of the thickness of chitosan and poly(PEGMA-co-PEGDMA) layers.

Measurement of Length of Single Layer Microstrips and Deflection of Folded Bilayer Microstrips

The lengths of the single layer microstrips in water were measured on the optical micrographs using an image analysis software, Image Tool (version 3.00, The University of Texas Health Science Center, San Antonio, TX). Five microstrips were randomly chosen for each type of microstrip to obtain the means and standard deviations. The deflection of the folded bilayer microstrips was obtained through measurement on those that happened to be on their sides vertically as shown in Figures

2C, 2D, and 2E. Measurements were performed on three microstrips for each type to obtain the means and standard deviations.

Determination of Swelling Ratio. Poly(PEGMA-co-PEGDMA) samples were prepared as disks of 4 mm in diameter and 0.9 mm in thickness. Volume in the unswollen state was calculated from the buoyant force exerted on samples immersed in water measured using a dynamic contact angle analyzer (DCA 332, Chan Instruments, Cerritos, CA). To obtain the volume of a sample in the swollen state, the sample was immersed in water for 3 h. Silicon oil, which is immiscible to water, was used as the medium to generate the buoyant force. The volume swelling ratio was calculated by dividing the swollen volume by the unswollen volume. Three samples were used for each type of hydrogel to obtain means and standard deviations.

Measurement of Elastic Modulus. The elastic moduli of the dry cross-linked chitosan and poly(PEGMA-co-PEGDMA) were measured using a Rheometrics System Analyzer (RSA III, TA Instruments-Waters LLC, New Castle, DE) at a displacement rate of 0.1 mm/s. Poly(PEGMA-co-PEGDMA) films with the same compositions and UV exposure conditions as those in the bilayer microstrips were prepared and cut into 5 mm by 30 mm rectangular strips with a thickness of 0.9 mm. The chitosan film was prepared by casting a solution of 1 wt % chitosan in 2 wt % acetic acid aqueous solution and cut into 5 mm by 30 mm rectangular strips with a thickness of 0.1 mm.

Results and Discussion

This hydrogel microstrip fabrication technique combines microtransfer molding,⁶ discontinuous dewetting,⁷ and sacrificial layer methods. Microtransfer molding is performed by filling microwells on a PDMS mold with a liquid precursor, pressing the mold against the substrate, and solidifying the precursor. This method is often plagued with a thin residual film between the produced microfeatures. Discontinuous dewetting is a technique used for filling a large number of microwells with a nonwetting liquid via the dewetting process of the liquid from the surface bearing the microwells. Using this technique, a liquid polymer precursor can be filled in the microwells but not around the surrounding area on a PDMS stamp. The problem of residue film associated with microtransfer molding is thus eliminated. Moreover, either a polymer solution or a reactive resin can be used as the polymer precursor. This method is especially suitable for fabricating laminated microstructures as exemplified in this study because no alignment is needed for adding new layers.

To produce bilayered microstrips, two different liquid precursors were filled and cross-linked in microwells successively. The two layers having different swelling ratios in water lead to self-folding of the bilayers from 2D plate-like shape to 3D structure. Adhesion between the two layers needs to be strong enough to withstand the delamination stress caused by the differential swelling. In the case of chitosan/poly(PEGMA-co-PEGDMA) bilayer, the PEGDMA and PEGMA molecules may diffuse into the chitosan layer and polymerize within, forming a semi-interpenetrating interface holding the two layers together during bending/curling. The degree of folding can be controlled by adjusting the composition of the layer with a higher swelling ratio or the primary swelling layer.

Figures 2A and 2B show plate-like, single-layer chitosan and poly(PEGMA-co-PEGDMA) microstrips in water, respectively. The length of the chitosan microstrips in water was $96.2 \pm 1.2 \text{ }\mu\text{m}$ (mean \pm SD, $n = 5$), and that of poly(PEGMA-co-PEGDMA) microstrips was $117.2 \pm 1.1 \text{ }\mu\text{m}$ (mean \pm SD, $n = 5$). Compared to the length of the microwells ($95 \text{ }\mu\text{m}$) the

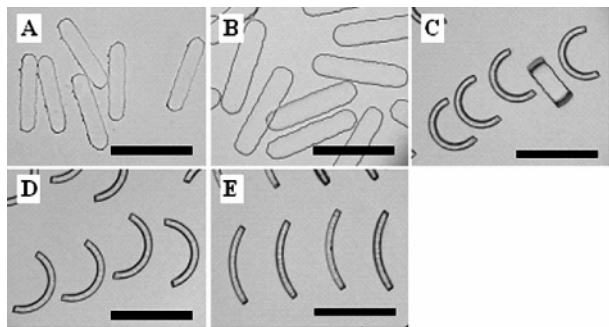


Figure 2. Optical micrographs of hydrogel microstrips in water: (A) single-layer chitosan microstrips; (B) single-layer poly(PEGMA-co-PEGDMA) microstrips with a PEGMA/PEGDMA weight ratio of 5:1; (C, D, E) curled microstrips made of chitosan/poly(PEGMA-co-PEGDMA) bilayer, with PEGMA/PEGDMA weight ratio of 5:1, 3:1, and 1:1, respectively. Scale bar = 100 μm .

chitosan microstrips showed little swelling, while the poly(PEGMA-co-PEGDMA) microstrips swelled significantly.

Figure 2C shows curled chitosan/poly(PEGMA-co-PEGDMA) bilayer microstrips. The PEGMA to PEGDMA ratio was 5:1. The thicknesses of the chitosan and poly(PEGMA-co-PEGDMA) in the microwells were $0.27 \pm 0.03 \mu\text{m}$ (mean \pm SD, $n = 10$), and $4.54 \pm 0.24 \mu\text{m}$ (mean \pm SD, $n = 10$), respectively.

To control the degree of folding, we prepared samples with different PEGMA/PEGDMA ratios of the poly(PEGMA-co-PEGDMA) layer in bilayer microstrips. Besides a 5:1 ratio, microstrips with ratios of 3:1 and 1:1 were also fabricated. They showed different degrees of curling in Figures 2D and 2E, respectively. A higher PEGMA/PEGDMA ratio led to a higher curvature of the self-folded microstrips because PEGDMA is a cross-linking agent that inhibits hydrogel swelling. Since the difference of swelling ratios between chitosan and poly(PEGMA-co-PEGDMA) is the driving force of self-folding, bilayer microstrips with a lower PEGDMA content would thus curl more.

The folding behavior of bilayered microstrips in medium can be treated as bending of a composite beam with linearly elastic deformation and no slip at the interface.⁸ The tip deflection, y_{max} , due to the swelling expansion can be expressed as eq 1:

$$y_{\text{max}} = 3(\gamma_b - \gamma_a)(\nu - \nu_0)(t_a + t_b) \frac{L^2}{t_b^2 K} = \frac{C}{2} L^2 \quad (1)$$

Here, subscripts a and b refer to the nonswelling and the swelling layers, respectively. γ is the swelling coefficient of expansion and t is the swollen layer thickness. ν_0 (equal to 1.0) is the volume-swelling ratio of bilayered structure at initial state. The volume-swelling ratio ν in equilibrium can be estimated by the volume-swelling ratios of layers a and b as shown in eq 2:

$$\nu = \frac{t_a}{t_a + t_b} \nu_a + \frac{t_b}{t_a + t_b} \nu_b \quad (2)$$

K and C are terms derived from the equivalent property of bilayered strips and are shown as follows:

$$K = 4 + 6 \frac{t_a}{t_b} + 4 \left(\frac{t_a}{t_b} \right)^2 + \frac{E_a}{E_b} \left(\frac{t_a}{t_b} \right)^3 + \frac{E_b t_a}{E_a t_b} \quad (3)$$

$$C = 6(\gamma_b - \gamma_a)(\nu - \nu_0)(t_a + t_b)/(t_b^2 K) \quad (4)$$

where, E is the elastic modulus, L is the modified half-length derived from the initial half-length of the microstrip L_0 as in eq 5:

$$L = L_0 \times \nu^{1/3} \quad (5)$$

By incorporating the swelling ratios, strip moduli, and strip thicknesses, the tip deflection for circularly folded microstrips can be given by eq 6:

$$y_{\text{max}} = \frac{3(\nu_b^{1/3} - 1) \left[\frac{t_a \nu_a}{t_a + t_b} + \frac{t_b \nu_b}{t_a + t_b} - 1 \right] (t_a + t_b) L^2}{(\nu_b - 1) t_b^2 \left[4 + 6 \frac{t_a}{t_b} + 4 \frac{t_a^2}{t_b^2} + \frac{E_a t_a^3}{E_b t_b^3} + \frac{E_b t_a}{E_a t_b} \right]} \quad (6)$$

The tensile tests for the dry gel samples were performed, and the elastic modulus of a swollen cross-linked structure, E_b , was calculated based on the following equation:⁹

$$E_b = A e^B X \nu_b^{-1/3} \quad (7)$$

where X is the PEGDMA fraction. The parameters A and B are determined by the measured swelling ratios and elastic moduli of poly(PEGMA-co-PEGDMA) samples ($A = 42477$, $B = 3.745$).

According to discontinuous dewetting, the geometry of a microwell is the major factor to determine the liquid volume filled in the microwell. Different liquids of similar properties filled in microwells are thus approximately of equal volumes. Since the chitosan solutions and the microwells used for preparing different types of microstrips are the same, the thicknesses of all chitosan films are assumed to be the same, i.e., $0.27 \mu\text{m}$. Since the viscosity and surface tension of the PEGMA/PEGDMA resins with different PEGMA/PEGDMA ratios are similar, the initial thicknesses of the poly(PEGMA-

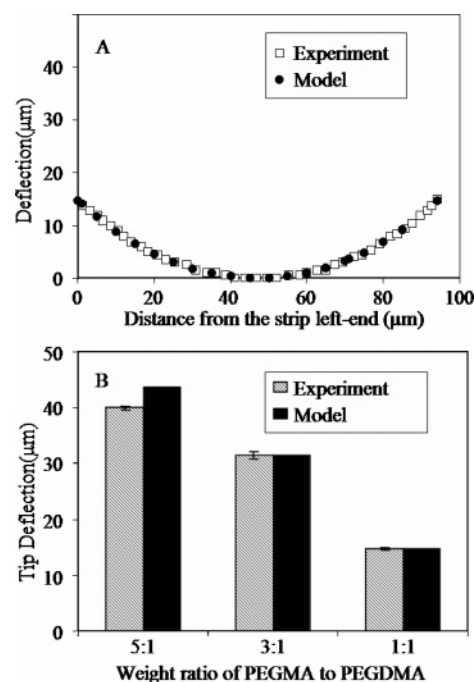


Figure 3. (A) Experimental and calculated results of curled bilayer microstrip with the higher-swelling layer made of a 1:1 PEGMA/PEGDMA weight ratio. (B) Experimental and calculated results of tip deflections of microstrips as a function of PEGMA/PEGDMA weight ratios of in the higher-swelling layer. Error bar = SD, $n = 3$.

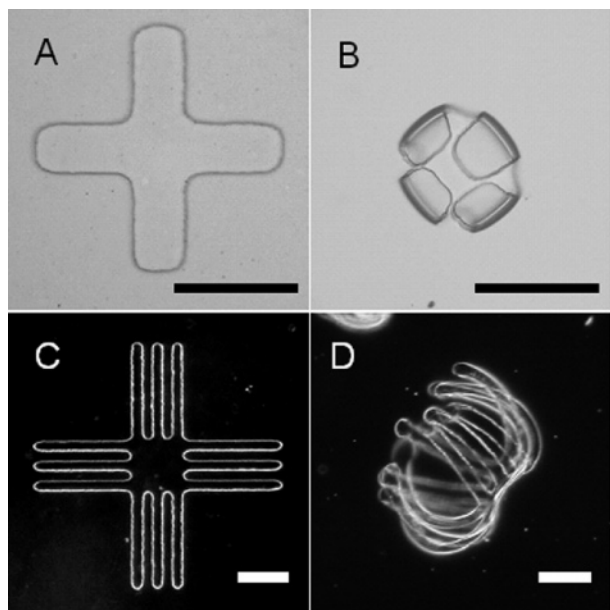


Figure 4. Optical micrographs of a cross-shaped microwell (A) and a 3D microstructure (B) made from the microwell, and a microwell with multiple extensions (C) and corresponding 3D microstructure (D). Scale bars = 100 μm .

co-PEGDMA) films are also assumed to be the same, i.e., 4.54 μm . However, the swollen thicknesses for different microstrips vary. We assume that the poly(PEGMA-*co*-PEGDMA) films in the bilayered microstrips swell freely in the thickness direction. The swollen thicknesses can thus be calculated from the initial film thickness and the volume-swelling ratios, which are obtained experimentally.

Since the chitosan layer is assumed nonswelling and its dry modulus, 8.25 MP, is an order of magnitude larger than that of the swelling layer, this value was used in the wet state. Substituting the measured and calculated parameters into the equations ($t_a = 0.27 \mu\text{m}$; $v_a = 1.0$; $t_b = 4.76 \mu\text{m}$, 4.94 μm , and 5.10 μm for samples with PEGMA/PEGDMA ratios of 1:1, 3:1, and 5:1, respectively; $v_b = 1.15$, 1.29, and 1.42 for samples with PEGMA-to-PEGDMA ratios of 1:1, 3:1, and 5:1, respectively; $L_0 = 47.5 \mu\text{m}$), the calculated self-folding of microstrips with 1:1 PEGMA/PEGDMA exhibits almost the same profile as the experimental result (Figure 3A). As shown in Figure 3B, the calculated and experimental tip deflections agree well for all PEGMA to PEGDMA ratios studied in this work.

3D microstructures with different hydrogels and more structural complexity can be produced by the same method. Figure

4A shows a cross-shaped microwell used to produce bilayers composed of chitosan and poly(PEGMA-*co*-PEGDMA) with a 5:1 weight ratio of PEGMA/PEGDMA. The self-folded 3D microstructure is shown in Figure 4B. Figure 4C shows a microwell with a more complicated shape. The two layers were made of poly(methacrylic acid) (PMAA) and poly(EGDMA), respectively. PMAA is a well-known pH-sensitive hydrogel. It formed the primary swelling layer in the bilayers. EGDMA is a widely used cross-linking agent, therefore the poly(EGDMA) layer has a very low swelling ratio. The EGDMA molecules might diffuse into the PMAA layer and polymerize within, creating a semi-interpenetrating interface that held the two layers together during curling. The self-folded 3D microstructure is shown in Figure 4D.

Conclusions

A simple soft-lithographic approach was used to produce hydrogel microlayers having well-defined sizes and shapes from a polymer solution and UV-curable resin. The bilayer microlayers can form 3D structures through self-folding induced by the differential swelling of the two layers in water. The curvature is a function of the cross-linking-agent fraction of the primary swelling layer. A model was established to describe the self-folding behavior of the microstrips that fit the experimental results well. The simple concept of this method and the ability to precisely control the 3-D architectures from 2-D precursors at a micrometer scale may find applications such as in the fabrication of next generation, intelligent drug delivery microdevices.

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