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Direct Observation of Dye-Containing Amphiphilic Copolymer in the Honeycomb-Patterned Film

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Summary: Honeycomb-patterned film was prepared by casting chloroform solution of hydrophobic polymer and dye-containing amphiphilic copolymer under humid conditions. The confocal laser scanning microscopy image shows that amphiphilic copolymer is localized around the pores of the honeycomb-patterned film.

Keywords: amphiphiles; dye-containing amphiphilic copolymer; honeycomb-patterned film; microstructure; self-organization

Introduction

Microporous structures are attractive materials due to their potentials as filtration membranes,[1] microreactors,[2] hydrophobic surfaces[3] and so on. Recently, polymer films with highly-ordered microporous structures have been reported by using bottom-up technologies including phase separation of block copolymers.[4–6]

We have reported self-organization method of preparation of hexagonally arranged microporous film (honeycomb-patterned film) by casting a solution of hydrophobic polymer and amphiphilic copolymer under humid conditions.[7–12,14] When the polymer solution is cast on the substrate, water droplets are condensed on the solution surface and packed hexagonally by the convection and capillary force during solvent evaporation. After evaporation of water droplets, highly-ordered porous array structure is formed in the polymer film. During water condensation, the amphiphilic copolymers are condensed at the water-solution interface and stabilize the water droplets.

Honeycomb-patterned films have been applied to hydrophobic surfaces[7,8] and cell culturing substrates.[9] Condensation of the amphiphilic copolymers influences the surface properties of the honeycomb-patterned films. The chemical composition of the surface affects the hydrophobicities and bioactivities of the honeycomb-patterned films. We have report that the amphiphilic copolymers were delocalized around the holes of the honeycomb-patterned films.[10–11]

In this report, fluorescence-labeled amphiphilic copolymer was newly synthesized to observe the distribution of the amphiphilic copolymer in honeycomb-patterned film by using confocal laser scanning microscopy (CLSM).

Experimental Part

Instrumentation

^{1}H NMR spectrum was measured in $CDCl_3$ (Wako) by using ECX-400 (JEOL). Trimethylshilane was used as a standard. The molecular weight was measured by Size Exclusion Chromatography (SEC) with using K-804, Shodex at 50 °C (polystyrene standards). Chloroform was used as the carrier solvent. And its flow rate was 1 ml/min. The ultraviolet-visible (UV-Vis) absorption spectrum was measured in chloroform

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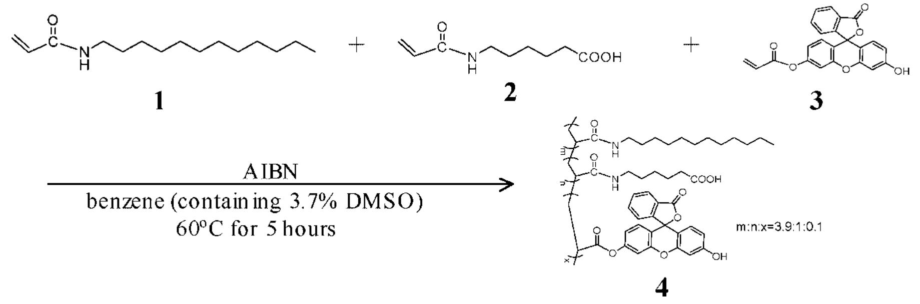

Scheme 1.

by using a UV-Vis spcetrophotometer (V-350, Jasco).

Synthesis of Dye-Containing Amphiphilic Copolymer

Monomers of *N*-dodecylacrylamide (**1**) and 6-acrylamide hexanoic acid (**2**) was prepared from the reaction with acryloyl chloride and amine.[12] Monomers including 9.8 mmol of **1** and 0.26 mmol of fluorescein -acrylate (**3**, Aldrich) were dissolved in 27 ml of benzene. Two point five mmol of **2** was dissolved in 1 ml of dimethylsulfoxide (DMSO). And then, these solutions were mixed. Recrystallized 2,2′- azobisisobutyronitrile (AIBN, Wako, 0.24 mmol) was added into the monomer solution and free-radical polymerization was performed at 60 °C for 5 hours with flow of N_2 gas. After cooling to the room temperature, the product was purified in 1.5 L of acetonitrile by reprecipitation. The white precipitate was collected by centrifugation (3000 rpm, 15 min). The product was dried in vacuo.

The yield of polymer was 88%. From the ^{1}H NMR spectrum, the signal due to the amide group was observed at 3.14 ppm.[13] The signals of olefins in monomers were disappeared in the spectrum of synthesized polymer. This result means that the monomers were successfully polymerized. From the SEC result, weight-average molecular weight (Mw) was 2.4×10^5. From the UV-Vis adsorption spectrum, absorptions of aromatic rings or ketone groups were observed less or equal 300 nm at UV region. And absorption of fluorescein was observed at 400–500 nm.

Preparation of Honeycomb-Patterned Film

Polystyrene (PSt, Mw $\sim 4.5 \times 10^4$, Aldrich) and obtained polymer were dissolved in chloroform at the polymer concentration of 4 mg/ml at their ratio of 10: 1. And 5 ml of the solution was cast on a Petri dish (ϕ 9 cm) under humid conditions (r.h. ~60%).

After evaporation of solvent, the film surface structures were observed by scanning electron microscopy (SEM, S-3500N, Hitachi). Fluorescence images were observed by CLSM (FV300, Olympus) with using Ar laser ($\lambda_{ex} = 488$ nm).

Results and Discussion

SEM images of the surface of the obtained films are shown in Figure 1(a). SEM images show that honeycomb-patterned films with hexagonally arranged pores were obtained by casting chloroform solution under humid conditions. After peeing the surfaces layers by using an adhesive tape, the films were observed by SEM (Figure 1 (b)). The top surfaces of the honeycomb-patterned films were supported by pillars, and honeycomb-patterned films had bi-layer structures.[14] The average pore size and the average height of the pillar were 5 μm and 4 μm, respectively.

Figure 2 (left) shows cross-sectional fluorescent images observed by CLSM. The film was observed at Z = 0 μm, 1 μm and 3 μm. The "Z" means the depth from the top layer of the honeycomb-patterned film.

At Z = 0 μm, fluorescence was observed along the edges of the holes. And triangle-shaped fluorescence was also observed

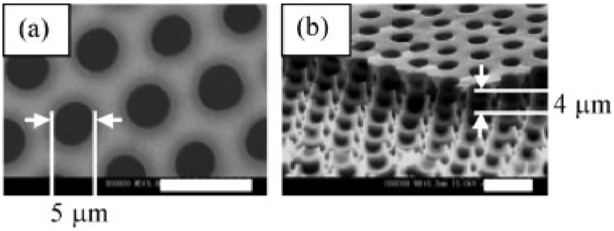

Figure 1.
SEM images of (a) top view and (b) side view of the film made from PSt and **4** (bar = 10 μm).

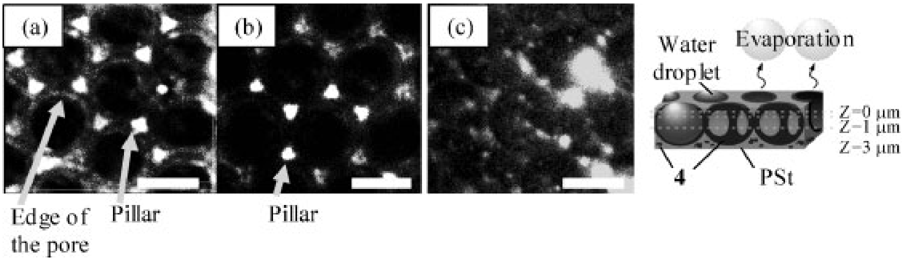

Figure 2.
(Left) CLSM images of the film;. (a) Z = 0 μm, (b) Z = 1 μm and (c) Z = 3 μm (bar = 5 μm). (Right) Schematic illustration of the honeycomb-patterned film.

around the pores. At Z = 1 μm, fluorescence was observed at the pillars. At Z = 3 μm, random fluorescence was observed. From Figure 2 (a) and (b), the amphiphilic copolymer was condensed along the edge of the pores and in the pillars. Figure 2 (c) shows the copolymer **4** molecules were phase-separated in the bottom layer of PSt. From these results, the model of the honeycomb-patterned film of PSt and **4** is shown in Figure 2 (right). When water droplets are formed on the polymer solution surface, amphiphilic copolymer condensed at the water-solution interface. Amphiphilic copolymer is localized around the pores after evaporation of water droplets.

Conclusion

Dye-containing amphiphilic copolymer was synthesized by free-radical polymerization. Honeycomb-patterned film was prepared by using the dye-containing amphiphilic copolymer and observed by using CLSM. The CLSM images directly revealed that amphiphilic copolymer was localized around the holes.

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[13] ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.14 (CH_2(NHCO)), 2.44(CH(CO), CH_2(CO)), 1.46–1.26 (CH_2), 0.895–0.862 (CH_3).

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