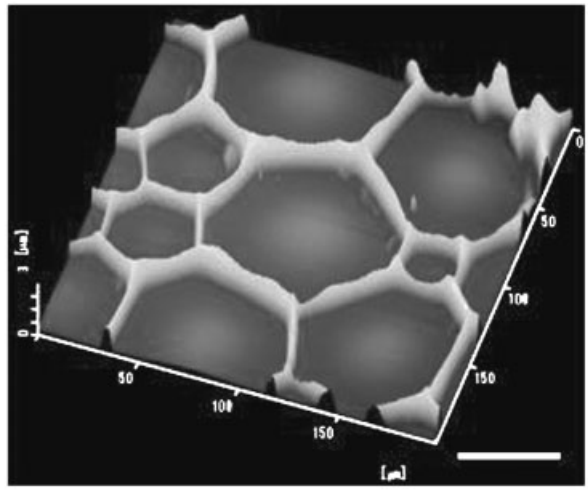
Summary: This paper reports for the first time on the fabrication of honeycomb-patterned cellulose films by casting water in oil emulsion of cellulose acetate onto a glass substrate and subsequent deacetylation treatment. The honeycomb pore size could be controlled from 1 to 100 μm under a saturated water vapor condition. Both cellulose and cellulose acetate films with honeycomb-pattern are expected to be a two-dimensional model of plant cell walls as well as of micro-wells for single cell cultivation.



Surface topographic image of a honeycomb-patterned cellulose film (scalebar: 50 μm).

Fabrication of Honeycomb-Patterned Cellulose Films

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Introduction

Microporous films of polymers have attracted much attention as functional materials in various fields such as microelectronics, biotechnology, photonics and scaffold for synthesis of composite materials.[1–6] They have been fabricated by photolithography, soft lithography and so on.[5–8]

Recently, it was reported that self-organization of hexagonal array of micropores (honeycomb-patterned films) was formed by casting dilute solutions of polymers on a solid substrate in a humid atmosphere[1–3,9] or simply by casting an emulsion of water in oil (W/O) on a substrate.[10,11] The porous structure of the films was molded by the shape of the water droplets after evaporation of the solvent in the polymer solution. Using this method, honeycomb-patterned films can be fabricated from polymer solutions with a controlled pore size, ranging from hundreds of nanometer to hundreds of micrometer, leading to novel materials with some characteristic features of such honeycomb-patterns.

Cellulose is the major biomacromolecule of the plant cell walls and has had a long history as a natural resource. A wood cell wall exists as a hollow tube along the stem direction having the hierarchical structure,[12–14] which plays an important role in the maintenance of the tensile strength and flexibility of wood as well as water pump for the biological system. Thus, the hollow tube structure comprising the wood cell and the wall is a typical example that exhibits a three-dimensionally built honeycomb architecture. If a honeycomb-patterned film is prepared from cellulose, it can be a template used for cultivation of individual cells at micro scale as well as a two-dimensional model structure of plant cell walls. In addition, as cellulose is a biosynthesized and biodegradable polymer, micro composites of the honeycomb-patterned cellulose film and another beneficial biomacromolecular materials may be also applied to medical usage.[15]

Here, we report for the first time on the fabrication of a honeycomb-patterned cellulose. First, a honeycomb-patterned cellulose acetate film was successfully fabricated, and then deacetylated to transform it to a cellulose film without changing the original honeycomb-pattern. Since not only cellulose but also cellulose acetate is a versatile

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polymer used for medical materials such as dialytic hollow fiber membranes and ultra filtration membranes, both the honeycomb-patterned cellulose and cellulose acetate films fabricated in this study are expected to be certain advanced functional materials.

Experimental Part

Materials

Commercial cellulose triacetate samples with 80–110 cP were provided by WAKO Chemicals Co. All solvents used were reagent-grade and used without further purification.

Preparation of W/O Emulsion

The W/O emulsion was prepared by modification of the method reported by Nishikawa et al.[11] 500 μL of super pure water (Milli-Q grade) was added to 5 mL of a chloroform solution of cellulose triacetate (0.5 $mg \cdot mL^{-1}$) in a 5 mL volumetric flask. The mixture was sonicated in a bath type sonifier for 10 min to disperse the water in the organic phase. The obtained suspension was milky turbid as an emulsion. The emulsion without emulsifier was shaken just before casting.

Preparation of honeycomb film

200 μL of the W/O emulsion above prepared was poured onto microscope glass slides as a substrate and the slide was placed in a closed box containing water at room temperature. In this way, the emulsion was dried up slowly under saturated water vapor to provide a film.

Deacetylation of the honeycomb film

The obtained films were deacetylated by soaking in aqueous 14% NH_4OH for 1, 6, 12, 24, 128, 176 and 273 h at room temperature, respectively. During the deacetylation process, the films were naturally peeled off from the glass substrate. Then the film was washed thoroughly with distilled water and stored in water, because it was hard to peel off the films chemically and physically once the honeycomb-patterned cellulose films were mounted on a glass substrate after the deacetylation treatment. Thus, for the following analyses, the wet films were picked up, mounted on a substrate and dried at room temperature.

Characterization of the honeycomb films

Infrared spectra were recorded with a JASCO FT/IR-620. All spectra were obtained using a microscopic attachment (MICRO-20). The spectra were the average of 32 scans recorded at a resolution of 2 cm^{-1} in the range from 4000 to 650 cm^{-1} with a MCT detector.

The surface morphology of the honeycomb films was observed by transmission optical microscopy (Leica DMRE; Leica Microsystems) and atomic force microscopy (AFM; Nanopics 2100; Seiko Instruments Inc.). The topographic features of the films were characterized also by AFM. Both the top and bottom surfaces of films were observed. Soon after the deacetylated films were naturally peeled off from the glass substrate by the NH_4OH treatment, a film to be observed for the top surface was re-mounted on a glass slide, whereas another film for observation of the bottom surface was also re-mounted on a glass slide. AFM was performed in air at room temperature, being controlled in damping mode with scan rate of 1–2 line/sec to observe 200 × 200 μm^2 areas. The AFM tip was commercial silicon tip, with a nominal radius >20 nm. The spring constant of the cantilever is 40 N/m (DFMPRC120). For observing surface topography of honeycomb-patterned cellulose films with AFM, film was deposited on a glass slide.

Result and Discussion

To obtain the honeycomb-patterned cellulose acetate film, W/O emulsion of cellulose acetate, temporary without emulsifier, was simply cast onto microscopic glass slides under saturated water vapor. In this process, as highly volatile chloroform was being evaporated, the cellulose acetate, dissolved in chloroform, began to be precipitated under a specific condition, which induces formation of micropores. Then, the remaining water droplets became a mold for fixing, to form superstructure of the precipitating cellulose acetate molecules. It should be noted that as water droplets evaporated slowly under this saturated water vapor, they could be present until the fixing of the superstructure was completed. The contribution of the droplets' presence was greatly effective as the mold. Therefore, the film preparation under saturated water vapor was the most influential factor to obtain stabilized honeycomb-patterned films.

Optical microscopic images of the honeycomb-patterned films with a different pattern size are shown in Figure 1. The

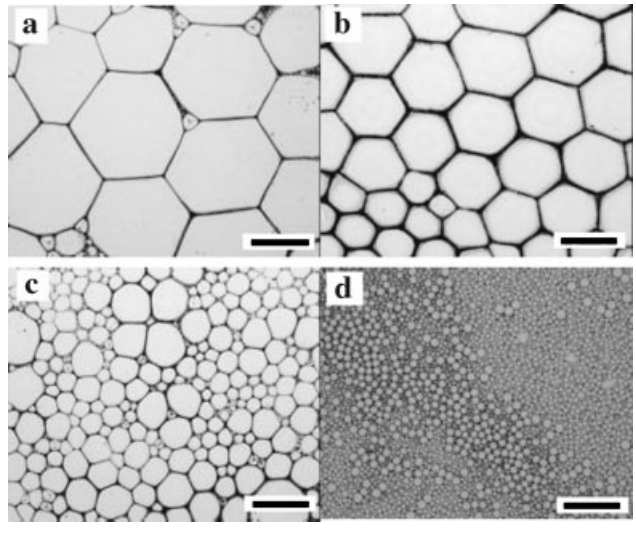

Figure 1. Micrograms of honeycomb-patterned cellulose acetate film. Dependence of the pore size on the retention time of the emulsion before pouring onto a substrate (scalebar: 50 μm): (a) 0 min, (b) 1 min, (c) 4 min, (d) 10 min.

cellulose acetate films were tough, but easily peeled off from the glass substrate. Honeycomb-patterned films having various microporous sizes ranged from 1 to 100 μm could be prepared depending on the preparation process. Nishikawa et al. fabricated honeycomb-patterned film whose pore size was controlled from 1 to a few μm using a humid atmosphere method.[2] However, our present study has made it possible to fabricate a wider range of the pore size of the honeycomb-pattern from 1 to 100 μm in diameter under a saturated water vapor atmosphere.

A reasonable explanation for the controlling of the pore size is as follows: W/O emulsion of cellulose triacetate was prepared by sonicating and shaking of water-chloroform mixture. After vigorous mixing, water droplets were at first dispersed homogeneously in the W/O emulsion. However, as the W/O emulsion was unstable without emulsifier, the relatively large water droplets were soon phase-separated, and aggregated to become larger. Such larger water droplets went up-wards to be a water phase as supernatant over the emulsion phase. On the other hand, the W/O emulsion including smaller water droplets, which were not phase-separated and still dispersed, was set under the water phase because of higher specific gravity of chloroform. The dispersed water droplets with a small size were stabilized by Brownian motion in the emulsion. While the aggregation of the larger sized water droplets, which lead to the water phase, occurred faster, the smaller droplets remaining in the W/O emulsion aggregated slowly. This means that a longer retention time after formation of the emulsion state would provide smaller water droplets in the emulsion. Therefore, depending on the retention time of the W/O emulsion after the mixing, the desired size of water droplets was allowed to attach on the glass substrate surface as a mold for a honeycomb structure. In this way, the retention time of the W/O emulsion before spreading on the substrates may be the most critical factor in order to control the pore size, since the pore sizes of cast films obtained from the W/O emulsion depend on the water droplet size as a mold.

A honeycomb-patterned cellulose film was obtained by deacetylation of the above cellulose acetate film. During this deacetylation process of the cellulose acetate film, infrared (IR) absorption bands at 1753 cm^{-1} due to C=O stretching vibration in the acetyl group was decreased while the band around 3400 cm^{-1} due to OH stretching vibration was increased upon increase of the deacetylation reaction time, as shown in Figure 2. In addition, IR absorption bands due to C–O–C antisymmetric vibration in the bridge oxygen of glycoside rings shifted from 1162 to 1158 cm^{-1}[16] in the treated sample (Inset in Figure 2). This indicates that the cellulose acetate in the film was completely deacetylated to be close to regenerated cellulose with the initial honeycomb-pattern as described later, and

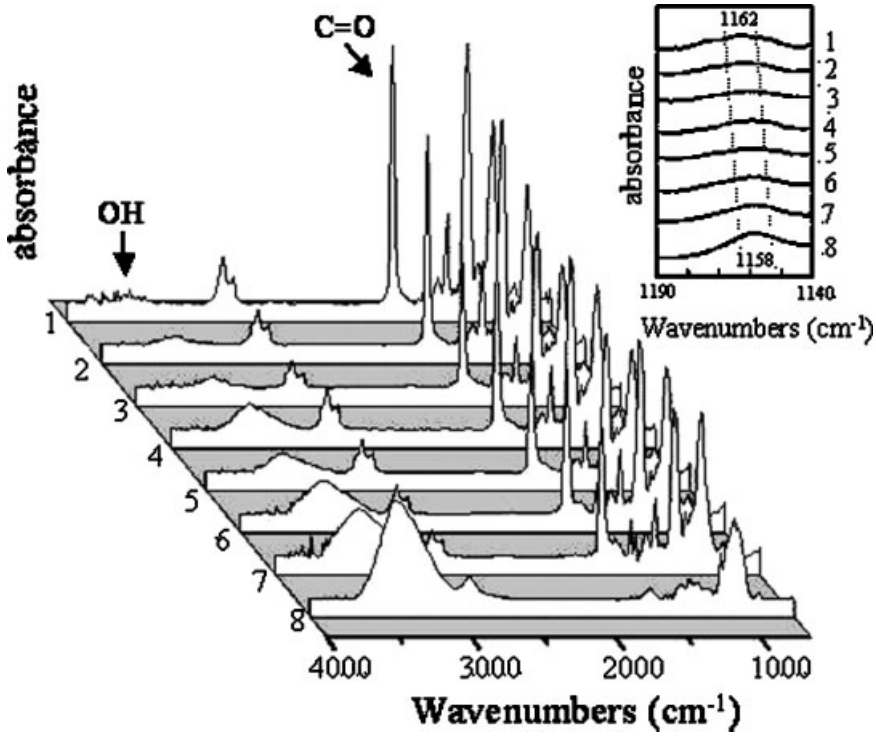

Figure 2. Change in the FT-IR spectra during the deacetylation process (from back to front). (1) 0 h, (2) 1 h, (3) 6 h, (4) 12 h, (5) 24 h, (6) 128 h, (7) 176 h, (8) 273 h. The inset in the Figure shows the shift of C–O–C antisymmetric vibration of glucoside ring.

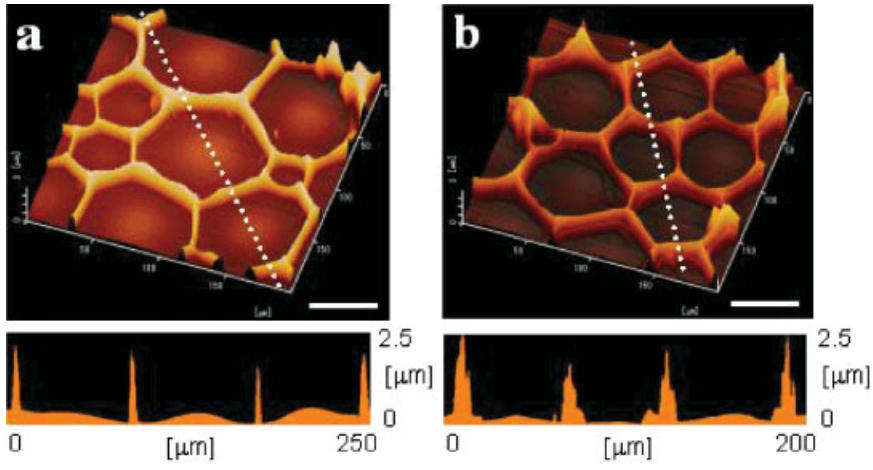

Figure 3. Surface topographic images of the honeycomb-patterned cellulose film. Cross sectional profiles were measured along the white dot line (scalebar: 50 μm): (a) top surface facing air, (b) bottom surface facing the glass substrate.

finally a honeycomb-patterned cellulose film could be prepared.

The surface topographic images of a honeycomb-patterned cellulose film were observed (Figure 3) using AFM. Nanopics provided by Seiko Instruments Inc. was employed in order to obtain the topographic images of films with such rough surfaces, otherwise impossible to be observed using a normal AFM equipped with a piezo-electric scanner. The machine was not composed of the piezo element but a voice coil motor. As it is a type of magnetic actuator, a broader-base scan is possible and Z range of sample is wider when compared with observation using a normal AFM. Therefore, a broad range of topography of the honeycomb-patterned films having large gaps in Z-axis could be observed stably. The images in Figure 3 proved that there was no significant change in the structure of the honeycomb-pattern through the deacetylation process. The film thickness and surface roughness were about 3 and 1 μm, respectively. The line width of the honeycomb-pattern was about 10 μm. The surface of the honeycomb-patterned cellulose film has two sides; the topside facing air (Figure 3(a)) and the bottom side facing glass (Figure 3(b)). Three-dimensional shapes on the surface of the honeycomb-patterned film exhibited different features in each side shown by the AFM. The surface roughness observed from the topside was relatively smooth when compared with that from the bottom side. Morphology of the films is probably dependent on how water droplets are adsorbed onto the surface of hydrophilic glass slides. Since water droplets on such hydrophilic substrates are easier to be spread, their function as a steady mold may become weaker. Thus, formation of the honeycomb pore at the bottom side is supposed to be influenced by the reduction of the function as a stable mold for micropores due to the strong interaction of water droplets on hydrophilic substrates. Accordingly, the topography of the two individual surfaces resulted in different features.

Conclusion

The pore size of the honeycomb-pattern in cellulose acetate films could be controlled from 1 to 100 μm under a saturated water vapor condition. Water droplets in the W/O emulsion play an effective role in molding the micropores under this specific condition. This is probably due to the interaction between the water droplets and the hydrophilic substrates during the formation process of the micropores. Then, the honeycomb-patterned cellulose film was obtained without deformation of the pattern and size of the structure after the deacetylation of the above cellulose acetate film, as already described above. This technique will be further applied to obtain such honeycomb-patterned film of other carbohydrate polymers such as chitin, dextran and sialyl Le^X. Both the honeycomb-patterned cellulose and cellulose acetate films thus prepared are expected to be a two-dimensional model of plant cell walls and micro wells for single cell cultivation.

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[1] T. Nishikawa, J. Nishida, R. Ookura, S.-I. Nishimura, S. Wada, T. Karino, M. Shimomura, *Mater. Sci. Eng. C* **1999**, *C8-9*, 495.

[2] T. Nishikawa, J. Nishida, R. Ookura, S.-I. Nishimura, S. Wada, T. Karino, M. Shimomura, *Mater. Sci. Eng. C* **1999**, *C10*, 141.
[3] N. Kurono, R. Shimada, T. Ishihara, M. Shimomura, *Mol. Cryst. Liq. Cryst.* **2002**, *377*, 285.
[4] C. S. Chen, M. Mrksich, S. Huang, G. M. Whitesides, D. E. Ingber, *Science* **1997**, *276*, 1425.
[5] E. Ostuni, C. S. Chen, D. E. Ingber, G. M. Whitesides, *Langmuir* **2001**, *17*, 2828.
[6] C. Miller, H. Shankes, A. Witt, G. Rutkowski, S. Mallapragada, *Biomaterials* **2001**, *22*, 1263.
[7] H. Sorribas, C. Padeste, L. Tiefenauer, *Biomaterials* **2002**, *23*, 893.
[8] A. Magnani, A. Priamo, D. Pasqui, R. Barbucci, *Mater. Sci. Eng. C* **2003**, *C23*, 315.
[9] G. Widawski, M. Rawiso, B. François, *Nature* **1994**, *369*, 387.
[10] A. Imhof, D. J. Pine, *Adv. Mater.* **1998**, *10*, 697.
[11] T. Nishikawa, J. Nishida, R. Ookura, S.-I. Nishimura, V. Scheumann, M. Zizlsperger, R. Lawall, W. Knoll, M. Shimomura, *Langmuir* **2000**, *16*, 1337.
[12] C. Brett, K. Waldron, "*Physiology and Biochemistry of Plant Cell Walls*", Unwin Hyman, London 1990.
[13] M. Knoll, E. Ruska, *Elektronen Mikroskop. Z. Phys.* **1932**, *78*, 318.
[14] M. McNeil, A. G. Darvill, S. C. Fry, P. Albersheim, *Annu. Rev. Biochem.* **1984**, *53*, 625.
[15] D. Klemm, D. Shumann, V. Udhandt, S. Mousch, *Prog. Polym. Sci.*, **2001**, *26*, 1561.
[16] C. Y. Liang, R. H. Marchessault, *J. Polym. Sci.* **1960**, *43*, 71.

