Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl19

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Version of record first published: 24 Sep 2006

To cite this article: Ryusuke Ookura, Jin Nishida, Takehiro Nishikawa & Masatsugu Shimomura (1999): Stabilization of Micropatterned Polymer Films as Artificial Extracellular Matrices for Tissue Engineering, Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 337:1, 461-464

To link to this article: http://dx.doi.org/10.1080/10587259908023477

Mol. Cryst. and Liq. Cryst., 1999, Vol. 337, pp. 461-464
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Stabilization of Micropatterned Polymer Films as Artificial Extracellular Matrices for Tissue Engineering

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Honeycomb-patterned polymer films are formed upon self-organization of water microspheres stabilized by amphiphilic polymers. The honeycomb films can work as cell culture substrates. The films composed of heparin-cationic lipid complex, however, gradually lost the honeycomb structure when immersed in culture medium. Crosslinking of the films was carried to stabilize the honeycomb pattern. A bisazido derivative was used as a crosslinker connecting the constituent polymer of the honeycomb films. Morphological study of the immersed films revealed that the crosslinked films maintained the honeycomb structure in the phosphate buffer solution.

Keywords: amphiphilic polymers; honeycomb films; photo-crosslinking; cell adhesion

INTRODUCTION

It has been noted that cells can respond to microtextures of material surface. For example, cells seeded on grooved surface with dimensions of micrometer are aligned along the groove structure[1]. Recently it was found that cells on micro-patterned adhesive sites respond to the size and density of adhesive sites with growth or apoptosis[2]. It is considered that these findings provide us with new perspectives in tissue engineering as well as in cell biology.

The patterned surfaces are usually prepared by photolithography. Re-

cently we have found that mesoscopic honeycomb-patterned films can be fabricated (Fig. 1) by a simple casting of a dilute solution of amphiphilic polymers under flow of moist air[3]. This method enables us to prepare and control morphologically and chemically patterned surfaces without using photolithography. Therefore, we may control the response of cells through the structure of honeycomb patterns. However, we found that the honeycomb patterns of some polymers are not stable in the culture medium. This suggests that the honeycomb patterns can not provide sufficiently constant stimuli to the cells.

Here we studied a photo-crosslinking of the honeycomb films composed of amphiphilic polyion-complexes to maintain the honeycomb structure in aqueous media. The crosslinked cast films were immersed in phosphate buffer, and the stability of crosslinked films was evaluated morphologically by means of optical microscopy and atomic force microscopy (AFM).

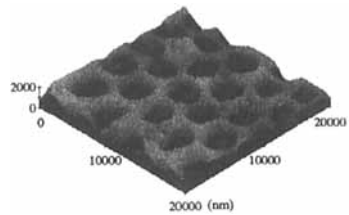


FIGURE 1. AFM image of a honeycomb-patterned film.

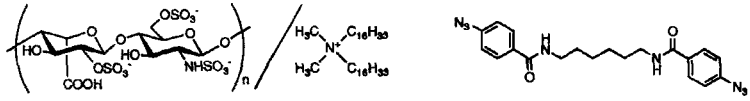
EXPERIMENTAL

Materials

Polyion-complex of heparin and cationic lipid, **1**, were prepared according to the method previously reported[4]. Photo-crosslinking agent **2** was obtained by the coupling reaction between 4-azidobenzoic acid and 1,6-diaminohexane using N-hydroxysuccinimide.

Sample preparation

Honeycomb-patterned films were prepared by casting dilute chloroform solution of **1** (1.0 mg/ml) under flow of moist air (90 ml/min). The humidity near the surface of the substrates was about 80%. Room temperature and humidity was kept at 20±1°C and 50±5% r. h., respectively.



1 2

FIGURE 2. The structure of polyion-complex (**1**) and crosslinking agent (**2**).

Photo-crosslinking reaction was run according to the following method. Five ml of chloroform solution of the photo-crosslinking agent **2** (0.25 μM) was added to 5 ml of chloroform solution of **1** (1.0 mg/ml). Honeycomb-patterned films were prepared from the mixture. The films were UV irradiated for 10 min using an UV lamp (UVP, 6 W) at a distance of 15 cm (intensity, 400 $\mu W/cm^2$).

Characterization of surface morphology

The honeycomb-patterned films were immersed into phosphate buffer (0.1 M, pH 7.3) at 37°C for 24 hours. After the immersion the films were washed with pure water to remove sodium salts, and were dried in vacuo at 40°C for 5 hours. The morphological changes of the honeycomb films were observed by optical microscopy (OLYMPUS BH2) and contact mode AFM (OLYMPUS NV-2500).

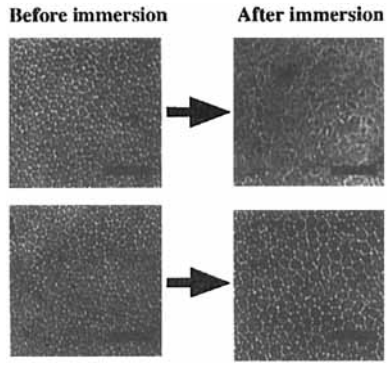


FIGURE 3. Optical micrographs of non-crosslinked (upper) and crosslinked honeycomb pattern (lower). Bar; 10 μm.

RESULTS AND DISCUSSION

Optical micrographs and AFM images of honeycomb films before and after the immersion

are shown in Fig. 3 and Fig. 4, respectively. The optical micrographs of non-crosslinked films show that honeycomb structure prepared from **1** was collapsed in a phosphate buffer solution 24 hours after immersing. On the other hand, honeycomb structures on the photo-crosslinked films were considerably maintained (Fig. 3 images in lower column).

The AFM images of the non-crosslinked films showed that the honeycomb structure disappeared and the surface roughness of the films decreased from 200 nm to 100 nm. The crosslinked films did not exhibit apparent surface morphological changes in the honeycomb structure. These results suggest that the crosslinking agent is useful for the stabilization of the honeycomb structure even in cell culture media.

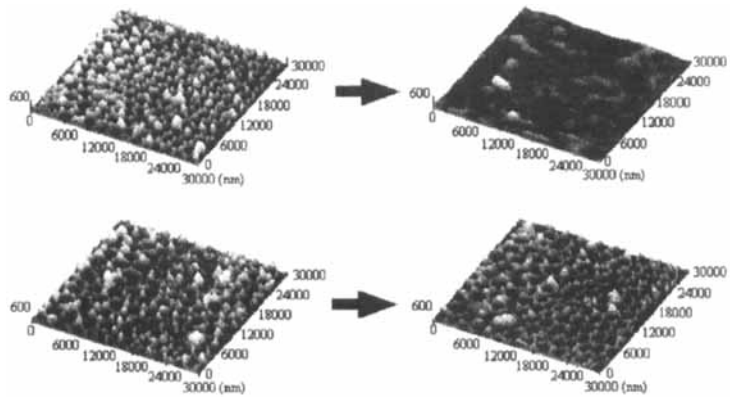


FIGURE 4. AFM images of immersed non-crosslinked (upper) and crosslinked honeycomb pattern (lower).

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