Honeycomb-patterned film providing a long-term hepatocyte survival and preservation of hepatospecific functions.

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[Introduction] Understanding for surface designing of substrate to regulate cell adhesion and growth in nano and micro scale is a critical issue in biomaterial science. The substrate must provide a suitable scaffold for cell attachment, proliferation, differentiation. Biodegradable porous polymers are a potential biomaterials for tissue engineering scaffolds. Recent experimental clinical studies demonstrated a bioartificial liver support system may be potential new therapeutic approaches as liver supports. Normal liver contains various populations of hepatocytes. Recent studies indicate that small-size fractions of hepatocytes in a normal liver, the so-called small hepatocytes, have proliferation potential *in vitro* and are expected as a potential cell source for liver supports. In this study, we describe the fabrication of highly regular patterned porous films (Honeycomb-patterned film) formed by a simply casting technique, and the culture of mature hepatocytes and small hepatocytes on the films. We evaluated the effect of the honeycomb-patterned films on the morphology, function and survival of the mature hepatocytes and small hepatocytes.

[Materials and Methods] The honeycomb-patterned films were prepared by casting poly (ε-caprolactone) (PCL) solution on glass substrate under humid air condition. The pore size of the patterned films used was 6, 12 and 16 μm. A flat film was prepared using a spin coater (1000 rpm, 30 second). Mature hepatocytes were isolated from 6- to 7-week-old male Wistar rats weighing 200–300 g by a modified two-step collagenase perfusion technique. Small hepatocytes were isolated by a Percoll density gradient solution from supernatant of cell suspension of mature hepatocytes. The hepatocytes were seeded onto polymer substrates that were immersed in the medium for over 6 h, and were then incubated at 37 °C in 95% air containing 5% CO_2. Cell yield and survival of the hepatocytes, and the morphologies of the hepatocytes were observed by using a scanning electron microscope (SEM) at 1 day, 3 days and 7 days after seeding. The differentiated hepatic functions (albumin production) were evaluated at 1 day, 3 days and 7 days after seeding.

[Results and Discussion] SEM images revealed that at 3 days in culture, mature and small hepatocytes on the flat films appeared to spread well, showing a typical monolayer morphology. On the other hand, spreading of adhered mature and small hepatocytes was restricted on the honeycomb-patterned films. At 7 days in culture, on the flat films, the attached hepatocytes were observed spread. Some of them detached from the films. A cell survival rate of the hepatocytes on the flat films decreased with increasing culture time (Fig 1). The result of 7 days culture on the flat film indicated that the flat film is an unsuitable scaffold for the hepatocytes. These results showed that the surface topography of the honeycomb-patterned film strongly affect the morphology, adhesion and spreading of the hepatocytes. Because the hepatocytes morphology affects the hepatic metabolic function, better and longer hepatic function based on the morphology of

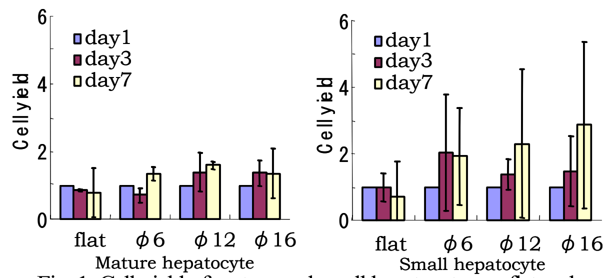

Fig. 1. Cell yield of mature and small hepatocytes on flat and Honeycomb-patterned film. On the honeycomb-patterned films, cell yield of small hepatocyte increased with culture time. Mean ± SD

hepatocytes on the honeycomb-patterned film was expected. At 3 days in culture, the albumin production volume of the mature hepatocytes on honeycomb-patterned films (pore size: 16 μm) was higher than that of the hepatocytes cultured on the flat films. On the other hand, albumin production volume of the small hepatocytes on the honeycomb-patterned films (pore size: 16 μm) and the flat film was similar. These results indicated that both the surface topography and the pore size of the honeycomb-patterned film strongly affected the hepatic metabolic function.

[Summary] The Honeycomb-patterned films have a strong influence on the cell adhesion, cell survival, and cell function of the hepatocytes. The patterned films may be potentially applied to tissue engineering scaffolds and biomedical devices.

[References]

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