

## Functional Evaluation of Cellular Populations with Different Size Prepared by Photocatalytic Lithography

K. Komori, J. Nada, I. Kameda, T. Tatsuma, and Y. Sakai  
Institute of Industrial Science, University of Tokyo  
Komaba, Meguro-ku, Tokyo 153-8505, Japan

### Introduction

To miniaturize cell-based biochips and biodevices, population of the cell should be minimized. However, it has been qualitatively recognized that a cellular population requires a certain number of cells to exhibit physiological functions and responses similar to those of corresponding cellular tissues. Although it is important to know the threshold number of cells, it has not yet been determined to the best of our knowledge.

In this study, we determine the threshold number for two-dimensional cellular populations of human liver cancer cell line Hep G2 and adult rat hepatocytes. To control the size of cellular populations, we employed photocatalytic lithography,<sup>1, 2</sup> which is a novel and convenient patterning technique based on photocatalytic remote oxidation of organic and inorganic compounds. A glass plate was coated with a non-cell-adhesive polymer, and the polymer was partially decomposed by the photocatalytic lithography. Cells were cultured in the thus-patterned cell-adhesive area with different size, and various sizes of two-dimensional cellular populations were prepared.

### Experimental

A TiO<sub>2</sub>-coated photomask (Dai Nippon Printing Co., Ltd., Fig. 1) was faced to a glass plate coated with a non-adhesive polymer, poly(2-methacryloxyethyl phosphorylcholine) (PMPC). The photomask was irradiated with UV light, so that reactive oxygen species were generated at the TiO<sub>2</sub> surface and diffused through the gas phase. The species oxidize and decompose the PMPC. As a result, the pattern of the photomask was transferred to the PMPC coating. The patterned substrate was treated with a 0.03% collagen solution, so that collagen was adsorbed onto the "decomposed aarea". Finally, a cell suspension was cast to the substrate and incubated.

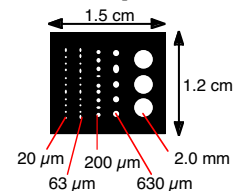


Fig. 1. The photomask used for the patterning

Albumin secretion activity and the intracellular activity of cytochrome P450 (CYP450, involved in hepatic detoxification) were measured as indexes for liver-specific functions of each cellular population by an immunostaining technique and ethoxyresorfin *O*-deethylase (EROD) assay, respectively.

### Results and Discussion

#### Hep G2 cells

Figure 2 shows micrographs of Hep G2 cell patterns obtained after 24-h incubation. The patterns were similar to those of the photomask (Fig. 1), indicating that the patterning was successful. The number of immobilized cells in each area was determined to be ca. 1 for 20 μm in diameter, 10 for 63 μm, 100 for 200 μm, 1 000 for 630 μm, and 10 000 for 2 mm area.

After 8-days incubation, the cellular populations of 63 μm in diameter or smaller were detached from the

substrate. This result suggests that autocrine and paracrine actions of various growth factors such as transforming growth factor were not sufficient due to dissipation of the factors from the small cellular populations. Thus, the activity of each cellular population was measured on day 7 of culture, before the detachment.

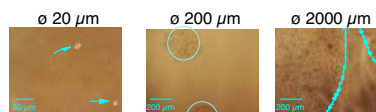


Fig. 2. Micrographs of patterned cellular populations.

The amount of albumin secreted from each cellular population was measured by an immunostaining technique. As a result, the albumin secretion per cell dramatically accelerated in the cellular populations of 630 μm in diameters or larger (Fig. 3, plot a). Intracellular activity of CYP450 was also measured by EROD assay. The activity also increased in the cellular populations of 630 μm in diameters or larger (Fig. 3, plot b), dramatically. These results might suggest that expression of transcription factors that enhance liver functions, such as hepatocyte nuclear factor, was facilitated in the sufficiently large populations since the signaling via cell cytoskeleton takes place at a sufficient level.

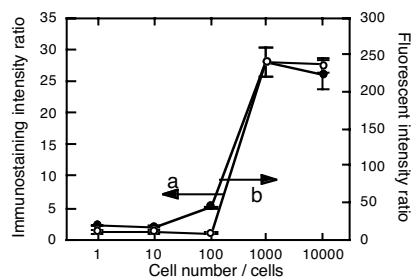


Fig. 3. Dependences of (a) the albumin secretion and (b) CYP450 activities on the size of Hep G2 of cellular populations.

#### Adult rat hepatocytes

Cellular patterns of adult rat hepatocytes could be observed after 6-h incubation. However, the cells in the cell-adhesive area of 20 μm in a diameter were detached after 1-day incubation. The other cellular populations were detached on day 5 of culture. Thus, the functional evaluation of each cellular population was performed on day 4 of culture.

The amount of albumin secretion and the concentration of CYP450 activity per cell dramatically increased in the cellular populations of 630 μm in diameter or larger. The tendencies were similar to those of Hep G2 cell.

In conclusion, the liver cells used in this work exhibit physiological functions and responses similar to those of a liver tissue when 1 000 of cells assemble into a two-dimensional population. Hence, at least 1 000 cells should be employed for the development of liver cell-based biochip and hepatic biodevices.

### References

1. T. Tatsuma, et al., *Langmuir*, **2002**, 18, 9632.
2. W. Kubo, et al., *J. Phys. Chem. B*, **2004**, 108, 3005.