Formation of hepatocyte spheroids with structural polarity and functional bile canaliculi using nanopillar sheets

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Introduction
Three-dimensional spheroids have been reported to maintain differentiated hepatocellular functions [1]. The previous research showed that the local geometric architecture of the substratum affected the cellular shape and functions [2]. From this standpoint, our objective was to elucidate the proper surface structure of culture device for spheroid formation. Therefore, we developed the nanopillar (NP) sheets with five kinds of pillar diameters and pitches, and applied for cell culture. We also examined the gene expression levels for the spheroids formed on the NP sheets.

Materials and Methods
Nanopillar sheet preparation
An NP structure was formed by pressing the nanomold onto the polystyrene film at 423 K and then releasing it at room temperature. The surface structures of the NP sheets with pillar diameters of 0.5 and 2.0 μm are illustrated in Fig. 1.

Hepatocyte isolation and culture
Hepatocytes were isolated from 6-week-old male specific viral pathogen free Wister rats (Charles River Japan Inc., Japan) by using a modified two-step in situ collagenase perfusion method. They were cultured using the NP sheets with pillar diameters of 0.18, 0.5, 1.0, 2.0, and 5.0 μm.

Results
Spheroids with a compact morphology were obtained using a sheet with a pillar diameter of 2.0 μm (Fig. 2). In contrast, monolayered hepatocytes and spheroids were intermingled using that of 500 nm. This results indicate 2.0 μm-NP sheet is suitable for spheroid formation. Bile canaliculi with well-developed microvilli those were in native tissue were observed in spheroids using 2.0 μm-NP sheet using TEM analysis (Data not shown). The expression level of MRP2 tended to increase with time except for the monolayer culture (Fig. 3). The expression at 72 h for spheroids cultured on the NP sheet with MatrigelTM overlay at 48 h postseeding (MG+), monolayer culture in collagen-coat dish (ML), and sandwich culture (SW). Statistical comparison was performed for 96-h samples. p<0.05 for MG - (¶) or MG + (*) versus SW (†).

Discussion and Conclusions
The present study demonstrated that the formations of highly functional spheroids were obtained using 2.0 μm-NP sheet, suggesting that it is practically possible to control the formation of spheroid only by optimizing the physical property of substrate surface. Our next challenge is controlling the diameter of spheroids, which may be effective in regulating their biological function. It is an essential factor to be considered in the development of highly-reproducible and reliable in vitro assay systems to evaluate the drug metabolism and toxicity.

References

Disclosures
No competing financial interests exist.