CONSTRUCTION OF HEPATIC LOBULE-LIKE 3D TISSUES UTILIZING CELL EMBEDDING HYDROGEL MICROFIBERS
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ABSTRACT
Here we propose a strategy for constructing hepatic lobule-like 3D tissues utilizing hydrogel microfibers as building blocks. Hydrogel microfibers with densely encapsulated hepatocytes were prepared using microfluidic devices, which were then coated with endothelial cell (EC) layers by suspension cultivation. Obtained microfibers with heterotypic cells were bundled and packed in a linear chamber. Cells were cultured under a perfusion condition, resulting in formation of relatively large, hepatic lobule-like tissues. The presented technique would provide useful models of liver tissues, which could be applicable to the cell based drug-screening assays and physiological study of liver cells.

KEYWORDS: Liver Tissue, Hydrogel Microfiber, Perfusion Culture

INTRODUCTION
Hepatocytes play central roles in drug metabolisms in liver, but they rapidly lose their differentiated characteristics and functions when isolated from the liver and cultured in vitro. Various approaches have been developed to mimic the in vivo liver microenvironment for the purpose of preserving the hepatocyte specific functions, including collagen-hydrogel sandwich cultivation [1], spheroid formation [2], and patterned co-culture with feeder cells [3]. We have previously developed hydrogel microfiber-based culture platform for hepatocytes; we fabricated hepatic micro-organoids composed of hepatocytes and non-parenchymal cells, which mimic the hepatic cord structures found in vivo [4]. Here, we propose a strategy to fabricate larger tissues closely mimicking the hepatic lobule structures, by bundling up liver cell-encapsulating hydrogel microfibers and culturing the encapsulated/adhered cells under a perfusion condition.

EXPERIMENTAL
The fabrication process of the liver lobule-mimicking tissues is shown in Figure 1. We used HepG2 cells as the model of hepatocytes. Initially hydrogel microfibers with densely encapsulated cells (HepG2) were prepared by using microfluidic devices. As the hydrogel material, we employed RGD peptide-conjugated alginate, which has cell-adhesive properties. ECs were cultured with the prepared hydrogel microfibers in non-cell-adhesive culture dishes to attach ECs to the surface of the hydrogel microfibers. Obtained fibers with two types of cells were then bundled up and packed into a fluidic chamber made of PDMS (width: 1 mm; depth: 1 mm; length: 15 mm). After cultivation for several days under a continuous perfusion condition, the bundle of the hydrogel microfibers was recovered from the fluidic chamber, and the cell viabilities and hepatocyte specific functions were quantitatively evaluated.

Figure 1: (a) Schematic image showing the fabrication process of the hepatic lobule-like tissue by using hydrogel microfibers prepared by microfluidic devices. (b) Cross-sectional image showing the microfiber bundle, mimicking the hepatic lobule-like tissue.
RESULTS AND DISCUSSION

By using the microfluidic devices, we first obtained HepG2 cell-encapsulating hydrogel microfibers at a production rate of ~6 m/min (Figure 2). The typical diameter of the hydrogel microfiber was 80-90 m, which was accurately controllable by adjusting the flow rates of the introduced solutions and/or the recovery speed of the roller. Next, we tried to attach ECs to the surface of the hydrogel microfibers. After several days of suspension cultivation of ECs with the hepatic cell-encapsulating hydrogel microfibers in non-cell-adhesive dishes, the hydrogel microfibers were successfully covered with ECs and complex microfibers with two types of cells were obtained as shown in Figure 3.

The hydrogel microfibers were then packed into the linear perfusion chamber, followed by the continuous introduction of the culture medium through the fiber bundle using a syringe pump (Figure 4). During this perfusion cultivation, the fiber bundle was stably maintained in the chamber without any breakage or medium leakage. After perfusion cultivation for 3 days, we were able to recover the liver lobule-like tissue from the perfusion chamber (Figure 4-d).

Finally we quantitatively evaluated the effects of perfusion flow rate on the cell viability and functions. As shown in Figure 5, we confirmed that both albumin secretion and cell viability were maintained high in a higher flow rate (15 L/min) condition. We found that not only the flow rate for perfusion but also the cell position (upstream/down-stream) within the formed tissues affected the expression of hepatocyte-specific functions.
functions. We also evaluated the expressions of hepatocyte-specific genes by using real-time RT-PCR. When HepG2 cells incorporated in hydrogel microfibers were cultured under the higher perfusion flow rate (15 L/min), the hepatocyte-specific functions, including albumin and cytochrome P450 expressions, were upregulated compared to those of the lower flow rate conditions (1 or 4 L/min). These results clearly demonstrated that the presented perfusion cultivation was advantageous in the sense that the 3D morphologies were reproduced.

CONCLUSIONS
We successfully constructed relatively large, mm-scale tissues mimicking hepatic lobules by using hydrogel microfibers as building blocks. The presented technique would be highly useful for preparing in vivo-like liver environment, which is potentially applicable to the hepatocyte-based drug screening assays and preparing bio-artificial extracorporeal liver devices.

ACKNOWLEDGMENTS
This study was supported in part by Grants-in-aid for Scientific Research (23106007 and 25750171) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

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