MICROFLUIDICS-BASED FORMATION OF HETEROGENEOUS HYDROGEL SHEETS FOR HIGH-DENSITY COCULTURE OF MULTIPLE CELL TYPES

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ABSTRACT

Microchannel-combined micronozzle devices have been developed to precisely produce heterogeneous hydrogel sheets for high-density coculture of multiple cell types. The hydrogel sheet, composed of multiple regions with different different physical stiffness, regulates the growth direction of incorporated cells, forming linear heterotypic organoids inside the hydrogel matrix. We successfully prepared stripe-shape and laminated hydrogels of 50–250 μm thickness and several-millimeter width. Additionally, hepatocytes and non-parenchymal cells (NPCs) were cultured at high densities, mimicking microstructures of heterotypic organoids found in the liver, which resulted in the enhanced hepatic functions.

KEYWORDS

Hydrogel, Coculture, Hepatocyte, Alginate

INTRODUCTION

Cell cultivation in a controlled three-dimensional microenviroments is an essential technology for various biomedical applications including tissue engineering, stem cell research, cell-based drug screening assay, and physiological studies of cells. Hydrogel matrices provide 3D environments for cells, and thus, researchers have proposed various types of heterogeneous hydrogel materials such as particles, fibers[1,2], and other complicated structures[3] by employing microfabrication and microfluidic techniques. We have developed microfluidic systems to produce physically/chemically anisotropic alginate hydrogel microfibers and applied them to guiding cell growth[1] and formation of heterotypic hepatic micro-organoids[2]. Here we propose a new microfluidic approach to prepare anisotropic hydrogel sheets, which would be more suitable for mimicking in vivo tissues and would be advantageous as unit structures for constructing larger tissues by stacking. As an application, we incorporated hepatocytes and non-parenchymal feeder cells to form arrays of heterotypic linear organoids, and examined if the coculture has the positive effects on the hepatocyte-specific functions.

EXPERIMENTAL SECTION

The microchannel system for preparing anisotropic hydrogel sheet incorporating different-type cells is shown in Fig. 1. By introducing sodium alginate (NaA) solutions with and without containing cells into the microchannel and by extruding the recombined streams into the outer gelation solution, we are able to obtain hydrogel sheets incorporating arrays of parallel regions with different cells at high densities.

In the experiment, we fabricated three- or four-layer PDMS microfluidic devices. Fig. 2 shows a microdevice for preparing the stripe-shape hydrogel sheets. There are three inlets; Inlet 1 for hepatocytes, Inlet 2 for NPCs, and Inlet 3 for the spacer solution. The flow with hepatocytes is divided into 16 streams, each of which was sandwiched by the flows with the NPCs. The spacer flow separates the flow with the NPCs cells. HepG2 and 3T3 cells were used as hepatocytes and NPCs, respectively. Propylene glycol alginate (PGA), a non-gelling derivative of alginate was used to prepare the soft regions, which guides/regulates the growth direction of cells. HepG2 and 3T3 cells were suspended in 0.3% NaA and 0.9% PGA solution at a density of 3–7×10^7 cells/mL, whereas 2.0% NaA solution was used to prepare the spacer region. The divided and recombined flows were extruded through a thin micronozzle (50 μm × 2.5 mm) into a bath with a gelation solution. To obtain straight hydrogel sheet with a uniform thickness, a roller with a diameter of ~10 mm was used.

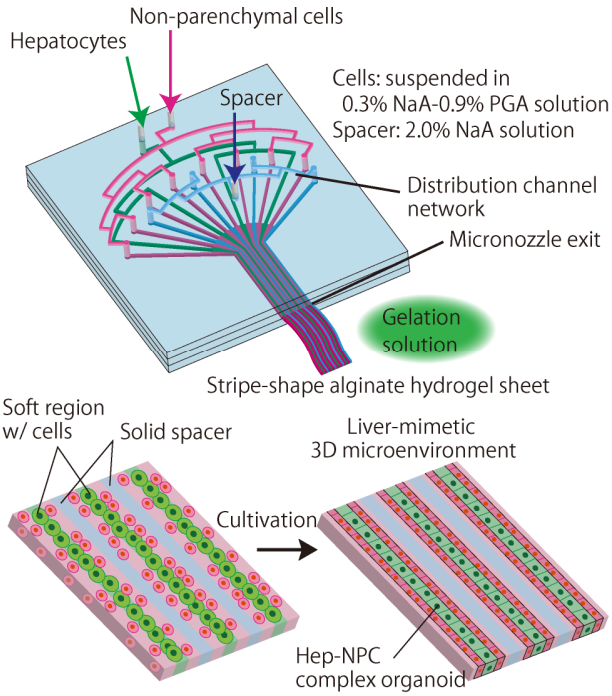

Figure 1: Schematic images showing the preparation procedure of the heterogeneous hydrogel sheet (top) and hepatic organoid formation in the soft/solid hydrogel sheet (bottom). NaA: sodium alginate, PGA: propylene glycol alginate.

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RESULTS AND DISCUSSION

First, we examined if the heterogeneous (stripe-shape or laminated) hydrogel sheets were precisely fabricated with controlled stripe width and hydrogel thickness. We prepared several types of microdevices for preparing stripe-shape hydrogel sheet composed of two different regions and three-layer laminated hydrogels sheets, and introduced 2% NaA solutions with different-color microbeads (diameter of < 1 μm). As shown in Figure 3, both the stripe-shape and laminated hydrogel sheets were obtained with a precisely controlled thickness of 50–250 μm and a width of ~2 mm. The hydrogel thickness was precisely adjustable by controlling the flow rate and/or the extension speed by the roller.

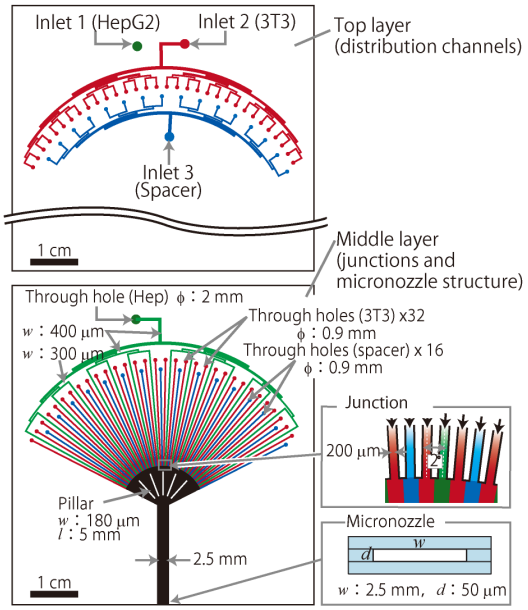

Figure 2: An example of microchannel design for preparing stripe-shape hydrogel sheets. This device was fabricated by layering three PDMS plates; top, middle, and flat bottom layers.

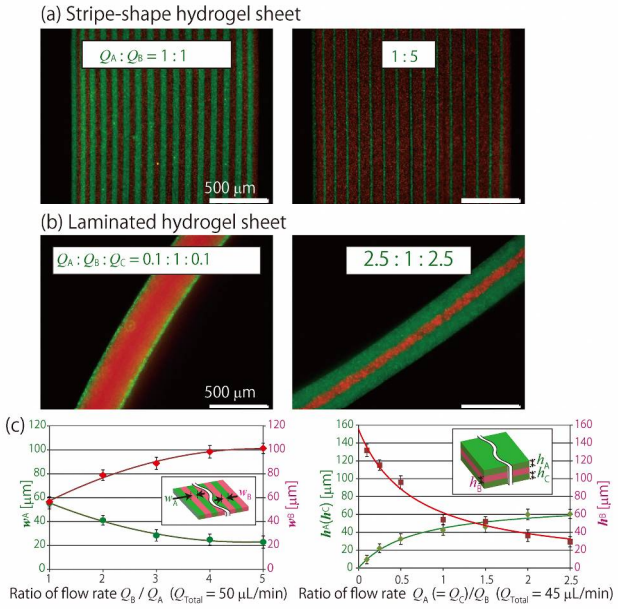

Figure 3: (a,b) Microscopic images of the prepared (a) stripe-shape and (b) laminated 3-layer alginate hydrogel sheets with a width of ~2 mm, incorporating fluorescent microbeads. Figure (b) shows the cross sectional images. (c) Relations between the flow-rate ratios and the width/thickness of each region of the stripe-shape/laminated hydrogel sheets.

Then, by using the microchannel system shown in Figure 2, we prepared hydrogel sheets incorporating HepG2 and 3T3 cells as models of hepatocytes and non-parenchymal cells, respectively, to mimic the sinusoidal structures of the liver. As shown in Fig. 4, HepG2 and 3T3 cells were focused into narrow streams in the microchannel by the spacer flow, because of the difference in the viscosities of the introduced solutions. Although totally 64 streams were combined in the microchannel with a width of 2.5 mm and then extruded into the outer gelation bath through the micronozzle, the positions of these two cell types were maintained throughout the gelation process (Fig. 4). After completing the gelation, we successfully obtained heterogeneous hydrogel sheets incorporating multiple cell types at high densities (Fig. 5 a,b). During cultivation, cells grew to form linear organoids (Fig. 5 c), because of the softness of the cell-containing region. We observed that the HepG2 cells located at the center of the organoid was surrounded by a layer of 3T3 cells after several days of cultivation (Fig. 5 d,e). Finally, we examined if the hepatic functions were enhanced in the coculture group, by measuring the amount of albumin secreted from HepG2 cells by ELISA. As shown in Fig. 6, albumin secretion from the coculture group was higher than that from the single culture of HepG2 cells in the hydrogel fiber, showing the effectiveness of the 3D coculture on the enhancement of the hepatic functions. Real-time RT-PCR assay also revealed the upregulation of several genes associated with differentiation functions of HepG2 cells.

CONCLUSIONS

A simple but highly versatile microfluidic system has been developed to prepare physically and chemically anisotropic hydrogel sheets including multiple cell types. Because of the presence of the solid spacer, cells in the soft regions formed restiform organoids with a width of ~100 μm and a length of up to several millimeters. We have successfully demonstrated that the hepatic cells cocultured with feeder cells showed the enhancement of the hepatocyte-specific functions compared with the single culture group. It would be possible to produce much more complicated and functional hydrogel materials by using the similar microchannel configurations, which possibly provides unique 3D cell cultivation platform for tissue engineering applications and stem cell research.

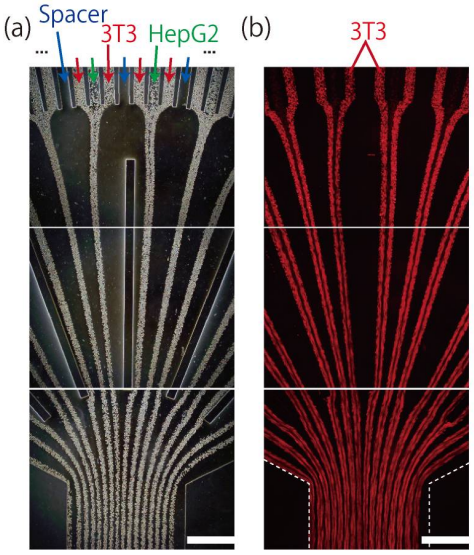

Figure 4: Two-types of cells (HepG2 and 3T3 cells) flowing through the microchannel. Cells were focused and concentrated into distinct lines because of the relatively low-viscosity of the cell suspension. 3T3 cells were stained withPKH26 red fluorescent dye.

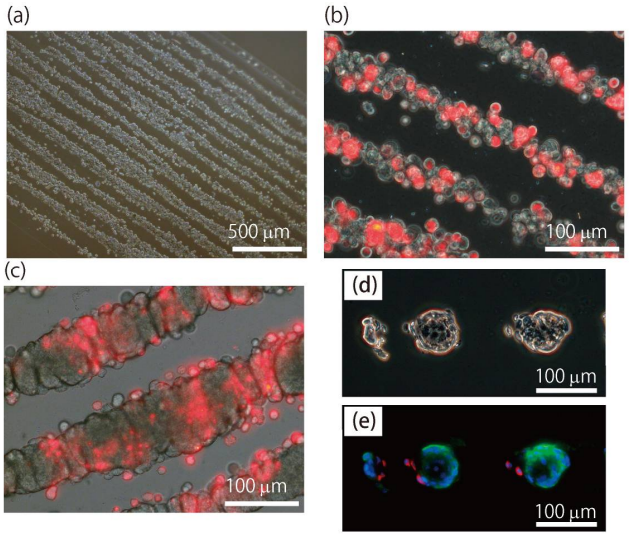

Figure 5: Coculture of HepG2 and 3T3 cells in the heterogeneous hydrogel sheets with a thickness of ~100 μm. (a,b) Hydrogel sheet at Day 0, (c) complex organoids formed at Day 7, and (d,e) cross sections of the organoids at Day 3. 3T3 cells were stained with red dye. In (e), albumin (green, HepG2) and nuclei (blue) were immunostained.

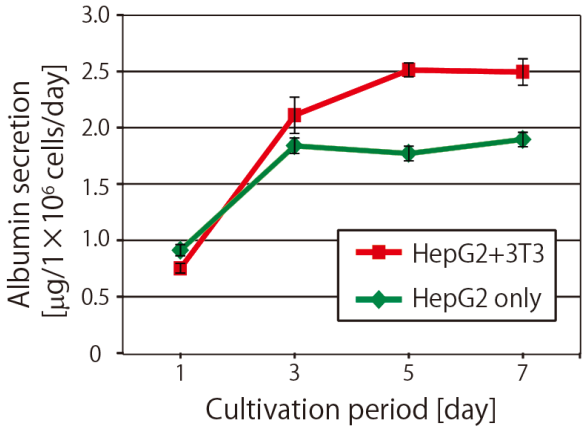

Figure 6: Comparison of albumin secretion from HepG2 cells in coculture and single culture groups in the heterogeneous hydrogel sheets, analyzed by ELISA.

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