RAPID FORMATION OF SIZE-CONTROLLED 3-DIMENSIONAL HETERO-SPHEROID USING MICRO-ROTATIONAL FLOW

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ABSTRACT

In this paper, we demonstrate formation of size-controlled 3-dimensional hetero-spheroids using a micro-fluidic device in approximately 2 min. The device collects cells in the center of a micro-chamber using micro-rotational flow as shown in Fig. 1. In previous work, we used this device to generate size-controlled HepG2 spheroids [1]. We applied it to form hetero-spheroids consisting of HepG2 and HUVEC. We experimentally found that the spheroid sizes could be controlled by the cell densities of the media independent of the ratio of HepG2 and HUVEC. Uniform dispersion of HepG2 and HUVEC in the formed spheroid was also experimentally verified.

KEYWORDS: Bio MEMS, Micro-fluidics, Micro-rotational Flow, Hetero-spheroid

INTRODUCTION

Cells in the human body aggregate together and interact with cells adjoining each other and the extracellular matrix. Several cell types, like hepatocytes [2], form spherical tissue called spheroid whose function is better than individual cells in vitro. For example, a spheroid based on hepatocytes performs about 500 liver-specific functions, such as the expression of new proteins and cell signaling, and increases their metabolic functions. Therefore, many kinds of device have been developed, that can form three-dimensional spheroids in order to improve the in vitro biological research like drag candidate testing [3].

In recent years, several co-culture approaches have also been adopted to more precisely investigate tissue functions and interaction between different cell types [4]. In particular, the function of spheroids consisting of more than one cell type, called hetero-spheroids [5], reveals to be closer to a tissue consisting of those cell types than spheroids that are composed of one cell type [6]. So hetero-spheroids is very useful for in vitro biological research.

However, the adhesion between different cell types is not as strong as that between the same cell type [7], so it is difficult to form a 3-dimensional hetero-cellular aggregate rapidly, which grow to a hetero-spheroid.

In our previous research, we developed chamber which forms a hepatic spheroid, that used micro-rotational flow to control the spheroid size [1]. In our device, a perfusion medium containing cells was injected into a micro-chamber which a micro-rotational flow was generated in. These cells were collected in the center of the micro-chamber, where they aggregated and formed a spherical cellular aggregate and finally grew to a spheroid. This chamber could create spheroids with diameters in the range of 130-430 μm with a standard deviation of approximately 15%. This chamber, which is arrayed, is superior to other micro-fluidic devices in that hepatic spheroids can be formed within 2 min and spheroid sizes can be controlled by altering the cell density in the medium without the necessity of changing the geometry of the chamber. In addition, this device can provides a space for spheroids to grow because the cells are not concentrated around the center of the chamber by solid structure of chamber but by hydrodynamic forces.

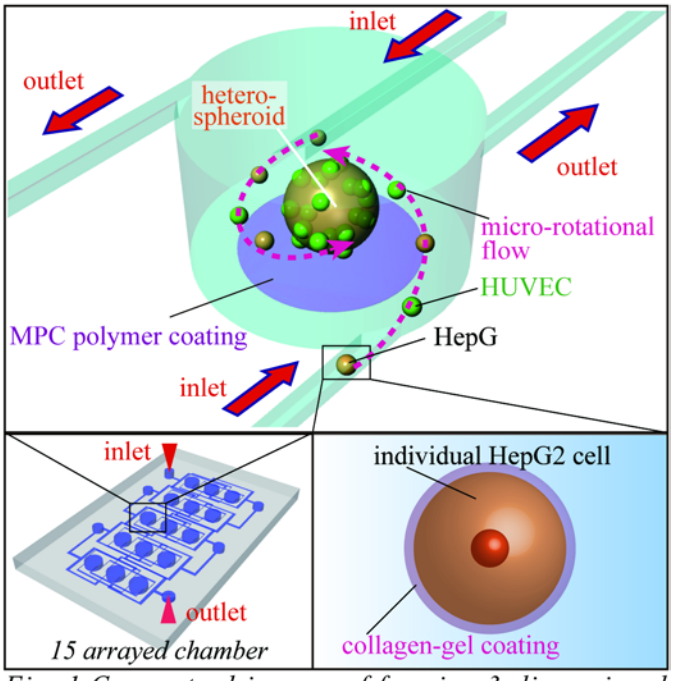

Fig. 1 Conceptual images of forming 3-dimensional hetero-spheroid using micro-rotational flow and coating collagen-gel on the surface of individual cell.

In this paper, we demonstrate rapid formation of mixed cellular aggregates composed of hepatocytes cells and endothelial cells, both of which are constituent of liver, using a later upgraded spheroid formation chamber (Fig. 1). We coated the bottom of the hetero-spheroid formation chamber with a 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer to prevent endothelial cells from adhesion to the bottom of the chamber and stabilize formation a hetero-cellular aggregates. In addition, in order to increase adhesion between these different cell types, the hepatocytes cells

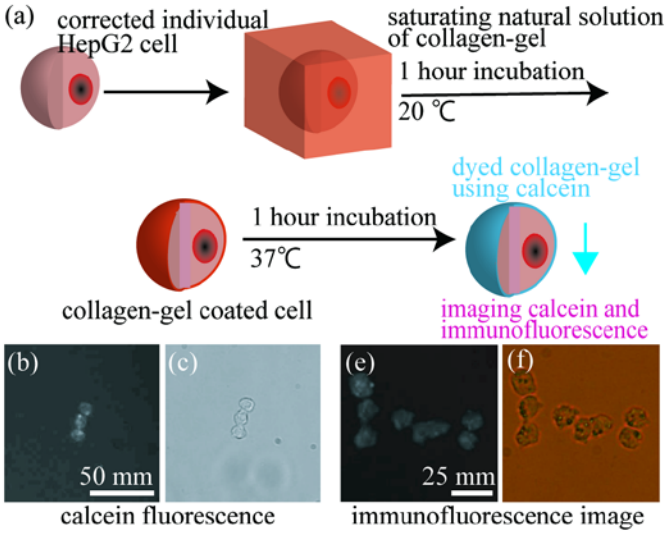

Fig. 2 Experimental flow of collagen-gel coating (a).the microscopic images of fluorescence of collagen-gel coating layer, calcein fluorescent image (b) and optical image (c),immunofluorescent image (e) and optical image (f).

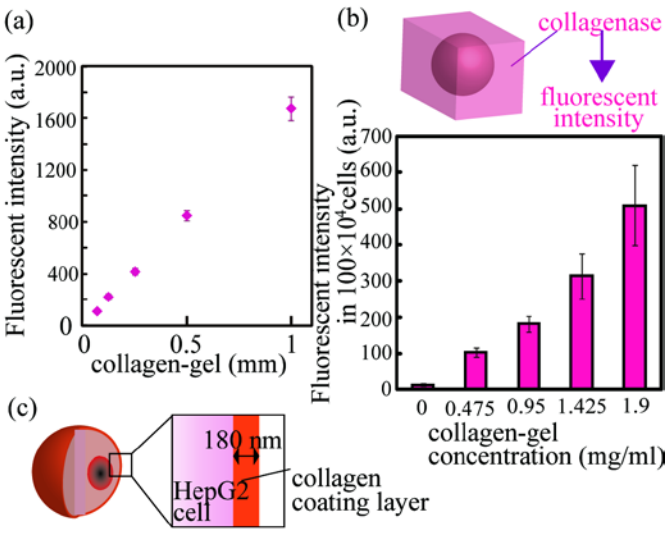

Fig.3 Thickness of the coated collagen. (a) Fluorescent intensity of collagen-gel with calcein as a function of thickness. (b) Fluorescent intensity of collagen-gel covering cells. (c) The thickness of the collagen-gel coating the cells was deduced to be 180 nm.

were coated with a collagen gel. Without this surface treatment, hepatocytes cells and endothelial cells did not bond to each other and did not form hetero-cellular aggregates. Furthermore, collagen coating on cell surfaces, suited to the cell collecting technique using micro-rotation flow, enabled the formation of mixed size-controlled hetero-cellular aggregates within 2 min. Our device can effectively form hetero-cellular aggregates and in turn, possibly hetero-spheroids, that is useful for application using hetero-spheroid.

EXPERIMENTAL

We demonstrate rapid formation of 3-dimensional hetero-spheroids composed of HepG2 and HUVEC using a microfluidic device that we developed, where coating collagen-gel on HepG2 cells to enhance the cell adhesion was crucial.

HepG2 cells were incubated in a neutral solution of collagen-gel at 20 ºC. After 1 hour of incubation, these cells were centrifuged and collected. A medium at 37 ºC was added to the cell suspension and the cells were incubated at 37 ºC for 1 hour to reconstitute a collagen-gel on the surface of the cells (Fig. 2a). Fluorescent images of collagen-gels, which were obtained using a calcein (Sigma, Japan) fluorescence mixed in collagen-gel and immunofluorescent technique using Alexa Fluor-conjugated anti-mouse IgG (Cell Signaling, Japan), verified that the collagen-gel successfully adhered to and covers the cell surface (Fig. 2b-f). Collagen-gel on cell surfaces was then treated by collagenase and the fluorescent intensity of the incorporated calcein was assessed using a spectrofluorometer (ex. 405 nm, em. 620 nm) (RF 1500, Shimadzu, Japan). Based on the results of gel extraction and calibration experiments (Fig.3), the thickness of the collagen-gel layer coated on the cells was found to be approximately 180 nm.

Since HUVEC are more likely to adhere to the micro-chamber walls than HepG2, in this study we modified the surfaces of the device with 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer to prevent cell adhesion by coating process using shadow mask and splay.

Applied the device as with our previous research [1], 3-dimensional hetero-spherical cellular aggregate consisting of HepG2 and HUVEC successfully is formed in approximately 2 min as shown in Fig. 4 a-d, where HUVEC cells were fluorescently labeled.

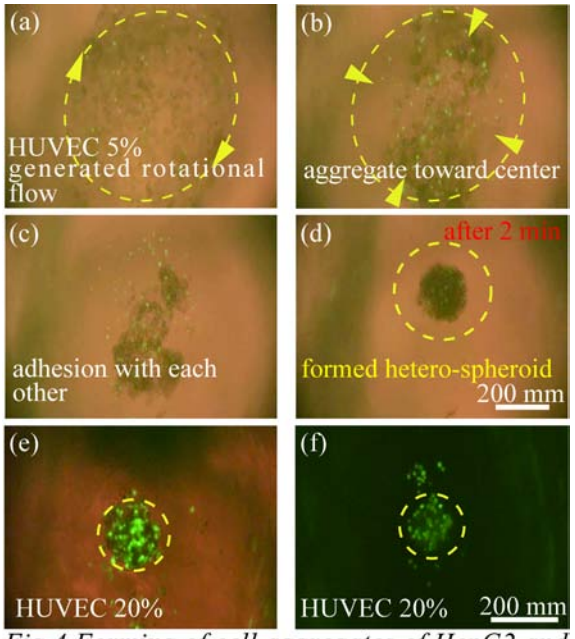

Fig.4 Forming of cell aggregates of HepG2 and HUVEC. HUVEC is labeled with calcein AM. (a-d) The spheroid was formed in 2 minutes. (e) An optical and fluorescent image and (f) a fluorescent image of the formed spheroid.

RESULTS AND DISCUSSION

The two species of cells were uniformly mixed and formed spheroids. The spheroid sizes were investigated as shown in Fig. 5. We could control the sizes by the cell densities of the media while the ratio of two species did not result in any significant differences. We investigated the distribution of HUVEC cells in the spheroid. We divided the spheroid into 4 regions such that each region has identical volume. We counted the cells contained in each region and found that the HUVEC cells were uniformly distributed in the formed spheroid (Fig. 6).

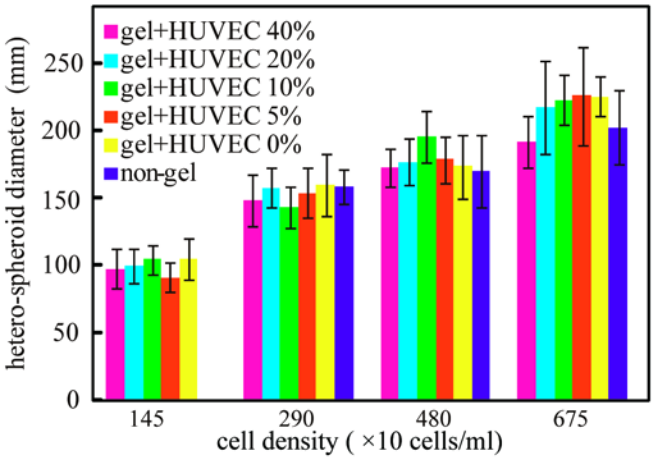

Fig. 5 Sizes of hetero-spheroids formed at 5 different blend ratios of collagen coated HepG2 and HUVEC as a function of cell densities. Sizes of HepG2 spheroid without gel coating was shown in the figure (non-gel) as a control.

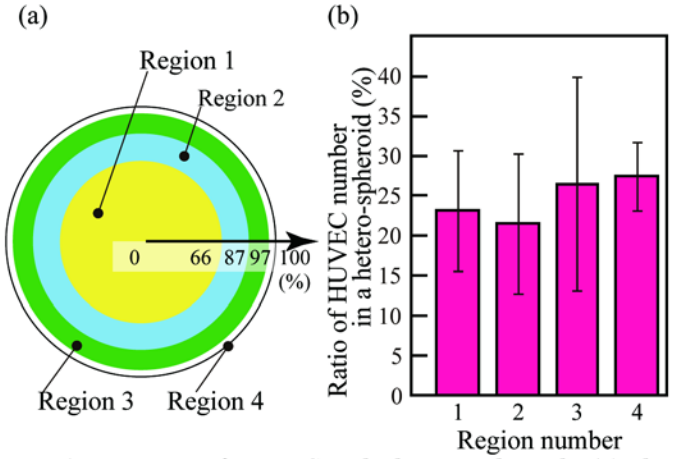

Fig. 6 Dispersion of HUVEC in the hetero-spheroids. (a) The spheroid was divided into 4 regions that have identical volumes. (b) Number of the cells in each region counted in fluorescent microscopic images. HUVEC was found to disperse uniformly in the spheroid.

CONCLUSION

In this research, We succeeded in rapid forming of 3-dimensional hetero-spherical cellular aggregate, using micro-rotational flow and collagen-gel coating. That size can controlled by total cell density injected into micro-chamber. we revealed that the dispersion of each cells in cellular aggregates is uniformly.

This rapid formation technique can be applied for hetero-spheroid based tissue engineering.

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