THREE DIMENSIONAL SPHEROIDS FORMATION PLATFORM USING MICRO-ROTATION FLOW

Hiroki Ota, Koji Deguchi, Ryosuke Yamamoto, Yutaka Kazoe, Koichi Hishida, Yohei Sato and Norihisa Miki

Keio University, Japan

ABSTRACT

Hepatocytes increase cell densities and acquire their liver-specific functions during the tissue formation from isolated cells. We developed three dimentional sphedoids formation platform using micro-rotation flow. Based on results of simulations and micro PIV, This chamber were designed to have a circular cylindrical shape with two inlets tangent to the circle at the bottom and two outlet channels at the top. The developed platform could not only form three dimensional spheroids, but also conrol sizes with micro meter scale only by a micro-rotation flow repeatedly. This platform will be applied to be new drug screening systems and hybrid artificial organs.

KEYWORDS: tissue engineering, micro-rotation, photolithography

INTRODUCTION

Hepatocytes are responsible for metabolic processes in the liver. During the tissue formation from isolated cells, they increase the cell density and acquire their liver-specific functions. Hence, tissue-based in vitro studies are strongly demanded for precise drug screening and a supportive device for an individual with liver failure. To date various spheroid-forming devices have been developed that use spinner culture [1], polyurethanefoam scaffold [2],and micropattering [3], however, those devices are not capable of controlling the three-dimensional architecture of spheroids.

We propose a three-dimensional spheroid forming platform that is capable of controlling size and three-dimensional shapes using a micro-rotation flow. The micro-flow in our device can control by a hydro dynamic force the sizes of spheroids and supply enough nutrition efficiently via circulation. High-controllable three-dimensional spheroids formation platform will not offer only new experimental systems that imitate tissues for biological research.

CHAMBER DESIGN AND SIMILATION

A designed micro chamber, as shown in Fig. 1, has a circular cylindrical shape with two inlet channels tangent to the circle at the bottom and two outlet channels at the top. The micro-rotation flow is generated by flowing fluids from two inlet channels at the bottom. The chamber was composed of poly-dimethylsiloxan (PDMS) formed by a photolithographic process.

We simulated the flows in the chambers with various shapes and sizes by FLUENT 6.3.26. The volume of the chamber was meshed using Tet/Hybrid elements by Gambit 2.4. The mesh data of the chamber created by Gambit 2.4 was imported to FLUENT 6.3.26. Rotation flow was generated in three-dimensional chamber models which had inlet and outlet channels at different heights near the wall and in the center

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of the chamber as shown in Fig.2. Furthermore, the channel height, the channel width, the chamber diameter and the influx velocity were found not to affect the generation of rotation flow.

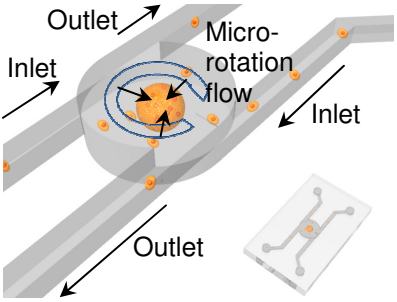

Figure 1 : The concept of the spheroid formation chamber.

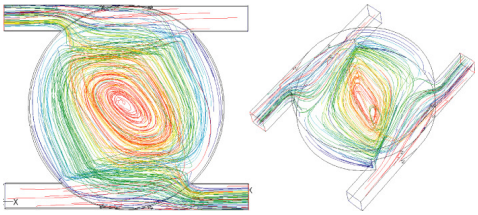

Figure 2 : Simulated fluid flow in the microchamber. In center of the chamber , rotation flow was generated.

EXPERIMENTAL

The developed platform was composed of a micro chamber and a perfusion system consisting of a reservoir, dampener and peristaltic pump to circulate cell culture medium constantly. Before starting experiments, air bubbles in a chamber and/or silicon tubes connected with elements were carefully removed.

We calculated velocity vectors in the 3 mm chamber by micro PIV. The fluorescent particles with a diameter of 2.0 μm were added into the media at 650×10^4 particles/ml. Velocity vectors in the chamber were calculated from the particle images taken by the cooled CCD camera, and the ensemble averaging technique was applied to the 100 instantaneous velocity vectors to avoid a measurement error associated.

Hepatocyte (HepG2) cultured in 75 cm^2 culture flask were detached. Temperature and pH of media circulating in the system were maintained by a thermostatic bath and circulating CO_2 gas during experiments. Firstly, the media containing cells were introduced into the chamber at a volumeric flow rate more than 1.1 ml/min, when the cells rotated at in the entire area of the chamber stably. Secondly, we decreased the flow rate down to an appropriate flow rate (0.39 ml/min) gradually over 2 min, when the cells near the center of the chamber were attracted towards the center to form a spheroid. Stationary images were taken by a CCD camera.

RESULTS AND DISCUSSION

A micro-rotation flow in an entire area of the chamber took place in a micro chamber 3000 μm in diameter at a volumetric flow rate of more than 0.287 ml/min (Fig. 3 (a-d)). Figure 3(e) shows flow velocities in x-axis direction along the y-axis crossing the center of the chamber at a volumetric flow rate of more than 0.287 ml/min. The velocities were found to be symmetric about the center and be stable.

The culture media containing human hepatocellular liver carcinoma cell were introduced into the chamber to form a spheroid. When a volumetric flow rate of the media circulating in the platform was 0.39 ml/min, cells adhered to each other to

form a spheroid in the chamber and kept rotating in the center. Spheroids 300 ±24 μm in diameter were repeatedly generated in nine out of ten experiments (Fig. 4).

CONCLUSIONS

In this research, we designed three dimensional chamber to generate micro-rotation flow according to simulation results. Micro PIV revealed micro-rotation flow in an entire area of the designed chamber. To conduct experiments, we developed platform composed of a micro chamber and channels made of PDMS and a perfusion system consisting of a reservoir, pulsation dampener, and peristaltic pump. The developed platform could not only form three dimensional spheroids, but also conrol sizes with micro meter scale only by a micro-rotation flow repeatedly.

We can applied this platform to various biological applications such as artificial liver, drug screening and regenerated medicine by arraying the chambers and inserting electrodes to measure biological parameters.

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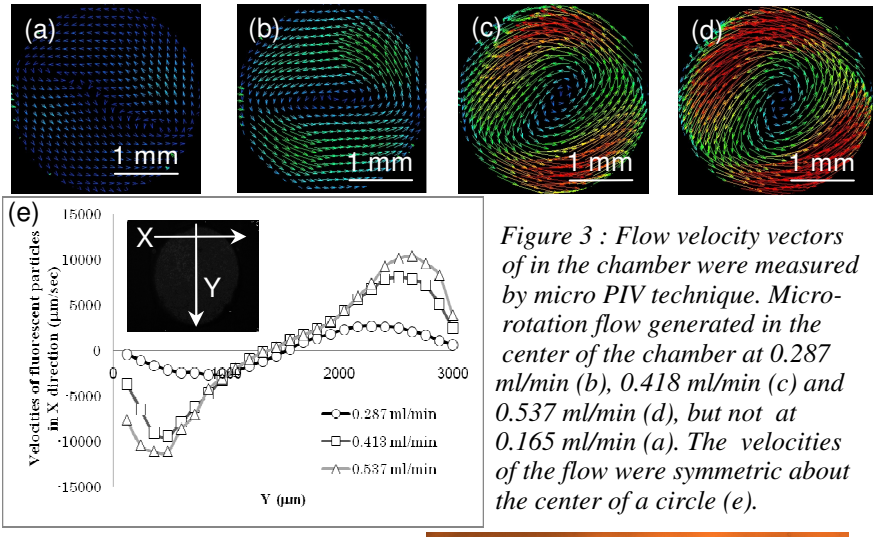

Figure 3 : Flow velocity vectors of in the chamber were measured by micro PIV technique. Micro-rotation flow generated in the center of the chamber at 0.287 ml/min (b), 0.418 ml/min (c) and 0.537 ml/min (d), but not at 0.165 ml/min (a). The velocities of the flow were symmetric about the center of a circle (e).

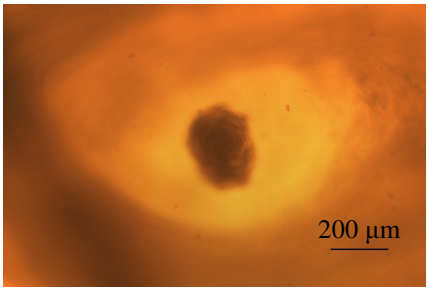

Figure 4 : Hepatocytes whirling in the chamber. Hepatocytes adhered to each other and