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Three-dimensional spheroid-forming lab-on-a-chip using micro-rotational flow

Hiroki Ota*, Ryosuke Yamamoto, Koji Deguchi, Yuta Tanaka, Yutaka Kazoe, Yohei Sato, Norihisa Miki

Department of Mechanical Engineering, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama, Kanagawa 223-8522, Japan

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ABSTRACT

We propose a spheroid-forming lab-on-a-chip that uses micro-rotational flow to control the size of three-dimensional hepatocyte spheroids. The developed device consists of a microchamber made of polydimethylsiloxane produced by soft lithography and a perfusion system consisting of a reservoir, a dampener, a chamber, a shredder channel, and a peristaltic pump to constantly circulate cell culture medium containing human hepatocellular liver carcinoma cells. Cells are attracted to the center of the chamber, where they aggregate and form spheroids. The size of the spheroids produced by this device can be controlled by varying the cell density and the flow conditions. Thus, spheroids of various sizes can be formed without altering the chamber design. The developed perfusion system permits cultures to be stored for a long time by suppressing sudden changes in the micro-rotation flow; this ability was verified by performing a live/dead assay. Hepatic spheroids with average diameters in the range 130–430 μm and with a standard deviation of less than 17.2% were successfully produced. Their sizes were found to depend on the chamber diameter and the cell density. The standard deviation of spheroids with average diameters between 150 and 200 μm was as low as 16.0%. Micro particle image velocimetry revealed a spheroid-forming region in the chamber. It also enabled the number of cells that form a spheroid to be estimated.

1. Introduction

Hepatocytes are responsible for metabolic and detoxification processes in the liver. During tissue formation from isolated cells, their cell density increases and they acquire more than 500 liver-specific functions, including the ability to express new proteins, cell signaling, enhancement of their metabolic activity, urea synthesis, and bile acid and albumin secretion [1,2]. Therefore, there is a great demand for *in vitro* hepatic spheroid-forming devices to acquire reliable and predictable *in vitro* data for drug screening and biological research. Consequently, *in vitro* hepatic spheroid-forming devices are required to form three-dimensional spheroids.

The functions of hepatocytes are related to their sizes [3]. Hepatocytes require more oxygen than other cell species. Hepatic spheroids are similar to tumor masses in that oxygen diffusion is limited to about 150–200 μm [4]. Hepatic spheroids with diameters over 500 μm exhibit a layered structure consisting of a necrotic core surrounded by viable cells [5,6]. Spheroid-forming devices need to have good size controllability in the range 150–500 μm.

Spheroid-forming devices have been developed that employ oscillatory agitation [7], a spinner culture [8], a NASA rotary cell culture system [9], a polyurethane foam scaffold [10], a rotating radial flow bioreactor [11] and a rotating disc bioreactor [12]. They are straightforward to use and can produce large numbers of spheroids. However, these devices are unable to control the sizes of individual spheroids and the devices are much larger than desired.

In recent years, microfluidic technologies have been developed that enable cells to be manipulated precisely on a micrometer scale and require only nanoliter to microliter reagent volumes to control the cell culture conditions [13–15]. Several spheroid-forming devices based on these technologies have been developed including devices that use micropatterning of a polyethylene glycol hydrogel substrate [16] or of an extracellular matrix [17], a microcontainer that traps cells [18], a microfluidic embryoid-body formation device [19] and a microfluidic spheroid-formation device that uses hydrodynamic trapping of cells [15,1]. These devices are capable of controlling the region in which cells aggregate and form spheroids so that the sizes and numbers of cells that make up the spheroids can be controlled.

We propose a three-dimensional spheroid-forming lab-on-a-chip that is capable of controlling the size of spheroids using a micro-rotational flow. Cells containing a perfusion medium are introduced to a microchamber in which a micro-rotational flow is generated. Cells are attracted to the center of the microchamber by the hydrodynamic force and they aggregate in the center forming spheroids. The device can create spheroids with diameters ranging from 150 to 430 μm with good accuracy. The device is superior to other microfluidic devices in that the spheroid size can be con-

* Corresponding author. Tel.: +81 45 566 1430; fax: +81 45 566 1495.
E-mail address: ota@miki.mech.keio.ac.jp (H. Ota).

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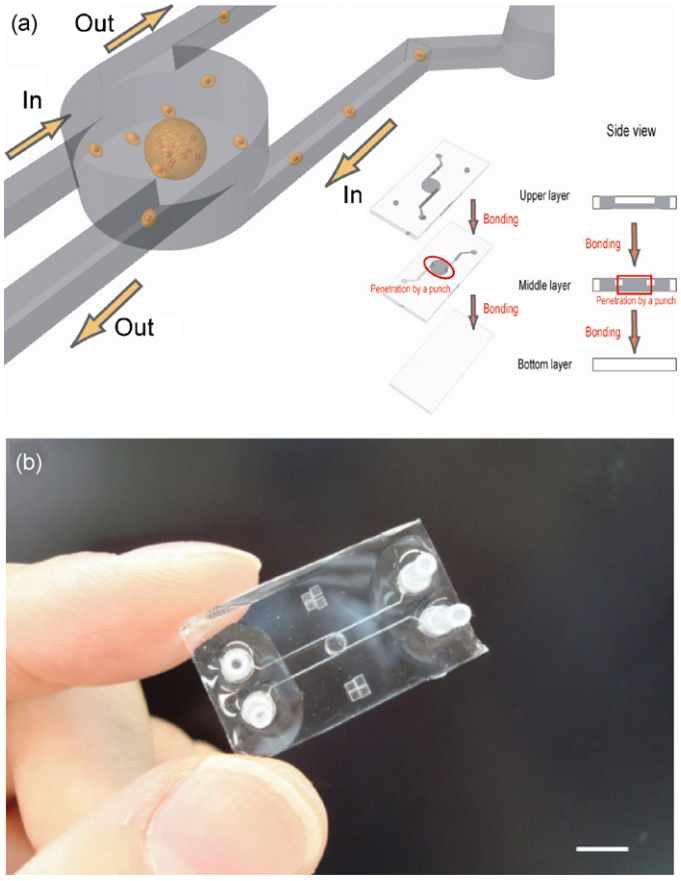

Fig. 1. (a) Schematic diagram of the spheroid-formation chamber fabricated by microlithography. It consists of three layers with two inlet channels tangential to the cylinder in the middle layer and two outlet channels tangential to the cylinder in the upper layer. (b) Photograph of the fabricated device (scale bar: 9 mm).

trolled by altering the cell density of the medium without the need to change the geometry of the device. The device provides a space for spheroids to grow since they are concentrated about the center of the chamber by hydrodynamic forces.

2. Experimental

2.1. Chamber design and fabrication

We used polydimethylsiloxane (PDMS) (Silpot 184 W/C, Dow Corning Corp.) as the device material. PDMS is commonly used as an artificial construct to mimic biological structures due to its low cost, manufacturability, and superior material properties such as high gas permeability and excellent biocompatibility [20]. Leclerc et al. were able to achieve enhanced hepatic functions by supplying sufficient oxygen from a layer sandwiched between PDMS chambers [21]. Our device supplies oxygen to the cells by the circulating culture medium as well as by diffusion through the PDMS walls.

The microchamber we designed (see Fig. 1) is a circular cylinder with two inlet channels that are tangential to the cylinder at the bottom and two outlet channels at the top. Micro-rotational flow is generated by fluid flowing from the two inlet channels. The chamber was formed by the following photolithographic process. First, a negative photoresist SU-8 (SU-8 10, MicroChem Corp.) was patterned to produce circular cylinders and channels with chamber geometries on a clean glass slide by photolithography. We used two asymmetric geometries for the molds. Degassed liquid PDMS was poured over the SU-8 mold to form a chamber and two channels, as shown in Fig. 1. The PDMS structures were cured on a hotplate at

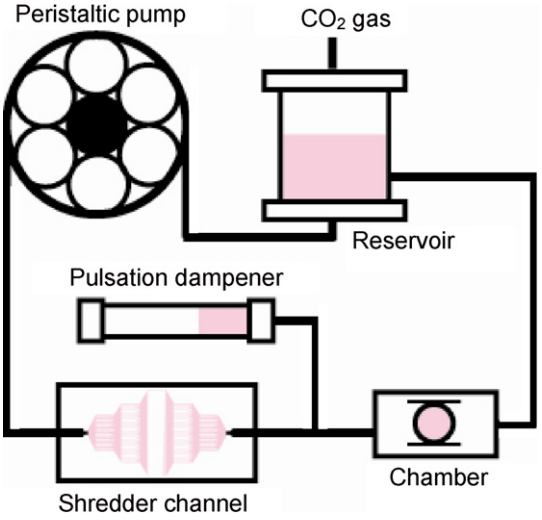

Fig. 2. Schematic diagram of the spheroid-formation device consisting of a PDMS microchamber and a perfusion system with a reservoir, a shredder channel, a pulsation dampener and a peristaltic pump.

65 °C for 6 h and then peeled off from the mold. The inlets and outlets in the upper layer and the inlets and the chamber in the middle layer were formed by a punch. After exposing the bonding surfaces of the PDMS structures to oxygen plasma, the upper and middle layers and the bottom cover were aligned and bonded. The channels were designed to be 100 μm in width and 100 μm in height. The chambers were 2 mm in height and 2–4 mm in diameter.

2.2. Cell culture and preparation

Human hepatocellular liver carcinoma cell line (HepG2) (DS Pharma Biomedical Co., Ltd.) was grown in minimum essential medium supplemented with 10% FBS (DS Pharma Biomedical Co., Ltd.) and 2 mM glutamic acid, 2 mM pyruvic acid and 1% non-essential amino acid (DS Pharma Biomedical Co., Ltd.) at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. Cells cultured in a 75 cm^2 culture flask were detached by ethylenediaminetetraacetic acid (EDTA) (DS Pharma Biomedical Co., Ltd.) and trypsin (DS Pharma Biomedical Co., Ltd.) and were subsequently cultured for 30 min in a medium containing 1000 PU/ml dispase (Sanko Junyaku Co., Ltd.). The cell densities were adjusted after collecting cells by centrifugation before performing experiments with them.

2.3. Set-up of the perfusion system

The three-dimensional spheroid-forming device needs to provide the rotating spheroid with a steady flow of cell suspension for a sufficiently long time [21]. This is challenging since both the culture medium and cells circulate in the system.

We developed an original perfusion system that consists of a peristaltic pump (SJ-1220, Atto Corp.), a reservoir, a pulsation dampener and a shredder channel to provide a steady flow of cell culture medium containing HepG2 to a rotating spheroid over a long time (see Fig. 2). The components in the system are described below:

2.3.1. Pump

The pump in the perfusion system needs to exchange old medium with fresh medium, prevent cell damage and maintain the desired flow speed so that micro-rotational flow is generated in the chamber. Several perfusion systems have been developed that can culture cells by perfusing only the culture medium by a syringe pump [22], a stir bar [23] and a peristaltic pump [20]. A

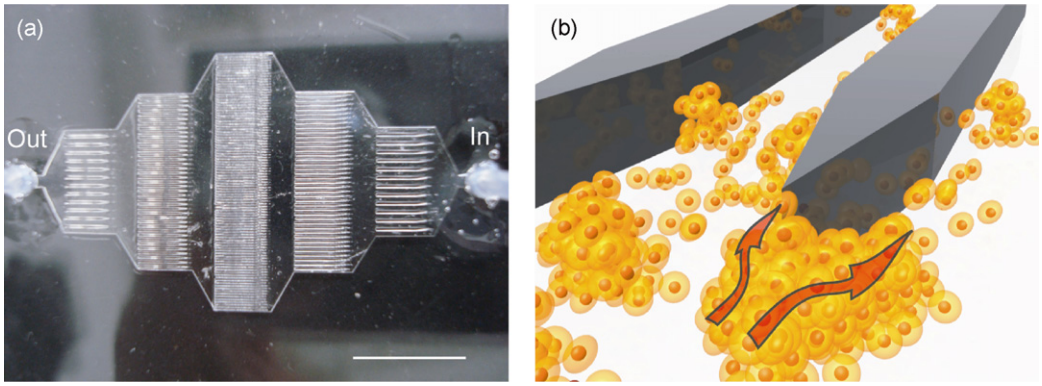

Fig. 3. (a) Photograph of the shredder channel fabricated by microlithography. The narrowest channel is 80 μm wide in the middle section (scale bar: 1 cm). (b) Large aggregations of cells are broken up by shear stress and/or trapped in the channel when aggregations of cells flow in the channel. This is to prevent the inlet and outlet channels of the chamber becoming clogged.

syringe pump is unable to hold enough medium for long-term cell culture nor can it exchange old medium with fresh medium. A stir bar may damage cells because it makes physical contact with cells and it cannot achieve a sufficiently high flow rate to generate micro-rotational flow in the chamber [23]. In terms of minimizing cell damage and achieving the desired flow speed, a peristaltic pump is the most suitable for our purposes.

2.3.2. Dampener

A peristaltic pump generates a pulsatile flow since it forces the liquid being pumped out of the tube by intermittently compressing the tube [24]. To prevent pulsatile flow, we connected a pulsation dampener to a peristaltic pump (see Fig. 2). An air pocket trapped in the dampener absorbs pulsations from the pump.

2.3.3. Reservoir

A small-volume (5 ml) tank was developed to ensure that the perfusion system was compact. The cells and culture medium to be circulated in the system are stored in the reservoir. Reagents that dye or chemically stimulate cells are supplied to the system from the reservoir. It has an outlet at its base to prevent cells from residing too long in the reservoir; otherwise cells will aggregate and form undesired spheroids in the reservoir.

2.3.4. Shredder channels

Occasionally cells aggregate and form spheroids in the tubes and reservoir. This can clog the channels and cause unstable perfusion flow. Spheroids either did not form at all or when they did form they did not remain in the chamber. This was because large cell aggregations clogged the channels and rendered the micro-rotation flow unstable. We used shredder channels to prevent large cell aggregates from entering the chamber (see Fig. 3). The narrowest channel was designed to be 80 μm in the central section, since the width of these channels needed to be narrower than the inlet and outlet channels (=100 μm) in the chamber to prevent large cell aggregations from entering the inlet and outlet channels. The shredder channels either break aggregates up by shear stress or, when the binding force of an aggregate is too strong to break the aggregate up, the aggregate becomes trapped in one of the channels.

2.3.5. Connections and tubes

The system requires a high-density cell suspension to form spheroids in the chamber. However, we observed that some cells settled to the bottom of tubes while circulating so that the cell density of the suspension decreased as perfusion of the culture continued. By downsizing the perfusion system and using narrow (1 mm diameter) tubes, we were able to maintain a constant cell density.

2.3.6. Cameras

Video images were acquired using a CCD camera (MTF-DFK, Nippon Roper Co., Ltd.) and stationary and fluorescence images were obtained by a CCD camera (Cool SNAP-cf, Nippon Roper Co., Ltd).

2.4. Live/dead cell viability assay

A spheriod that had formed was left to rotate for one day and it was then stained by 4 μM calcein–acetoxymethyl ester (AM) and 8 μM ethidium homodimer-1 (EthD-1) by exchanging the circulating medium with a medium that contained these two dyes. This was done to detect living cells.

2.5. Micro particle image velocimetry system for flow structure analysis

Fluorescent polystyrene particles with a diameter of 2.0 μm (G0200, Moritex Corp.) were used as tracer particles. They had peaks in the absorption and emission spectra of 505 and 515 nm, respectively.

Cells detached by EDTA and trypsin were cultured for 30 min in a medium containing 1000 PU/ml dispase (Sanko Junyaku Co., Ltd.). The cell density was adjusted to 690×10^4 cells/ml after collecting the cells by centrifugation. We used a 3-mm-diameter chamber for this experiment. Fluorescent particles were then added to this cell suspension at a concentration of 650×10^4 particles/ml. The optical system for micro particle image velocimetry (PIV) consisted of an inverted fluorescence microscope, an objective lens (Nikon Corp., Plan Fluor, 4) and a cooled CCD camera (Phantom v 7.1, Vision Research, Inc.). A mercury lamp provided excitation light to the microchannel after the light had passed through an excitation filter (450–490 nm) and a dichroic filter that reflects wavelengths below 505 nm. Particle images were captured by the CCD camera through the objective lens after the light had passed through an emission filter that transmits wavelengths longer than 520 nm. Velocity vectors were calculated from the particle images, and the ensemble averaging technique was applied to 100 instantaneous velocity vectors to reduce measurement errors.

3. Results and discussion

3.1. Spheroid formation

When the medium was introduced into the chamber at a volumetric flow rate of 1.1 ml/min, micro-rotational flow was observed throughout the chamber (see Fig. 4(a)). The volumetric flow rate

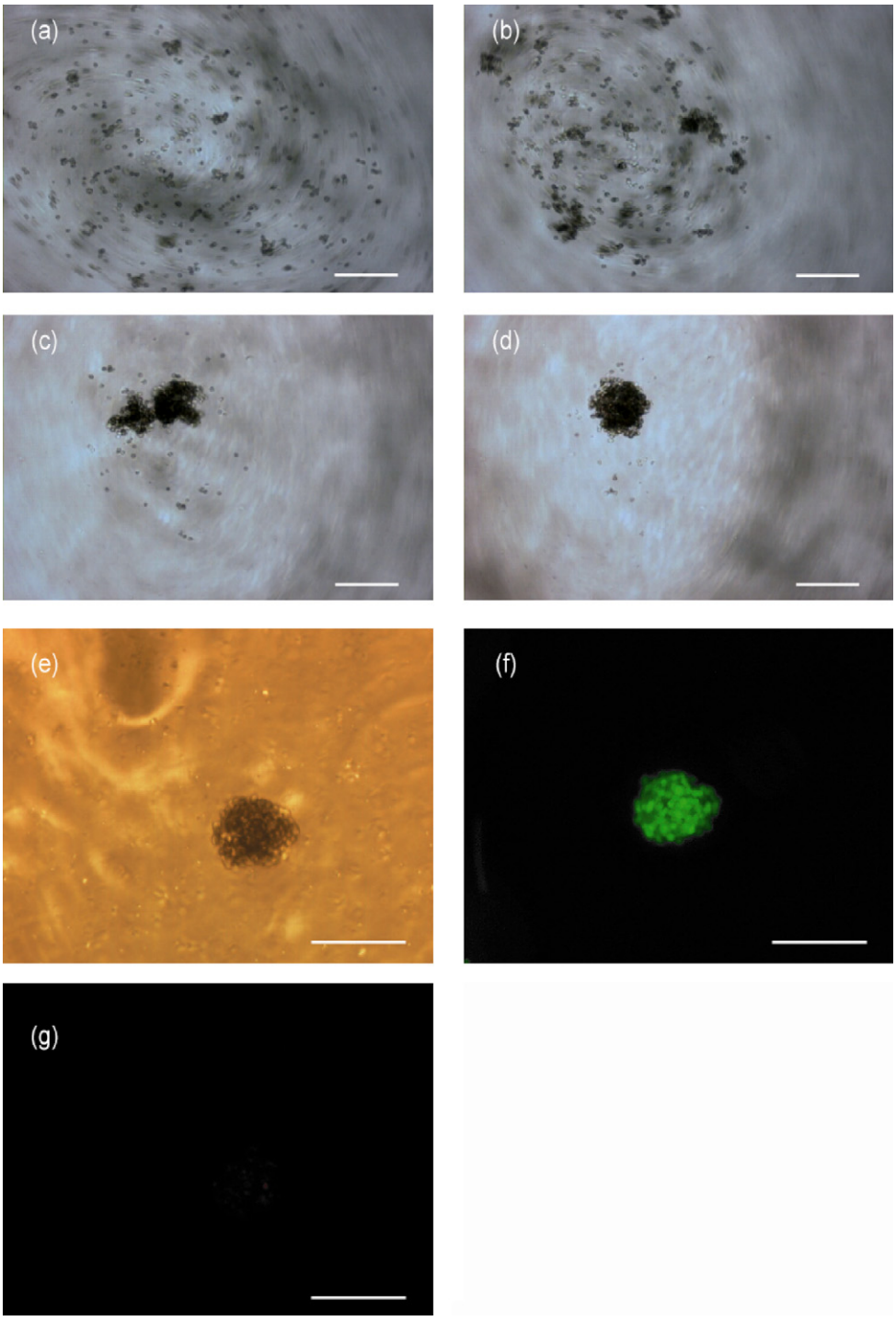

Fig. 4. (a–(d) Process of cell aggregation in the chamber. Cells rotated around the entire area of the chamber stably at a flow rate greater than 1.1 ml/min at (a) 0 s. When the flow rate was reduced to 0.4 ml/min, they started to aggregate in the center of the chamber and formed a spheroid after 120 s. (b), (c) and (d) show the aggregation of cells after (b) 30 s (c) 60 s and (d) 120 s, respectively. After culture for 24 h, the spheroid was stained with calcein AM and EthD-1 to verify the viability of the cells. (e), (f) and (g) respectively show an optical image and fluorescence images of calcein AM and EthD-1 (scale bar: 200 μm).

was then reduced until a small region, in which the velocity was low compared to other regions, was formed at the center of the chamber (see Fig. 4(b)). In our experiments, the volumetric flow rate at which spheroids were formed was approximately 0.4 ± 0.05 ml/min. The flow rate varied between individual devices; we conjecture that this is due to manufacturing variations, especially those in the assembly of the inlets and outlets.

The cells gradually aggregated and formed spheroids over approximately 120 s. These spheroids remained in the chamber and continued to spin (see Figs. 4(c) and (d)). The developed perfusion system provided stable flow to the chamber during the experiments and produced a long-lived culture (>1 day). Fig. 4(e)–(g) respectively show an optical image and fluorescence images of a formed spheroid stained with calcein AM and EthD-1 after one day. No fluorescence was observed from the EthD-1 in the cells, but fluorescence was observed from the calcein AM (compare Fig. 4(f) and (g)). These images demonstrate that cells in the spheroid lived in the chamber for one day.

The center region where the spheroid was formed was confined by micro-rotational flow and no cells flowed into this region from outside. Consequently, the spheroid size is determined by the number of cells in the confined region, which is given by the product of the cell density and the volume of the region, which is in turn determined by the chamber diameter.

Fig. 5 shows the spheroid diameter as a function of the cell density and chamber diameter. We could consistently control the diameter of the spheroids in the range 130–430 μm and the ratio of the standard deviation to the mean was less than 17.2%. Spheroids with diameters ranging from 145 to 432 μm were successfully formed in a 4-mm-diameter chamber by varying only the cell density from 6.6% to 12.0%. The ability to control the size of spheroids without altering the design of the device is highly beneficial for users. Spheroids could not be formed in chambers that were less than 2 mm in diameter, possibly due to the low throughput or volume. A cell density of greater than 480×10^4 cells/ml was necessary to form spheroids in a 2.5-mm-diameter chamber while a cell density of greater than 280×10^4 cells/ml was necessary for a 3.0-mm-diameter chamber. The standard deviation was within 13.2% for spheroids with diameters in the range 150–200 μm, which has been reported to be the optimum range for spheroid sizes [4].

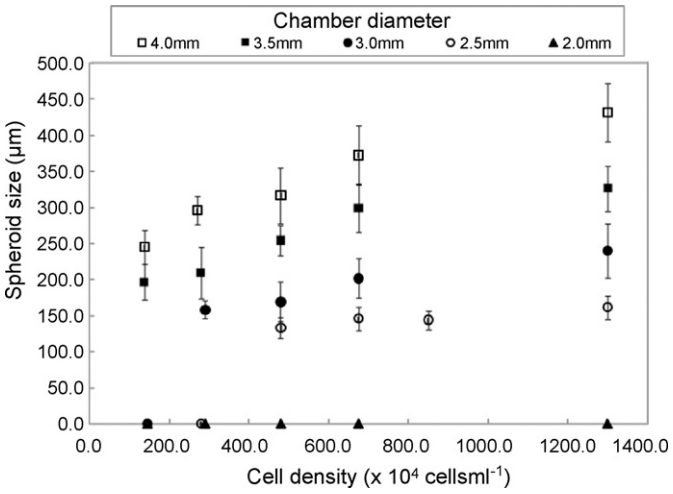

Fig. 5. Spheroid sizes as a function of cell density for five different chamber diameters.

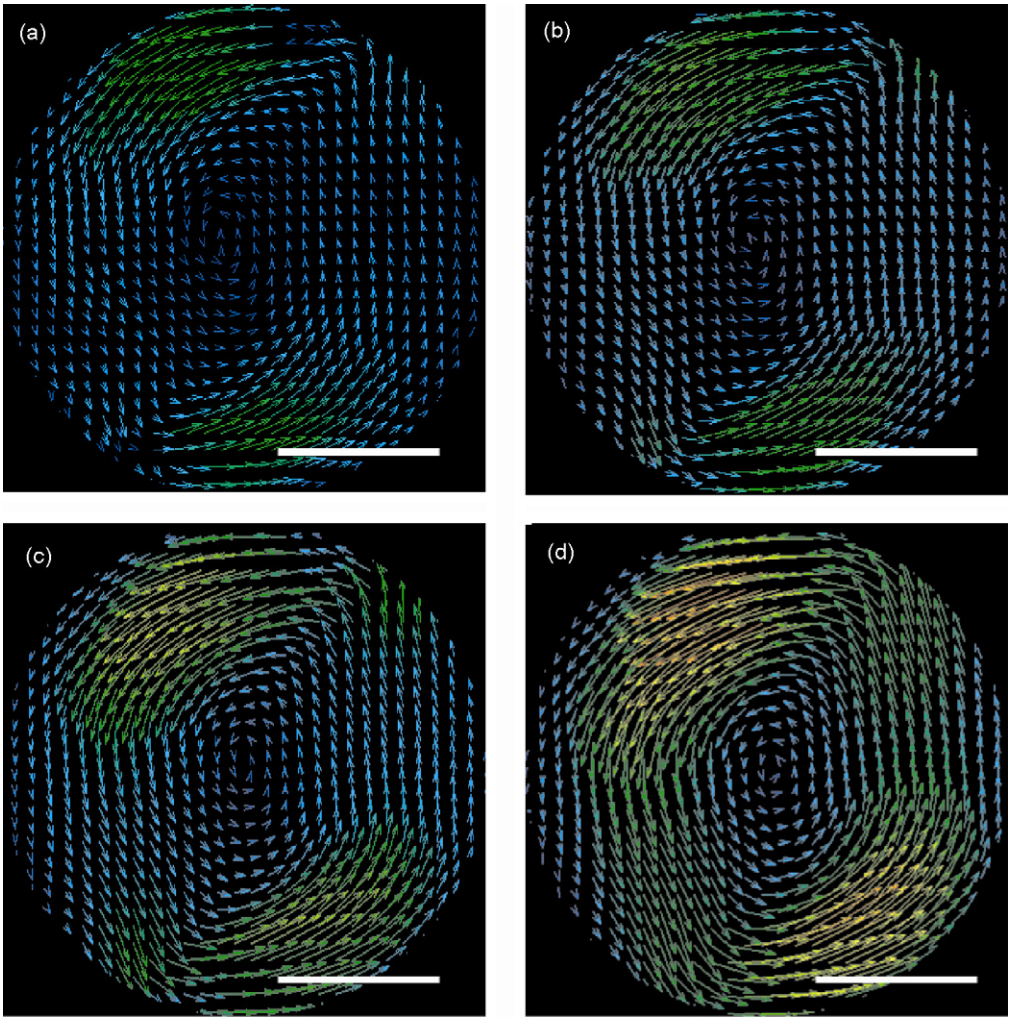

Fig. 6. Velocity vector distribution obtained by micro PIV in the chamber in which a three-dimensional spheroid is rotating when the flow rate was 0.4 ml/min at distances of (a) 10 μm, (b) 50 μm, (c) 100 μm and (d) 200 μm from the base of the chamber (scale bar: 1 mm).

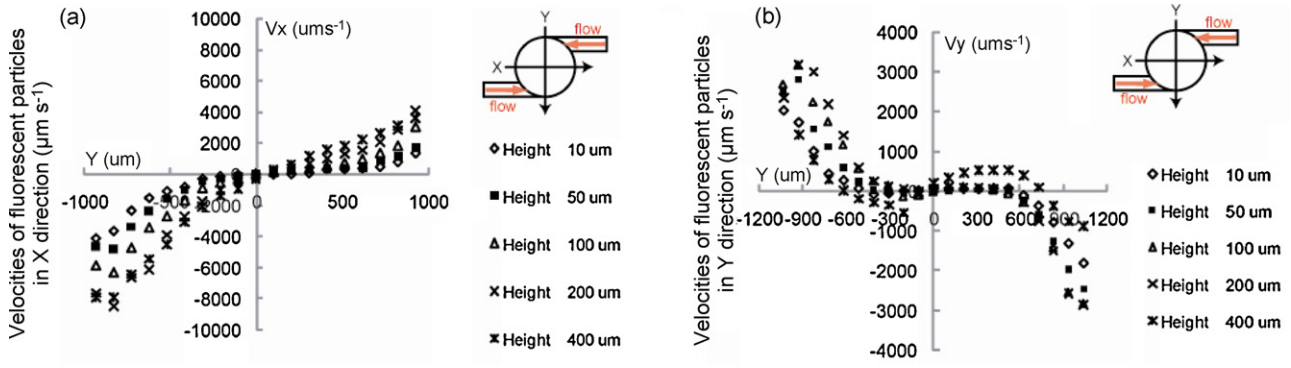

Fig. 7. (a) Flow velocities in the *x*-axis direction as a function of distance along the *y*-axis crossing the center of the chamber at 0.4 ml/min at heights between 10 μm and 400 μm. The origin represents the center of the chamber. The positive direction of the *x*-axis represents the direction to the right of the horizontal axis in the images of the velocity vector distributions in the chamber. The positive direction of the *y*-axis represents the down direction of the vertical axis in the images. (b) Flow velocities in the *y*-axis direction with distance along the *y*-axis crossing the center of the chamber at 0.4 ml/min at heights between 10 μm and 400 μm. The origin and axes of the graph are the same as those in (a).

3.2. Measurement of flow velocity vectors in the chamber

We measured velocity vectors in a 3.0-mm-diameter chamber by micro PIV at a volumetric flow rate of 0.4 ml/min when a three-dimensional spheroid rotated in the chamber. Fig. 6(a)–(d) show the micro-rotational flows generated in the chamber at heights of 10, 50, 100 and 200 μm from the base of the chamber, respectively. Fig. 6(c) and (d) clearly reveal confined regions of about 1 mm in diameter in the center of the chamber (where the flow velocity is much lower than in the outer regions). Fig. 7(a) and (b) respectively show flow velocities in the *x* and *y*-axis directions as functions of distance along the *y*-axis. Fig. 7(a) indicates that the flow velocity parallel to the *x*-axis was greater at the edge of the chamber than at the center and that micro-rotational flow was generated in the chamber. Fig. 7(b) shows that the flow velocity parallel to the *y*-axis was almost zero for $y = \pm 500$ μm and at heights below 200 μm. This indicates that the confined region was formed below a height of 200 μm from the base of the chamber. Micro PIV verified that micro-rotational flow is generated in the chamber and that the confined region has approximate dimensions of ϕ1000 μm × 200 μm. Given that the HepG2 cells are 10–15 μm in size, the number of cells in the spheroid was estimated to be approximately 1000. Under these conditions, the spheroid formed was 180 μm in diameter. The filling rate of cells in the spheroid is estimated to be about 50%. Since spheroids have been reported to contain some gaps [25], this estimated filling rate appears to be reasonable.

4. Conclusion

In this study, we developed a lab-on-a-chip that is capable of forming 3-D spheroids hydrodynamically with good size controllability. The perfusion system used consists of a reservoir, a pulsation dampener, a shredder channel and a peristaltic pump and it enabled long-term culture of a spheroid for periods of over one day. A live/dead viability assay verified that almost all the cells in the spheroid (<180 μm) were alive. The diameter of hepatic spheroids can be controlled in the range 130–430 μm (150–200 μm) with a standard deviation of less than 17.2% (13.2%) by varying the chamber diameter and cell density. Spheroids with diameters ranging from 245 to 432 μm were created by the same device by varying only the cell density, demonstrating the advantages of the proposed device. Micro PIV revealed hydrodynamically confined regions where a spheroid forms and resides and enabled the number of cells in a spheroid to be estimated. This device has the potential to be a technological innovation in biology by forming a bridge between cell research and tissue research.

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References

[1] M.Y. Zhang, P.J. Lee, P.J. Hung, T. Johnson, L.P. Lee, M.R. Mofrad, Biomed. Microdevices 10 (2008) 117–121.
[2] J. Fukuda, K. Okamura, K. Nakazawa, H. Ijima, Y. Yamashita, M. Shimada, K. Shirabe, E. Tsujita, K. Sugimachi, K. Funatsu, Cell Transplant. 12 (2003) 51–58.
[3] L.G. Griffith, M.A. Swartz, Nat. Rev. Mol. Cell Biol. 7 (2006) 211–224.
[4] E. Curcio, S. Salerno, G. Barbieri, L. De Bartolo, E. Drioli, A. Bader, Biomaterials 28 (2007) 5487–5497.
[5] J. Alvarez-Pérez, P. Ballesteros, S. Cerdán, MAGMA 18 (2005) 293–301.
[6] W. Mueller-Klieser, Biophys. J. 46 (1984) 343–348.
[7] S.L. Nyberg, E.S. Baskin-Bey, W. Kremers, M. Prieto, M.L. Henry, M.D. Stegall, Liver Transplantation 11 (2005) 901–910.
[8] M. Wartenberg, F. Dönmez, F.C. Ling, H. Acker, J. Hescheler, H. Sauer, FASEB J. 15 (2001) 995–1005.
[9] M. Ingram, G.B. Techy, R. Saroufeem, O. Yazan, K.S. Narayan, T.J. Goodwin, G.F. Spaulding, In Vitro Cell Dev. Biol. Anim. 33 (1997) 459–466.
[10] K. Monastyrskaya, E.B. Babiychuk, J.C. Schittny, U. Rescher, V. Gerke, H.G. Mannherz, A. Draeger, Cell Mol. Life Sci. 60 (2003) 2702–2709.
[11] G.A. Thouas, J. Sheridan, K. Hourigan, J. Biomed. Biotechnol. 9 (2007) 32754.
[12] M. Miyazawa, T. Torii, Y. Toshimitsu, K. Okada, I. Koyama, J. Gastroenterol. Hepatol. 22 (2007) 1959–1964.
[13] J.P. Shelby, D.S. Lim, J.S. Kuo, D.T. Chiu, Nature 425 (2003) 38.
[14] J.M. Karp, J. Yeh, G. Eng, J. Fukuda, J. Blumling, K.Y. Suh, J. Cheng, A. Mahdavi, J. Borenstein, R. Langer, A. Khademhosseini, Lab Chip 7 (2007) 786–794.
[15] L.Y. Wu, D. Di Carlo, L.P. Lee, Biomed. Microdevices 10 (2008) 197–202.
[16] H. Otsuka, A. Hirano, Y. Nagasaki, T. Okano, Y. Horiike, K. Kataoka, ChemBioChem. 5 (2004) 850–855.
[17] S.R. Khetani, S.N. Bhatia, Nat. Biotechnol. 26 (2008) 120–126.
[18] E. Eschbach, S.S. Chatterjee, M. Nöldner, E. Gottwald, H. Dertinger, K.F. Weibezahn, G. Knedlitschek, J. Cell Biochem. 95 (2000) 243–255.
[19] Y.S. Torisawa, B.H. Chueh, D. Huh, P. Ramamurthy, T.M. Roth, K.F. Barald, S. Takayama, Lab Chip 7 (2007) 770–776.
[20] E. Leclerc, Y. Sakai, T. Fujii, Biotechnol. Progr. 20 (2004) 750–755.
[21] R.Z. Lin, L.F. Chou, C.C. Chien, H.Y. Chang, Cell Tissue Res. 324 (2006) 411–422.
[22] J.W. Allen, S.R. Khetani, S.N. Bhatia, Toxicol Sci. 84 (2005) 110–119.
[23] H. Kimura, T. Yamamoto, H. Sakai, Y. Sakai, T. Fujii, Lab Chip 8 (2008) 741–746.
[24] I.L. Garcia, P. Vinas, M.H. Cordoba, J. Anal. At. Spectrom. 9 (1994) 553–561.
[25] J. Fukuda, Y. Sakai, K. Nakazawa, Biomaterials 27 (2006) 1061–1070.

Biographies

Hiroki Ota received Master of Engineering from Keio university Graduate School of Science and Technology in 2007. He belongs to PhD program for School of Integrated Design Engineering, Graduate School of Science and Technology, Keio University. His research interest was abnormal endothelial permeability regulated by RhoA activation in master degree program. Now, He is developing microfluidic platform that can form size-controlled three dimension micro-hepatic spheroids with microfabrication technique in doctor course.

Ryosuke Yamamoto is a graduate student of Faculty of Science and Technology at Keio University. He received his BE in mechanical engineering from Keio University

in 2009. Currently his research focuses on the design and fabrication of MEMS-based cell handling devices.

Koji Deguchi received his BE and ME in mechanical engineering from Keio University in 2007 and 2009, respectively. His master thesis focused on Cell handling with microfluidic. Since 2009 he has been with Fuji Xerox Corporation.

Yuta Tanaka is a graduate student of Faculty of Science and Technology at Keio University. As an undergraduate, he involved in a measurement of wall-zeta potential in the micro fluidic device. He is presently working on a clarification of the relationship between the wall-zeta potential and the electroosmotic flow using simultaneous measurement technique.

Yutaka Kazoe received his BE, ME and PhD degrees from Keio University in 2004, 2006 and 2008, respectively. He has been a research fellow of JSPS and a visiting scholar in Keio University from 2008, and a postdoctoral fellow in Georgia Institute of Technology from 2009. His current researches are as follow: nanoscale laser induced fluorescence imaging, nano- and micron-resolution velocimetries for investigating microchannel flows, and development of nanoparticle separation technique using AC dielectrophoresis.

Yohei Sato is an Associate Professor in the Department of System Design Engineering at Keio University. His current research interests are mainly divided into three parts: nanoscale laser induced fluorescence, optical manipulation of micro-scale objects, and nanoparticle separation on μTAS.

Norihisa Miki received his BE, ME and PhD degrees in mechanical engineering from the University of Tokyo in 1996, 1998 and 2001, respectively. From 2001 to 2004 he was a postdoctroal associate and then a research engineer in the Department of Aeronautics and Astronautics at the Massachusetts Institute of Technology, where he worked on the development of a button-size silicon micro gas turbine engine. In 2004 he joined the Department of Mechanical Engineering at Keio University, Kanagawa, Japan, as an assistant professor. His principal fields of interests include MEMS-based human interface, microfabrication technology, microTAS, bio and chemical sensors, and power MEMS. Prof. Miki is a member of IEEE.