CONTINUOUS MYELOMA CELL CULTURE IN STORAGE CHAMBER BASED ON DROPLET FUSION-DIVISION

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ABSTRACT

This paper reports a continuous cell culture chip that employs droplet microfluidics to encapsulate suspension cells in a droplet and the cell droplet is passively stored in a storage chamber during the long-period cell culture process. The proposed system provides a closed micro-environment for cell culture which has advantages of cell droplet isolation to avoid cross contamination, simple droplet manipulation for fusion-division cycles and easy integration for further cell-based analytical techniques. In the demonstrated experiment, myeloma cells are successfully cultured using the developed integrated droplet cell culture system.

KEYWORDS: Droplet generation, Droplet storage, Cell culture, Fusion-division.

INTRODUCTION

Cell culture on a chip is one of the major area in cell-based Lab-on-chip system. However, most of the developed systems are focused on adherent cell, such as microfluidic cell-culture array with integrated concentration gradient generator [1], integrated microfluidic system using hydrogel [2] or peptide microgel [3] as a 3D scarfold etc. Previously, droplet-based cell culture has been demonstrated for suspended cell [4] but the cells are continuously cultured by droplet fusion-division process without being able to track the generation of cell batches. In addition, cell droplets are stored in reservoir which have the possibilities for droplet fusion and contamination. To avoid these, we proposed an integrated microfluidic system in which cell droplets are passively stored in the microchip [5] for long-period cell culture. The proposed system provides a closed micro-environment for cell culture which has advantages of cell droplet issolation to avoid cross contamination, simple droplet manipulation for fusion-division cycles and easy integration for further cell-based analytical techniques.

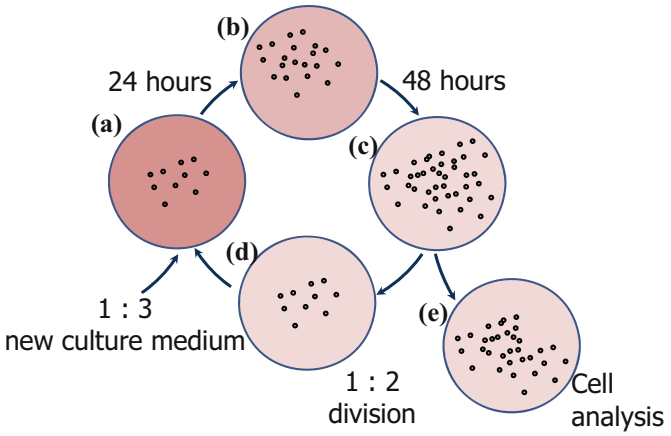


Figure 1: Schematic illustration of cell culture protocol for myelomas.

Figure 1 shows the conventional cell culture protocol for myelomas (P3X63Ag8.653). Initially, myelomas (2×10^5 cells/ml) are seeded in the flask and stored in 5% CO_2 at 37°C for 2 days (Fig. 1a-c). The myelomas are split in 1 : 2, in which the 1st part of the myelomas are added with new culture medium for subculture (Fig. 1d) and the 2nd part of the myelomas are extracted for cell analysis (Fig. 1e). In the droplet-based cell culture system, the complete cell culture protocol are integrated which consists of (1) the storage of cell droplet, (2) the replenishment

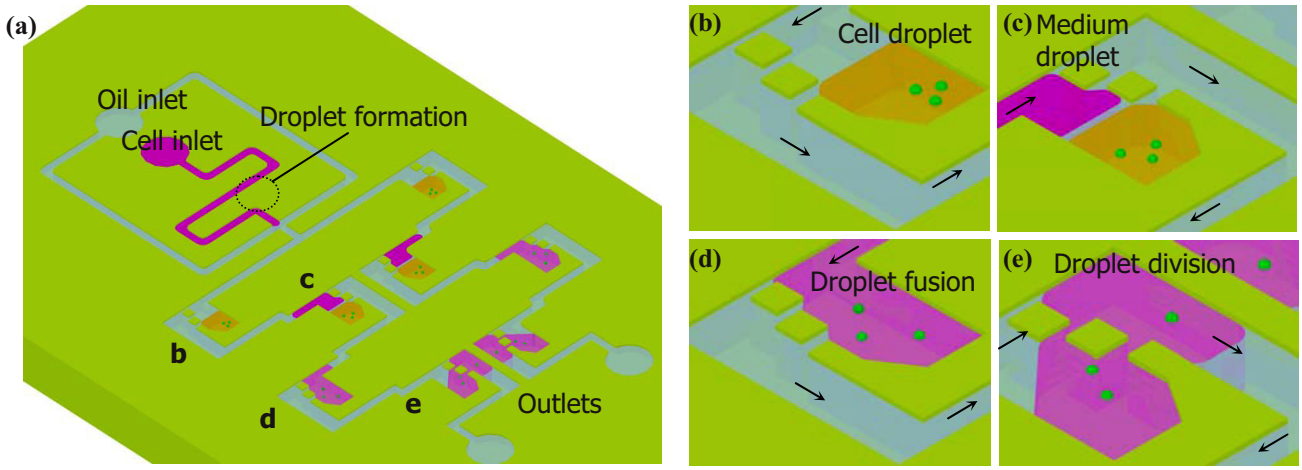


Figure 2: Schematic illustrations of (a) the droplet-based cell culture micro-array system, and (b-e) droplet fusion-division cycle.

of culture medium, (3) the division of droplet with cultured cells, and (4) the repeating steps for subsequent culture cycle.

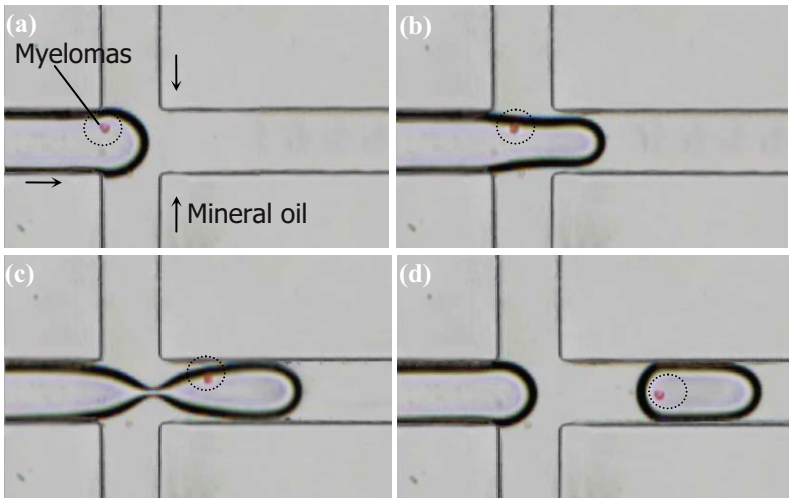


Figure 3: Microphotos show the encapsulation of single myeloma (colored in red) in a medium droplet.

DESIGN AND FABRICATION

Figure 2 shows the schematic illustration of the proposed microfluidic system which has an array of storage chambers. Myelomas are encapsulated in a culture medium droplet by mineral oil (1% SPAN 80) using flow focusing mechanism. Cell droplets are stored in the storage chamber (Fig. 2b). When the myelomas are ready to be split, a new medium droplet is flow to the storage chamber. The new medium droplet filled the chamber and merged with the cell droplet (Fig. 2c and 2d). The merged droplets are split by the flow (Fig. 2e) and restored to the original size. As a result, the replenishment of culture medium and the splitting of cells are performed concurrently.

The microfluidic chip was fabricated in polydimethylsiloxane (PDMS) by using a standard soft lithography technique. Unlike normal chip application, the fabricated chip has to be further processed to ensure its biocompatibiltiy for cell culture. After being fabricated, the microchannel is injected with 70% ethanol and the whole device is immersed in the ethanol solution for one hour. Subsequently, the chip is put into a Class II biosafety cabinet under UV light for 24 hours. Then, cell culture medium is introduced into the microchannel and the chip is placed into the incubator for 1 hours before it it ready for cell seeding.

EXPERIMENTAL RESULTS AND DISCUSSIONS

Figure 3 illustrates the encapsulation of myelomas in medium-in-mineral oil droplet by flow-focusing mechanism. As the myeloma flow is emerged into the flow-focusing junction, the myeloma flow is pinched by the two side slows of mineral oil. Subsequently, a culture medium droplet with suspended myelomas is formed as shown in Fig. 3d.

Figure 4 shows the droplet storage mechanism. When the medium droplet enters the storage chamber, the flow becomes slower and the chamber is filled with the culture medium as shown in Fig. 4a. The cell culture medium prepared consists of RPMI 1640 with 10% FBS, 1% penicillin/streptomycin, 1% sodium pyruvate, 1% L-Glutamine and 1% non-essential amino acid (Invitrogen). When the whole chamber is filled with the culture medium as shown in Fig. 4b, the mineral oil flow is blocked by the medium droplet and pressure is built up. The medium droplet is split when the pressure is substantially high as shown in Fig. 4c. Part of the medium droplet is trapped in the storage chamber as shown in Fig. 4d. Therefore, encapsulated myeloma cells can be incubated in the storage chamber to monitor its cell division cycle and new medium droplet can be injected into the storage chamber during the cell culture process.

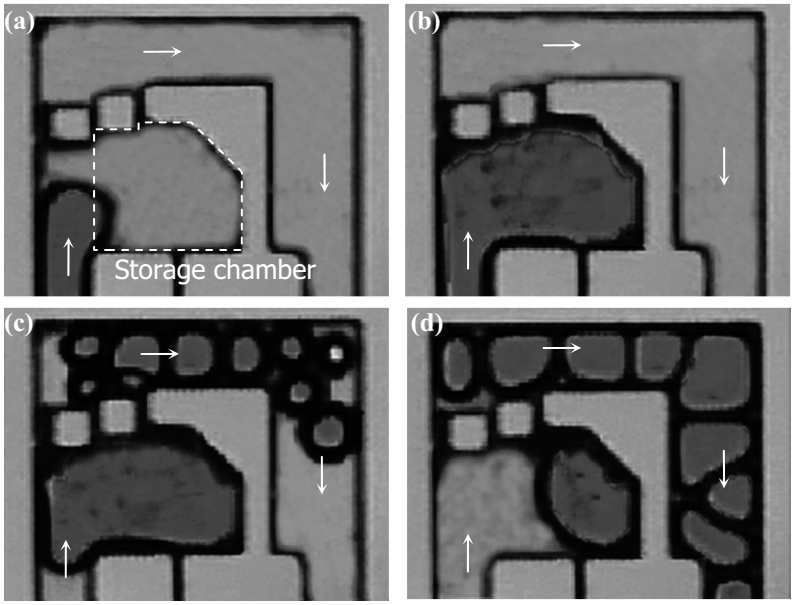


Figure 4: Microphotos show the medium droplet (darkened) being (a) injected into the storage chamber, (b) fully filled the chamber, (c) forced through the narrow passages and split into smaller droplets, and (d) trapped in the chamber.

Figure 5 shows the fusion-division cycle of the cell culture medium droplet with the myeloma cell droplet. First, myeloma cell droplet is stored in the storage chamber after the cells are encapsulated in a medium-in-mineral oil droplet as shown in Fig. 5a. After the culture medium has been consumed by the myeloma cells, a new medium droplet is injected into the microchannel. The new medium droplet enters and fills the storage chamber as shown in Fig. 5b. Subsequently, the medium droplet and the myeloma cell droplet are merged as shown in Fig. 5c. Since the merged medium and myeloma cell droplet is blocking the flow in the microchannel, pressure is building up. When the built up pressure is substantially high, the merged droplet is started to split as shown in Fig. 5d.

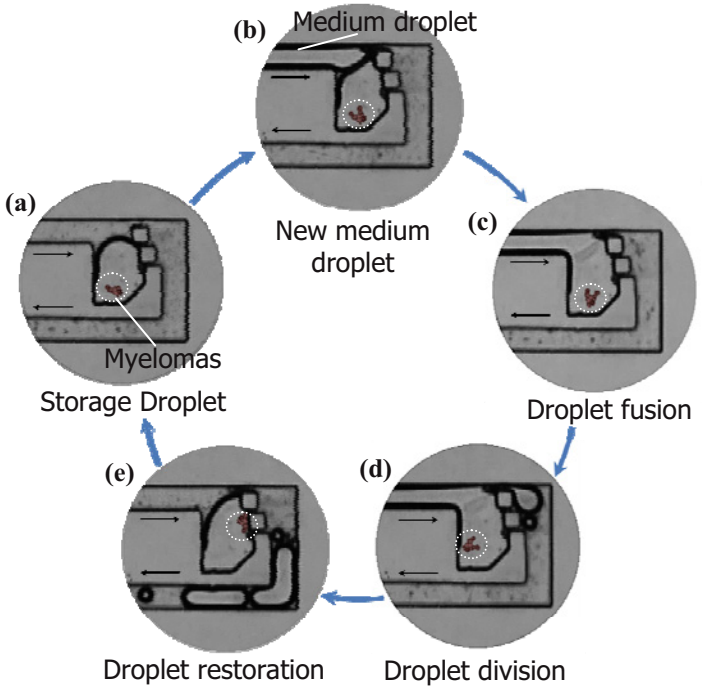
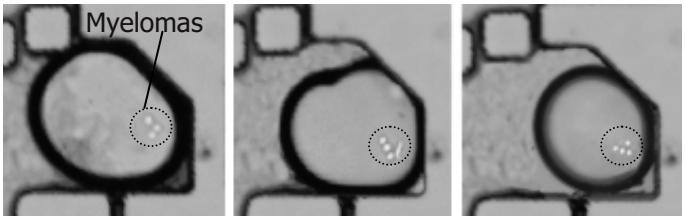


Figure 5: Microphotos of the cell culture cycle in the storage chamber. (a) Myeloma (colored in red) encapsulated droplet stored in the storage chamber, (b) new medium droplet is injected into the chamber, (c) droplet fusion occurs, (d) droplet division occurs, and (e) the myeloma cell droplet is restored to the original size.

The merged droplet is split until it is restored to the original size as shown in Fig. 5e.

Figure 6 shows the division of myeloma cell after it has been stored in the storage chamber for 20 hrs. A myeloma is successfully undergone cell division after 20 hrs of incubation.



(a) t = 0 **(b) t = 19 hrs** **(c) t = 20 hrs**

Figure 6: Microphotos show the division of myelomas in the storage droplet with (a) 3 myelomas, (b) one of the myeloma undergoes cell division, and (c) 4 myelomas.

CONCLUSIONS

In conclusion, a droplet-based continuous myeloma cell culture system is developed in which cell droplets are stored in the storage chamber. The replenishment of culture medium and the splitting of cultured cells are performed by the droplet fusion-division process. In future, cultured cells can be delivered to the downstream with integrated analytical techniques for cell analyses or drug screening.

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REFERENCES

[1] P. J. Hung, P. J. Lee, P. Sabounchi, R. Lin and L. P. Lee, Continuous perfusion microfluidic cell culture array for high-throughput cell-based assays, Biotechnology and Bioengineering, **89**, 1 (2005).

[2] J. Flueckiger and K. C. Cheung, Integrated microfluidic systems for cell culture and characterization, IEEE 14th International Mixed-Signals, Sensors and Systems Test Workshop, Canada, 1 (2008).

[3] Y. Tsuda, Y. Morimoto and S. Takeuchi, Monodisperse cell-encapsulating peptide microgel beads for 3D cell culture, Langmuir, **26**, 2645 (2010).

[4] T. Ichii, H. Suzuki and T. Yomo, Droplet-based continuous cell culture system by repeating fusion-division cycles, The 13th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS), South Korea, 558 (2009).

[5] H. Boukellal, S. Selimović, Y. Jia, G. Cristobal and S. Fraden, Simple, robust storage drops and fluids in a microfluidic device, Lab on a Chip, **9**, 331 (2009).

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