

GRAVITY-ORIENTED MICROFLUIDIC DEVICE FOR CELL SPHEROID FORMATION

Kangsun Lee¹, Choong Kim², Jaehoon Bang², Youngeun Kim², Seungha Lee²,
Byungwook Ahn¹, Ji Yoon Kang², and Kwang W. Oh^{1*}

¹SMALL Lab, University at Buffalo, The State University of New York (SUNY at Buffalo), Buffalo, NY, USA

²Nano-Bio Research Center, Korea Institute of Science and Technology (KIST), Korea

ABSTRACT

In this study, we propose a simple method to form cell spheroids in a single-layered microfluidic device by employing gravity force. Simple device fabrication and straightforward loading of cells with manual pipetting facilitate the formation of 3D spheroids. To demonstrate the simple and effective method, two cell lines of MCF-7 cell (human breast adenocarcinoma cell line) and P-19 EC cell (embryonal carcinoma cell line) were applied to form 3D cell spheroids with the proposed gravity-oriented PDMS microfluidic device. With the model cell lines, uniform and well-conditioned cell spheroids were formed. Thus, we expect the proposed method will give valuable chances to study 3D cell culture models.

KEYWORDS: Gravity oriented microfluidic device, 3D spheroid, embryonic body (EB), cell cytotoxicity

INTRODUCTION

Formation of 3D cell spheroids is an essential tool to study or mimic *in-vivo* environment, specifically for embryonic body (EB) and cancer stem cells. 3D cell spheroids have been formed on a micromachined PEG (Poly ethylene glycol) microwell array [1] or in a complex microfluidic device with a membrane having porous structure [2]. In this study, we present a simple gravity-oriented microfluidic device for the 3D cell spheroid formation. The advantage of this method is multifold, including easy fabrication due to a single-layered device design, pipette-based straightforward sample loading, and efficient geometric locking for the formed spheroids. All these are useful for post-processes such as chemical treatments or co-culturing (heterogeneous culturing) without loss of the spheroids. Moreover, a massive number of cell spheroids can be formed by an extended parallel microchannel configuration. Using the proposed method, we have successfully formed uniform and well-conditioned cell spheroids with two model cell lines.

WORKING PRINCIPLE / DESIGN RULE

Fig. 1 shows the schematic of the proposed method. First, cells were loaded into a main microchannel coated with BSA (bovine serum albumin), to prevent cell attachment on the surface, using manual pipetting. After cell loading, the device was lifted and positioned vertically, which can trap the loaded cells into each pocket by gravity force. After 10-min incubation, most of cells were settled into the pocket wells. In the design of the proposed device, round-shaped side walls alleviated the attachment of cells onto the wall surface. Moreover, they enhanced clustering of the sediment cells at one point in the pocket. The cells remaining in the main microchannel were flushed out with buffer medium and the cells in the pocket were not affected due to their isolated structure. After one day, the cell spheroids were well formed in the pocket. The neck structures play a role in preventing the release of the cell spheroids into the main microchannel, which can be helpful in monitoring the behavior of the cell spheroids, and further staining for immunological analysis.

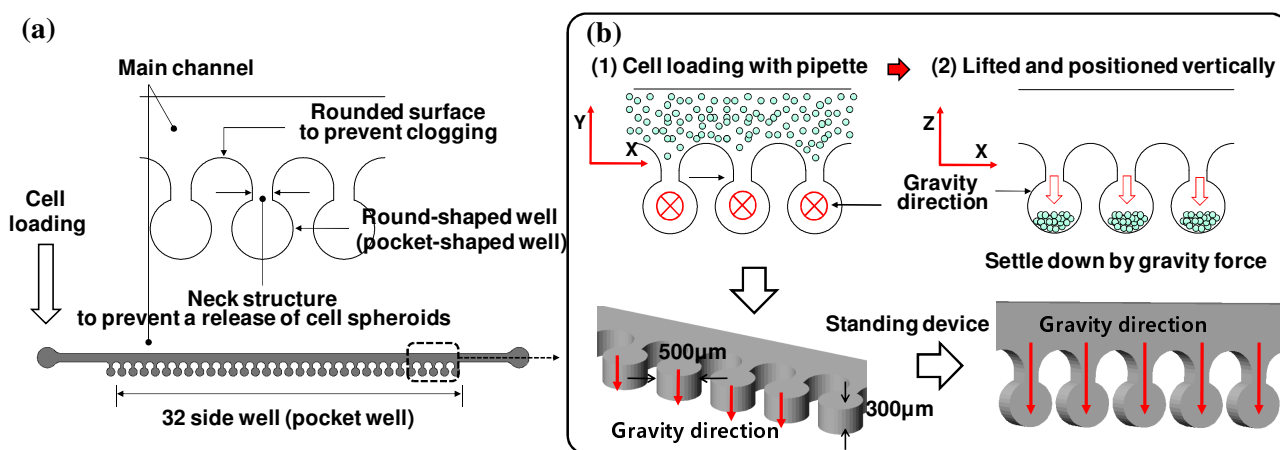


Fig. 1 The proposed method to form cell spheroids in a single-layered PDMS device by employing gravity force. (a) The geometry of the proposed device that includes pocket wells and neck structures, and (b) the protocol for cell loading and spheroid formation.

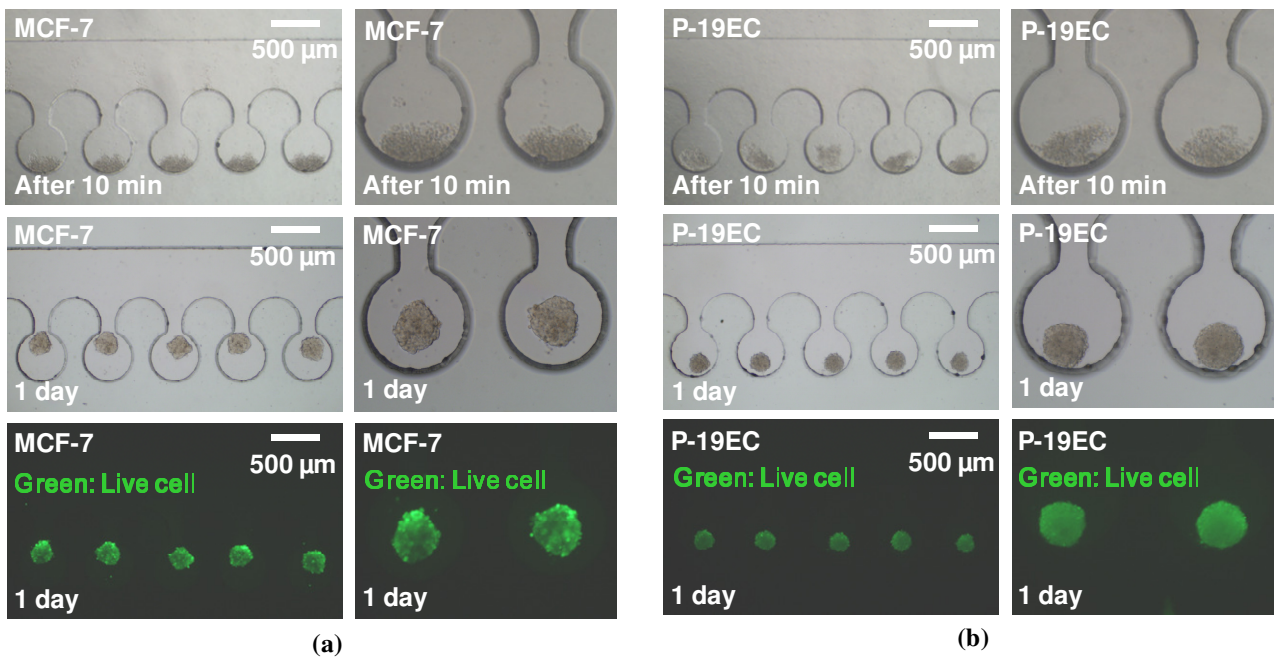


Fig. 2 The morphologies of the loaded cells and their spheroids after 10-min and 1-day, and the fluorescence images of immuno-staining for cell viability. (a) MCF-7 cancer cell (human breast adenocarcinoma cell line) and (b) P-19EC cell (embryonal carcinoma cell line).

EXPERIMENTAL

The proposed PDMS device was fabricated by a conventional soft-lithography method. A master mold was patterned with SU-8 photoresist to form the desirable patterns, as shown in Fig. 1. Using the SU-8 mold, a PDMS layer was molded and sterilized by an autoclaving process. The autoclaved, patterned PDMS layer was irreversibly bonded to a flat PDMS substrate. Next, the microchannel was filled with a solution of 3% BSA to prevent the attachment of the cells on the microchannel. After overnight incubation, the microchannel was flushed out by medium for MCF-7 and P-19EC cells. Cells (MCF-7 and P-19EC) with a concentration of 2×10^6 cells/mL were loaded into the inlet by manual pipetting. After loading the cells, the device was lifted and positioned vertically for 10 min, in order to induce a settling effect of the loaded cells due to gravity. Then nutritive medium was provided for one day culture.

RESULTS AND DISCUSSION

Fig. 2 shows the images of cells trapped in the pocket after 10 min. The levels of cells accumulated in each pocket were uniform. After one day, the aggregated cells formed 3D cell spheroids. Using a conventional “hanging droplet” method, the aggregation time of cells is normally more than one day and the size distribution of spheroids is wide-spread [3]. However, by employing the proposed device, the time was less than one day and the size distribution was relatively narrow (Fig. 3). Also, to investigate cell viability for the spheroids, immuno-staining was performed. As shown in Fig. 2, excellent cell viability was obtained.

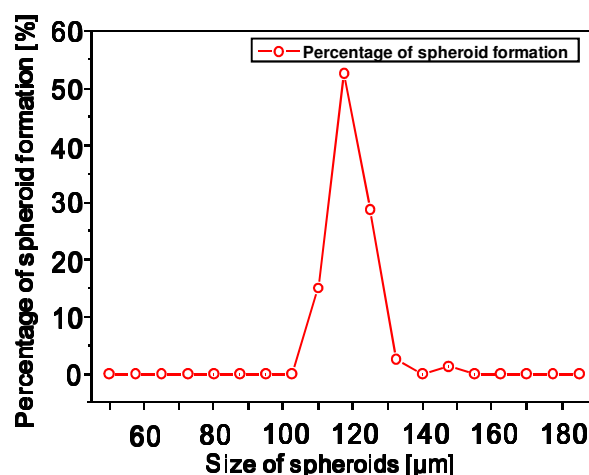


Fig. 3 Size distribution of cell spheroids of P-19EC cells (embryonal carcinoma cell line) in the wells.

Future Works

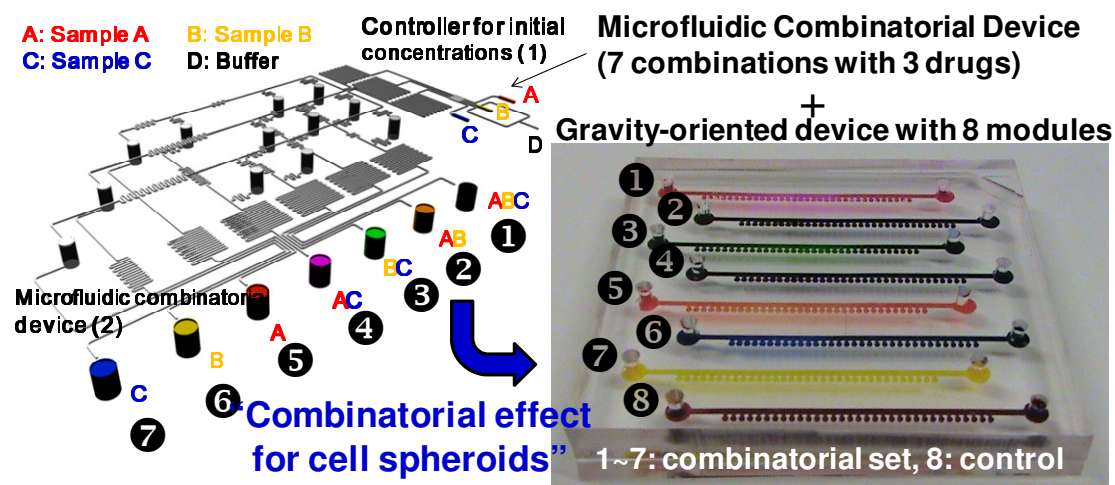


Fig. 4 The schematic of a microfluidic combinatorial device for 7 kinds of combinations with 3 drugs and a gravity-oriented device with 8 modules for cell spheroids.

As shown in Fig. 2 and Fig. 3, the proposed method can provide relatively uniform sized spheroids with excellent viability. In particular, the procedure is very simple, thus we think the device has a great potential to be coupled with other devices. For instance, we propose to couple the combinatorial dilution device developed by our group [4] with the proposed device as shown in Fig. 4. This could expand the horizons of current cancer and stem cell research.

CONCLUSION

We have demonstrated a simple and effective method to form 3D cell spheroids with a gravity-oriented single-layered microfluidic device. With two cell lines, uniform and well-conditioned cell spheroids were formed. Thus, we expect the proposed method will give valuable chances to study 3D cell culture models. We plan to study combinatorial effects of drugs for cancer or stem cell spheroids by integrating the proposed device with a suitable combinatorial dilution generator.

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REFERENCES

- [1] Y. S. Hwang, B. G. Chung, D. Ortmanna, N. Hattoria, H. Moellera, and A. Khademhosseini, "Microwell-mediated control of embryoid body size regulates embryonic stem cell fate via differential expression of WNT5a and WNT11," *PNAS*, vol. 106, pp. 16978-16983, 2009.
- [2] A. Y. Hsiao, Y. Torisawa, Y. Tung, S. Sud, R. S. Taichman, K. J. Pienta, and S. Takayama, "Microfluidic system for formation of PC-3 prostate cancer co-culture spheroids," *Biomaterials*, vol. 30, pp. 3020-3027, 2009.
- [3] C. Kim, I. H. Lee, K. Lee, S. Ryu, S. H. Lee, K. Lee, J. Lee, J. Y. Kang, and T. S. Kim, "Multi-Well Chip for Forming a Uniform Embryoid Body in a Tiny Droplet with Mouse Embryonic Stem Cells," *Bioscience, Biotechnology, and Biochemistry*, vol. 71, pp. 2985-2991, 2007.
- [4] K. Lee, C. Kim, G. Jung, T. S. Kim, J. Y. Kang, and K. W. Oh, "Microfluidic network-based combinatorial dilution device for high throughput screening and optimization," *Microfluidics and Nanofluidics*, vol. 8, pp. 677-684, 2010.

CONTACT

Kwang W. Oh (kwangoh@buffalo.edu)