DEVELOPMENT OF A MICROFLUIDIC HANGING DROPLET PLATFORM FOR 3D CELL CULTURE

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ABSTRACT

This paper reports a method for the automatic generation of hanging drops (HDs) for sustained three-dimensional (3D) cell culture. HDs emerge automatically through openings at the bottom of a microfluidic channel, which provides a means of and control over the mass transport into and out of the HD. We demonstrated the automatic formation of an array of HDs of various sizes using a microfluidic device and the loading of polystyrene beads and HL60 leukemic cells into HDs.

KEYWORDS: hanging drops, microfluidics, stem cells, three-dimensional cell culture

INTRODUCTION

The HD culture method is widely used for the formation of *in vitro* 3D aggregation of cells, avoiding the cellular distortion typically seen in the two-dimensional cell culture. The conventional HD technique consists of placing a small drop of medium and cells on a plastic substrate, inverting the substrate, and incubating for the desired length of time. However, the size of the HD is restrained by gravity, and hence only a limited number of cells can be sustained without cumbersome periodic replenishment. Approaches to modified HD method include hollow spheres [1], 384-well HD culture plates [2], microwell structures [3]. In the first two methods, the contents in the HD cannot be exchanged easily. In the microwell system, the substrate must be optimized to support 3D cell growth without cell adhesion.

In this study, we present a new microfluidic HD platform that enables the control of HD formation, cell loading, media exchange and retrieval of cells without pipette-based manual labor. A cross-section of the operation is shown in Figure 1. When the microchannel is filled, pedant drops form and hang from openings at the bottom. Cells are loaded into the microchannel and docked in the HD. Untrapped cells can be rinsed off. Different extracellular solutions can be introduced into the HD through the microchannel with the precise temporal and spatial control. Cells cultured in the HD can be either harvested directly from the HD using a micropipette or collected at the outlet by applying suction to the microchannel.

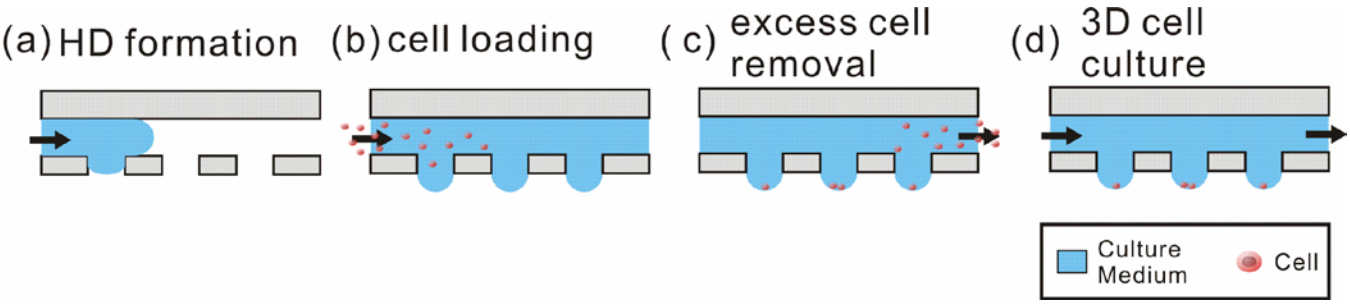

Figure 1: A cross-section of the operation of the microfluidic HD platform. (a) The microfluidic channel is filled and droplets are formed and hanging at the openings. (b) Cells are driven by the flow and docked in the HD. (c) Untrapped cells are rinsed off. (d) Cells are incubated and their extracellular solution can be modulated through the microchannel while gas can also be exchanged directly through the HD that is open to air.

EXPERIMENTAL

The device consists a microfluidic channel incorporating openings at its bottom and is fabricated in polydimethyl-siloxane (PDMS) on glass substrates using soft lithographic [4] and PDMS membrane transferring techniques [5]. Briefly, master wafers with two-layer features were made using conventional photolithography with a negative photoresist (SU-8, Microchem Inc., Newton, MA). The masters were then used as molds, on which PDMS prepolymer was poured. A fluoropolymer coated polyester (PE) sheet (Scotchpark™ 1002 Release Liner, 3M™, St. Paul, MN) and poly(methyl methacrylate) plates were applied and pressed against the master to squeeze out excess PDMS. The PDMS was allowed to cure in a conventional oven at 65 °C for 24 h. Once cured, PDMS features along with the adhered PE sheet were removed from the master. The PDMS layer was bonded to a glass substrate after both being treated with oxygen plasma and the PE sheet was peeled off.

HDs were generated at openings at the bottom of the PDMS microchannel when solutions were introduced into the microchannel at a controlled flow rate by using a syringe pump. The relationship of the diameter of the opening and the burst pressure was evaluated as the flow rate was increased until the HD becomes flattened.

To seed cells or beads, cell or bead suspension was flowed through the channel and the flow was stopped for 10 min to allow the cells or beads to settle into HDs. Excess cells were removed by restarting the flow.

RESULTS AND DISCUSSION

We have developed a microfluidic device for the automatic generation an easy fluidic access. HDs were formed at the bottom of the microchannel with openings of various sizes, ranging from 50 μm to 500 μm in diameter as shown in Figure 2.

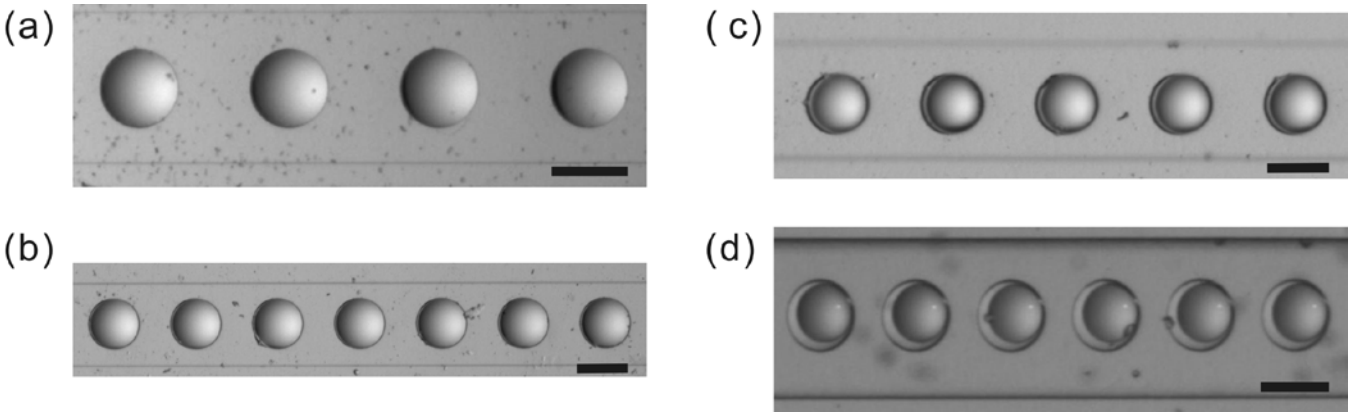

Figure 2: Automatic formation of an array of hanging droplets of various sizes at the bottom of the microfluidic HD chip (scale bars, (a) 500 μm, (b) 200 μm, (b) 100 μm, (c) 50 μm).

The relationship of the diameter of the opening and the burst pressure, at which the HD becomes flattened, was evaluated and is shown in Figure 3. As a qualitative assessment considering only convective transport, the maximal rate to replenish the solution in the HD can be calculated as the diameter of the opening divided by the mean velocity in the microchannel. The solution at the opening could be replenished for more than 50 times in a second even for the openings as large as 500 μm in diameter, which is adequate for typical cell cultures.

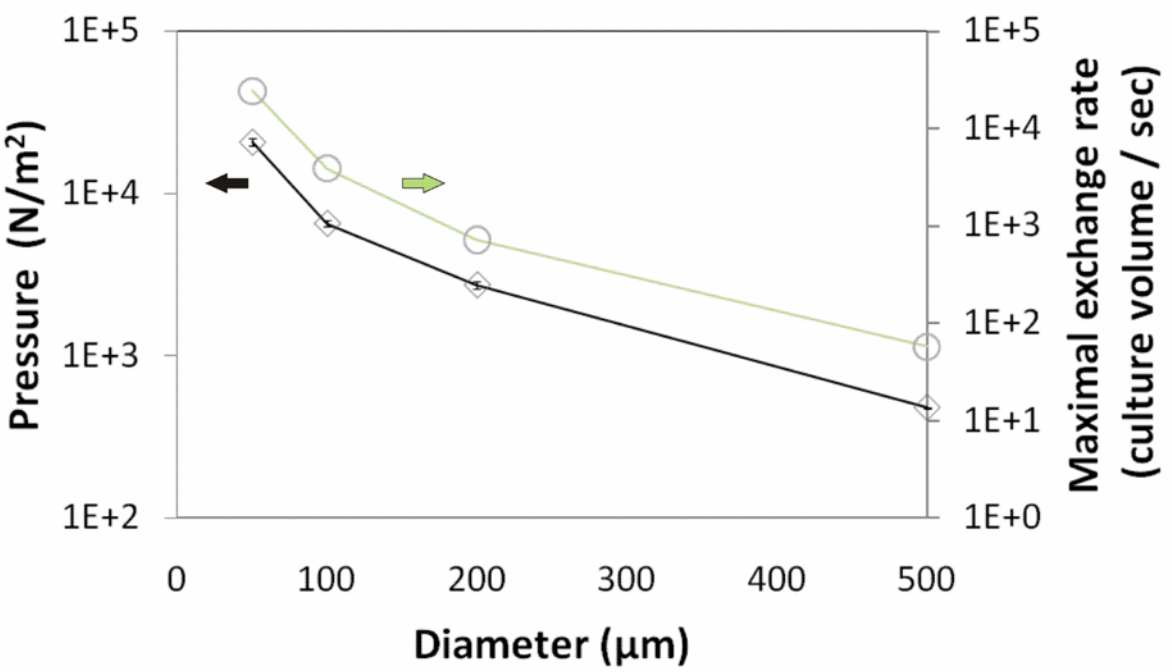

Figure 3: Dependence of burst pressure of the hanging droplet on the diameter of the opening (◇). Each data point was repeated in four devices spanning different diameters of openings; error bars represent standard deviations (mean ± std, n = 4). The maximal exchange rate (○) was calculated as the diameter of the opening divided by the mean velocity in the microchannel, which is an over estimation of the maximal rate to replenish the solution in the hanging droplet, assuming the diffusion of mass is instantaneous.

Polystyrene beads of 10 μm in diameter and HL60 leukemic cells were successfully loaded into HDs as shown in Figure 4(a) and 4(b), respectively.

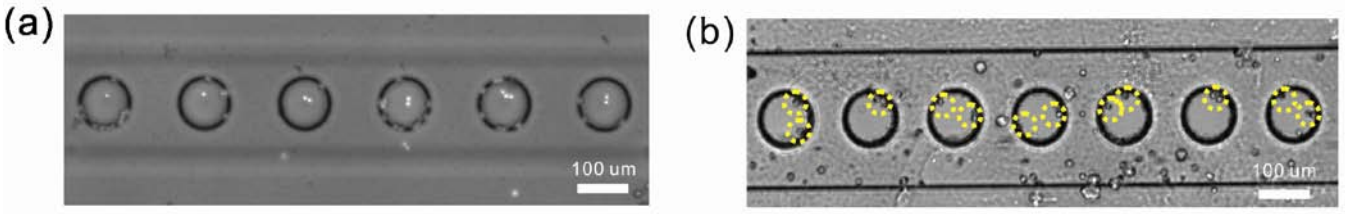

Figure 4: Loading of (a) fluorescent polystyrene beads and (b) HL60 leukemic cells into hanging droplets.

CONCLUSION

In conclusion, we invent and demonstrate a simple microfluidic HD cell culture platform. The integration of microfluidic channel to the HD allows automation, and high-throughput, long-term 3D cell culture, which is expected to be beneficial for the studies, such as stem cell differentiation, regenerative medicine, tissue engineering, drug screening and developmental biology.

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