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Abstract

This paper reports, for the first time, an optimized microfluidic droplet device for fabricating 3D microtissues and studying the cell behaviors in 3D microtissues. It has been found by properly selecting the size of the microchambers on the microfluidic device and choosing an optimal concentration of collagen (2 mg/ml) to fabricate microtissues, the behaviors of cells in the microtissues can be essentially the same as those of cells in a conventional cell culture system. The normal cell spreading and division in the microtissue have been observed, and the cell migration speed is ~14.1 μm/hr, close to that of 17.3 μm/hr in a macroscale tissue. All these experimental results suggest the microfluidic droplet device might provide a new avenue to replace other approaches to fabricate 3D microtissues and study cell behaviors.

Keywords

Microfluidic chip, 3D microtissue, cell growth, cell migration

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Studies of cell behaviors in 3D microtissues in a microfluidic device: growth and migration

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***Abstract*—This paper reports, *for the first time*, an optimized microfluidic droplet device for fabricating 3D microtissues and studying the cell behaviors in 3D microtissues. It has been found by properly selecting the size of the microchambers on the microfluidic device and choosing an optimal concentration of collagen (2 mg/ml) to fabricate microtissues, the behaviors of cells in the microtissues can be essentially the same as those of cells in a conventional cell culture system. The normal cell spreading and division in the microtissue have been observed, and the cell migration speed is ~14.1 μm/hr, close to that of 17.3 μm/hr in a macroscale tissue. All these experimental results suggest the microfluidic droplet device might provide a new avenue to replace other approaches to fabricate 3D microtissues and study cell behaviors.**

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I. INTRODUCTION

Traditional cell culture system, which can only offer 2 dimensional (2D) tissues and cannot isolate single cells or groups of cells, is difficult to be used as a technical platform to uncover how cells respond to the extracellular matrix (ECM) and how cells communicate [1-2].

Basically the following features are required for an ideal platform for high throughput studies of cell-ECM interactions and cell-cell communication: (i) the platform can realize the encapsulation of cells in an ECM similar to that in the body. The ECM should be 3 dimensional (3D); (ii) the platform can achieve the isolation of single cells or groups of cells in order to control the cell-cell communication. This implies confining cells by providing barriers between the cell environment and the surroundings; (iii) the platform must allow building microenvironments that are sufficiently small such as microtissues. Cells often communicate through the secretion of soluble molecules, so volumes between 10^3-10^4 fold larger than the cell are appropriate to ensure that the secreted molecule concentration is sufficiently high; and (iv) the platform can rapidly generate a large number of cell-encapsulated microtissues in parallel in a cost-effective manner for high throughput studies.

To this end, recently we have developed and used a droplet microfluidic device (coined as *first generation device*) to fabricate 3D microtissues and study the cell migration [3-4]. However, it was found that the cell migration speed inside the microtissue, which has a volume of ~600 pL, is ~ 6 μm/hr and much lower than that of the same type of cells when they are in the macroscale tissue of ~17.3 μm/hr [4]. Furthermore, due to the size/volume limitation of the microtissue, the proper growth of the cells cannot be observed. Basically at this volume size range, the behaviors of cells are different from those of cells when they are at a large scale tissue environment.

In order to study the cells' behaviors in microfluidic devices with the aforementioned features, meanwhile in order to ensure their behaviors are essentially the same as those in the large scale environment, in this effort, we modify the dimensions of the microfluidic device (coined as *second generation device*) and evaluate the cells' behaviors for the first time.

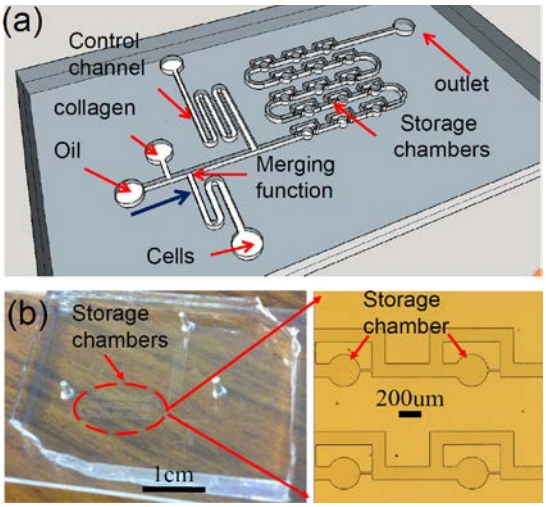

Fig. 1. (a) Sketch of the droplet microfluidic device for generating 3D microtissues: Each storage chamber (a cylinder with a radius of 180 μm and height of 30 or 80 μm) has one 3D microtissue containing single or multiple cells; (b) photo of a fabricated chip with 15 storage chambers; (c) optical micrography showing the storage chambers.

II. DESCRIPTION OF THE MICROFLUIDIC DEVICE

A droplet microfluidic device for generating arrayed microtissues is given in Fig. 1a. Filtered silicone oil is used as the continuous flow phase and the carrier fluid. Along the flowing direction of the fluids as illustrated in Fig. 1a, this device consists of a T-shape droplet generator, a liquid-droplet

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merger, a serpentine control-channel (c-channel), and the droplet storage-chambers (chambers). The droplet generator forms cell-laden collagen droplets. The c-channel is designed to prevent any air bubbles or non-uniform droplets from entering and occupying the chambers at the beginning of the operation of the device [3-4]. Once the uniform droplet generation is established, the c-channel is closed, and the outlet of the chambers is open. As a result, the droplets will flow toward the chambers, thereby entering and occupying them one by one. Compared to the *first generation device* [4], the dimensions of the *second generation devices* have been scaled up (Fig.1b-c). Specifically, the diameter of the chamber increases from 120 µm to 360 µm, while the width of the flowing channel increases from 50 µm to 150 µm. The height of the chamber has been designed as 30 µm and 80 µm, respectively for two types of the second generation devices.

III. Device Fabrication and Experimental Procedure

Device fabrication

The device is fabricated using a soft lithography process. Briefly, a SU-8 mold (30 µm and 80 µm thick) of the device is formed on a silicon substrate. Polydimethylsiloxane (PDMS) is then casted on the mold, followed by 1.5 hr of curing at the temperature of 65 °C. Finally, the PDMS microfluidic layer is peeled off from the mold, and then bonded with a glass substrate after oxygen plasma treatment for 10 s. The input and output holes are made in the PDMS layer for the delivery of the samples to the chip, followed by assembling input and output tubing (Upchurch Scientific, Inc., Oak Harbor, DC, USA),

3D microtissues fabrication procedure

The device is firstly soaked in PBS buffer solution (pH-7.4) in incubator (FISHER SCIENTIFIC-ISOTEMP 3530) overnight [3]. Silicone oil (SIGMA-ALDRICH) is used as the fluid carrier. The cell (MDA-MB-231 cells) loading in the collagen droplets is based on Poisson distribution during the experiments. No surfactant is used to facilitate the droplet stability. The collagen flowing input tube and syringe are submerged into a cold water tank (0~2 °C) to avoid fast polymerization. After the droplets are in the chambers, the device is flipped over regularly until the collagen is fully polymerized, thereby making sure the cells are in the middle of the storage chamber (along the z-axis), namely the cells in the 3D-matrix.

IV. Results and Discussion

Cell spreading in 3D microtissue

Representative images showing the measured cell spreading in first generation device and the second generation device are given in Fig. 2. As seen clearly, the cells are still alive but do not show apparent spreading after 24-hour incubation in the first generation device. Basically the shape of the cell remains roughly round 24 hours later. In contrast, the spreading of cells in the second generation is very obvious. The morphologies of the cells become essentially the same as those of cells grown inside the large scale tissue in a conventional cell culture system, indicating as far as the dimensions are selected properly, the microtissue can provide similar biological environment for cells to that of macroscale tissue. The volume of the microtissue in the second generation device is ~8140 pL in comparison with the volume of ~600 pL for the first generation device.

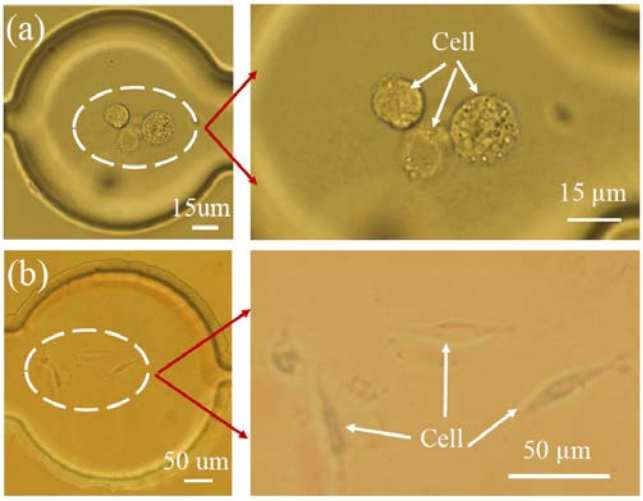

Fig. 2. Cell spreading in 3D matrix within (a) small chamber of ~600 pL volume; (b) larger chamber of ~8140 pL volume after 24-hour incubation.

Cell division in 3D microtissue

Another important observation of the second generation device is the cell division procedure as shown in Fig. 3. In the first generation device, the cell division was not observed, suggesting the size limitation of the tissue, resulting in the abnormal growth of the cells [4]. As shown clearly in the second generation device, the cell becomes round shape in Fig. 3c. then cell division occurs in Fig. 3d. After the two daughter cells are formed in Fig. 3e, they grow normally in Fig. 3f.

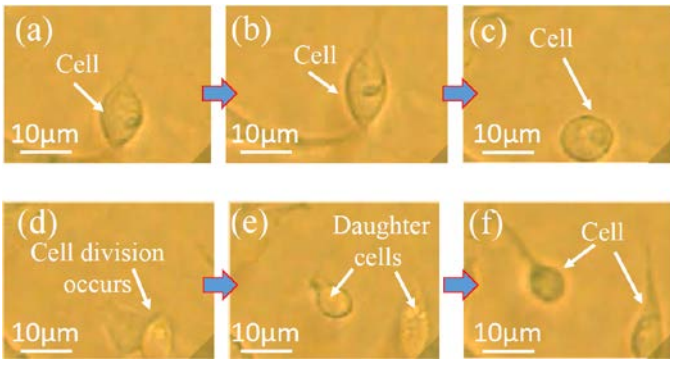

Fig. 3. Optical micrographies showing the cell spreading and growth procedure in a 3D microtissue

Cell migration trajectory and speed in 3D microtissue

The *videos* of the cells' migration inside microtissue have been recorded using an optical microscope [4]. Representative migration trajectories are given in Fig. 4a. Compared to those in first generation devices, the range of the migration of cells has been significantly increased. For instance, the cell migration is within an area of 3 µm × 7 µm for the first generation device,

while the cell migration area becomes 8 µm × 14 µm for the second generation device. Also the cell migration speed has been calculated based on the videos as shown in Fig. 4b.

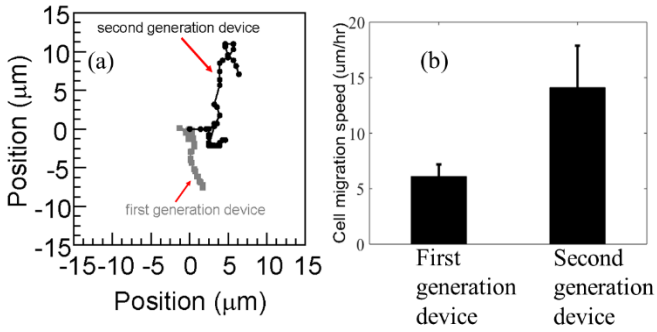

Fig. 4. Comparison: Cell migration trajectory (a) and migration speed (b) inside the microtissue fabricated in the first generation device and the second generation device.

Collagen concentration (tissue fiber density) effect on cell migration speed: Different concentrations of collagen have been used to fabricate microtissues, resulting in microtissues with different densities. The measured cell migration speeds are summarized in Fig. 5. It has been found that the lower collagen densities (1 mg/ml and 2 mg/ml) show an improved cellular migration speed over the higher collagen densities (3 mg/ml). This is due to the thick gel having more fibers to restrict the pore size within the matrix. An increased density of fibers would require more MMP activity to increase pore size and allow for higher freedom of movement. Note that collagen fibers must be dense enough for the cell to grow on, but not too dense to limit the cell migration speed. Experiments have found that 2mg/ml is an optimal concentration for collagen because most of cell spreading and division are only observed at this value.

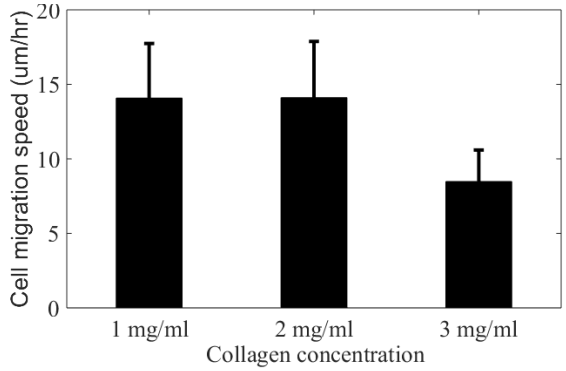

Fig. 5. Collagen concentration (tissue fiver density) effect on cell migration speed. 2mg/ml used as a standard concentration since most of cell spreading occurs and cell division is only observed at this concentration.

Microtissue thickness (volume) effect on cell migration speed: In order to understand the cell speed influenced by the microtissue thickness (i.e. volume), devices with chamber thickness of 30 µm and 80 µm are fabricated, while the chamber diameter remains 360 µm for both types of devices. As shown in Fig. 6, the average cell migration speed in 30 µm thick microtissue is ~7.1 µm/hr, which is much lower than that in 80 µm thick microtissue of ~14.1 µm/hr. This indicates the microtissue thickness (volume) indeed affects the cells' behavior. For comparison, the measured cell migration speed in a slab of 360 µm thick tissue is ~17.3 µm/hr, very closed to the device with 80 µm thick microtissue.

For the device with an increased chamber height, and thus increased volume of microtissue, the cells migrate faster as a result of experiencing less edge effects, cellular waste taking longer to accumulate, and oxygen depletion within the gel requiring more time to affect the cells.

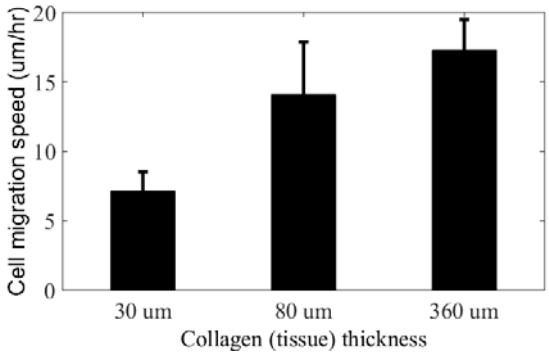

Fig. 6. Thickness (volume) effect on the migration speed: the 2nd generation devices with chamber heights of 30 m and 80 µm. A large gel with a height of 360 µm. The cell migration speed in 30 µm thick microtissue is half of that in 80 µm thick microtissue, which is close to the migration speed in the 360 µm thick tissue.

These experiments indicate by adjusting the dimensions of our microfluidic device, the cells' behaviors in microtissue in a microfluidic device can be similar to that in the tissue fabricated in a conventional cell culture system. But the microfluidic device offers many advantages over the conventional cell culture system as mentioned in the Introduction section.

In summary, in this effort, we have demonstrated the possibility to use microfluidic devices to fabricate microtissues of *proper* sizes/volumes for studying the *normal* cells' behaviors including growth and migration, which are similar to those of cells in a large scale tissue. This technical platform opens a new avenue to study the behaviors of single cell and/or a group of cells in a in vivo –mimicking 3D microtissue environment.

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