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A gel-free multi-well microfluidic device utilizing surface tension for cell culturing

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ABSTRACT

This paper reports a gel-free multi-well microfluidic chip for cell cultures. Polydimethylsiloxane (PDMS) material was used because of its hydrophobic and gas-permeable features. Surface tension and fluidic channel resistance simplified the procedure of cell injection into the system. The shear stress and the mass-transfer perfusion medium reached a balance through the multi-row square-pillar microstructure and the driven pressure. The cell seeding was completed in 10-mm scaled culture wells in less than 6 s using the microstructure composed of PDMS. The adjustable initial cell density and length of the culture area facilitate its use in desired biological experiments. To verify whether the operation caused cell damage or not, the biological index of supernatants with glutamic oxaloacetic transaminase (GOT) and blood urea nitrogen (BUN) were monitored for 4 days. The 1–4-mm-long multi-rows square-pillar microstructure can sustain HA22T cells for 5 days and still maintain cell viability for up to 90% of seeded cells.

1. Introduction

A bioartificial liver device (BAL) can serve as a supportive device for patients with acute liver failure prior to undergoing liver transplant and liver regeneration treatment [1–3]. Hollow fiber is a technology commonly used for clinically assessing BAL devices [4,5]. This technology requires a large number of liver cells (approximately 10^{10} cells) with a large surface area equivalent to 10 m^2 [2,6]. Furthermore, liver cells are extremely sensitive and demand a balance in not only oxygen gradient concentration but also the metabolic medium [7–11]. Therefore, achieving a balance of a high mass-transfer rate and low shear stress conditions between cells is a major engineering problem for in vitro liver models [6,12–14].

Microfluidic cell culture systems offer several advantages for cell research [15–18]. The microflow channel within the device is small, which allows the rapid diffusion of nutrients. This low shear stress and high mass-transfer rate environment is similar to the in vivo cellular microenvironment [19]. To enhance cell functionality, the cells are cultured from a two-dimensional (2D) cell culture to a three-dimensional (3D) cell culture, which is sustained in all directions by the cells or the extracellular matrix (ECM) [14,20–23]. For these microfluidic cell culture devices, the cell culture well dimension is of the micrometer scale. Most of them are unable to sustain the large cell number required by BALs. Therefore, BAL research on microfluidic liver-cell culture systems must increase the cell culture region and enhance the functions of the continuous injection of the multi-well area and the cell seeding process. The cell-seeding method must be simple and easy to operate. In addition, the shear stress and mass-transfer balance for a large-area cell culture must be considered to achieve high cell viability.

Previous gel-free cell-seeding methods for microfluidic cell culture systems can be divided into 4 categories: (i) the gap of the microstructure is less than the cell diameter [24–27]; (ii) the gap of the single row of the microstructure is larger than the cell diameter [28–30]; (iii) the device contains an active control valve [31–33]; and (iv) the gas-permeable material property is incorporated into the system design [34]. Types (i) and (ii) cell-seeding designs provide a facile operation and repeatable results. However, type (i) has a small gap, resulting in a low mass-transfer rate and rendering it incapable of sustaining large-area cell cultures. The type (ii) cell-seeding design requires a pre-mixture of condensed biomaterials that can raise the complexity of the operating procedure and reduce the mass-transfer rate. The medium cannot diffuse through a condensed ECM because the diffusion force is not strong enough [35]. Therefore, a large cell culture area and the type (ii) cell seeding design are combined under high velocity. If the proposed gel-free design can be applied to the type (ii) cell seeding design in a large cell culture area, the large gap of the microstructure can

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raise the mass-transfer rate and reduce the operational complexity of the designed system. Although applying the gel-free design can solve a number of problems associated with the type (ii) design for large cell culture areas, the functionality of the gel-free design might be limited under the requirements of reducing shear stress and maintaining a stable cell-culturing operation. This is a major conflict in engineering design.

The goal of this study is to resolve these issues in the microstructure-type cell-seeding method and raise the cell-seeding scale to the millimeter range. We also want to achieve efficient cell seeding under low shear stress, a high mass-transfer rate, and uniform cell density, and still maintain a simple method of operation. Polydimethylsiloxane (PDMS) material is hydrophobic and gas-permeable. Surface tension and fluidic channel resistance simplified the operation of cell injection into the system. The shear stress and mass-transfer perfusion medium reach a balance through the multi-row square-pillar microstructure and by using the pressure balance method. Simulation and biological experiments on cultivated HA22T cell lines in the designed device were performed to verify the feasibility of this design. This simplified cell injection method can be adjusted for different cell lines. During the degassing process, the pressure of 4.2 psi was used to force air bubbles out of the system, to test the effect of the degassing process for possible damages induced on the cells. Biological indicators glutamic oxaloacetic transaminase (GOT) and blood urea nitrogen (BUN) were measured to assess the level of damage on cells from the increased pressure.

2. Methods

2.1. Device fabrication process

Microfluidic devices with the multi-row square-pillar microstructure were designed using AutoCAD software (Autodesk, USA) and fabricated with PDMS (Sylgard 184, Dow Corning) by using soft lithography [36,37]. The height of the top channel and bottom channel was 60 μm. The microfluidic cell culture device contains 2 inlets and 2 outlets, as shown in Fig. 1(a). The cell culture wells, with a width of 1 mm and varying lengths from 1 to 10 mm, were located between the top and bottom channels. When red ink was simultaneously injected into the top and bottom channels, air was trapped within the multi-row square-pillar microstructure by the surface tension force, as shown in Fig. 1(b). A 40 μm × 40 μm multi-layer microstructure with a 15–25 μm gap was situated above the cell culture well (Fig. 1(c) and (d)). SU8 photoresist templates were fabricated using the standard photolithography process. The microfluidic channels were then obtained by molding PDMS onto the SU8 photoresist templates. The PDMS structures were plasma-treated in oxygen plasma for 30 s (30 W, 13.5 MHz, 100 sccm) for irreversible bonding to the glass cover slips before connecting to the fluidic components of a Luer-lock fitting (Upchurch, USA).

2.2. Cell-seeding process

The fabricated microfluidic devices were sterilized by using an autoclave in 2 ATM at 120 °C. Type I rat tail collagen (BD Biosciences) dissolved in 0.02 N acid was injected into the cell culture chambers in the microfluidic device and placed in an incubator at 37 °C. After 1 h, the cell culture wells in the microfluidic device were washed 3 times. Then, the cells and the medium were placed in a centrifuge tube and prepared for cell seeding.

The operational procedures for the cell-seeding method are as follows: The cells were suspended and pre-mixed by using a pipette in the cell medium. Thereafter, 20 μl of the medium with suspended cells was drawn with a pipette and rapidly injected into the inlet of the bottom channel. This step was performed with ease and did not alter the pre-mixed cell concentration. The cells in the bottom channel were washed away by the slow medium flow, which did not block the bottom channel during the process.

Once the cells were attached to the glass substrate for 4–6 h, they remained biologically active. The slow medium flow maintained cell viability, and the slow flow rate was maintained via gravity. The cells and the medium were introduced through Port A, whereas Port C was left open (Fig. 1). Because the cross-section of the cell culture region was larger than that of the connecting Channel C, the flow resistance in the cell culture region was less than the flow resistance at the connecting Channel C. The cells and the medium spontaneously flowed into the cell culture area because of differential flow resistance. The air, because of its smaller molecular size, was expelled to either Port B or Port D through the hydrophobic microchannels, where almost no resistance was encountered for the gas molecules (Fig. 2(a)). The gaps between microchannels were 15 μm wide, which is similar to or slightly larger than the diameter of most cells types. When the cells and the medium filled the culture area, surface tension in the hydrophobic microstructure stopped the flow toward Port B and Port C (Fig. 2(b)). Therefore, if the cells and the medium continued to perfuse into the chip, the cells and the medium that flowed into the next cell chamber would repeat the cycle until the cell array was filled with the cells and the medium (Fig. 2(a) and (b)). At this point, discarded cells remaining in the bottom channel must be removed. The medium was then slowly injected from Port A (Fig. 2(c)) to Port C to wash out the non-viable cells and to provide the cells with a sufficient amount of fresh medium through mass transfer. The process was carried out with the help of gravity until cells attached onto the substrate (Fig. 2(d)).

2.3. Degassing process

The proposed cell-seeding method utilized the hydrophobic and gas permeability characteristics of PDMS material [38,39]. As mentioned in the previous section, the cells were trapped in the cell culture area because of surface tension from the hydrophobic microstructure. In the degassing process, the cell culture medium was first delivered into Channel BD (Fig. 2(e)). The air pressure was raised to 4.2 psi in this device to diffuse micro-bubbles within the microstructure through the PDMS membrane.

Fig. 3 shows the experimental setup of the degassing process for measuring the removal time from the microchannel. An air tank in the pre-mixed 5% CO_2 was connected to the pressure regulator and filtered to provide a good pressure source. The precise air pressure-to-flow ratio in the device was adjusted by the mass flow controller (MFC). The pressure sensor in the MFC measured the corresponding air pressure. The feedback control of the MFC achieved the set pressure and removed air bubbles through a personal computer that communicated with the MFC. To prevent bacteria from entering the chip, pressurized air was passed through a 0.2 μm filter. Closing the 3 valves connected to the 3 inlets (A, C, D) and joining the 4.2 psi air pressure to the other top-channel inlet (B) enable the air bubbles trapped within the microstructure to be thoroughly degassed (Fig. 2(f)).

2.4. Perfusion setup

After the top and bottom channels were filled with the medium and degassed, a peristaltic pump was used to transfer the medium from the medium reservoir to the front of the supply channel. The schematic diagram of the flow perfusion apparatus is shown in Fig. 4. The tube was then attached to the inlet at one end and fixed to the outlet on the supernatant reservoir at the other. The purpose