FABRICATION OF A CIRCULAR PDMS MICROCHANNEL TO CONSTRUCT 3D CULTURE MICROSYSTEM

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ABSTRACT

In this study, we described the fabrication method for microfluidic channels with circular-cross sectional geometries by reflow phenomenon. The photoresist reflow leads to shape change from rectangular to semicircular, and we optimized the reflow conditions by using positive photoresist to form the perfect cylindrical micropatterns. The resultant circular micropattern was used as a template for generating PDMS circular microchannels. The channel routes could be easily modified for mimicking the vascular network. To generate a 3D endothelial cell layer, we cultured the HUVECs inside the PDMS circular microchannels, and monitored the adhesion, proliferation, and alignment of HUVEC to the direction of the microchannel.

KEYWORDS

Polydimethylsiloxane (PDMS), circular microchannels, cell culture, reflow of photoresist, Human umbilical vein endothelial cell (HUVEC).

INTRODUCTION

The application of microfabrication technology has expanded from simple biomolecular separation to more complicated genomic, proteomic, and cellular analyses.[1] In particular, the ability of microtechnology for precise control of time and space in microenvironments provides an excellent platform for cell biology and tissue engineering.[2, 3] Microfluidic-based biomimetic devices have demonstrated more reliable and high-throughput drug screening capability compared with the conventional static 2-D cell culture system.[4] Research on the construction of an artificial microvascular system on a chip is a good example. The microfabrication technology can provide a micro-scale vascular system ranging from capillaries to large arteries similar to the organizational complexity of in vivo blood vessel architectures. In this study, we demonstrated a simple but efficient fabrication method to provide a circular microchannel with a goal of mimicking the microvascular system.

EXPERIMENT

Figure 1 shows the fabrication scheme for the circular microchannels by using soft lithography combined with a reflow phenomenon of a thick positive photoresist. A positive photoresist was spin-coated on the Si wafer. To increase the height, the photoresist was spin-coated twice to form a double layer of resist (Figure 1(A)). To pattern the rectangular shape, the double layered positive photoresist on the Si wafer was exposed to UV-light, post-baked, and developed (Figure 1(B)). To form the circular patterns, the rectangular shaped positive photoresist was heated at 105 °C for 5 min to cause the reflow phenomenon, converting the rectangular shape to a circular shape (Figure 1(C)). A PDMS prepolymer and PDMS curing agent, mixed at a volume ratio of 10 : 1, were poured into the convex half-circular photoresist on a Si wafer, and cured in an oven (Figure 1(D)). The cured PDMS was then carefully peeled off from the Si wafer, and the resultant PDMS microchannel shape was a concave circle (Figure 1(E)). Two half-circular PDMS layers were exposed to O_2 plasma for 2 min to generate hydroxyl groups and oxygen radicals on the surface, and then permanently bonded to produce a nearly perfect circular PDMS microchannel (Fig. 1(F)). The channel dimensions were controlled by tuning the thickness of the positive photoresist, and various shaped microchannels, such as straight, S-curve, X-, Y-, and T-shapes, were generated through the photomask designs.

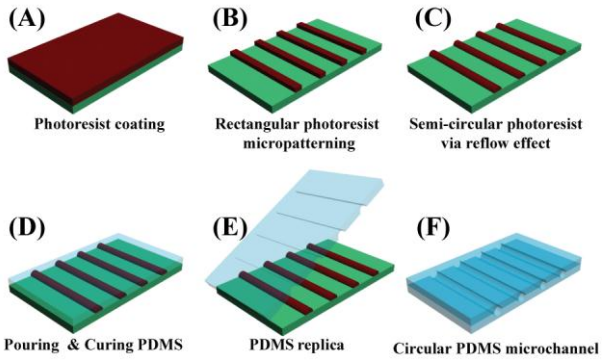

Figure 1. Fabrication scheme for circular PDMS microchannels by combining soft lithography and reflow phenomenon of a thick positive photoresist.

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HUVECs of passage number 2~4 were cultured and grown in a humidified 5 % CO_2 incubator at 37˚C. After confluency, the cells were trypsinized with 0.25 % trypsin and 0.05 % EDTA for 3 min, and then concentrated by centrifugation and resuspended with the complete medium to adjust the cell concentration to 2 X 10^6 cell/mL. The circular PDMS microfluidic channel (diameter of 250 μm) was sterilized with 70 % ethanol and exposed to UV overnight. The surface of the circular microchannel was coated with 0.1 mg/mL fibronectin by incubation at 37˚C for 1 hr. After washing with Dulbecco's phosphate-buffered saline (DPBS), the harvested HUVECs were introduced into the microchannel by using a micro-syringe pump with a flow rate of 14 nL/sec. After 10 min incubation in a humidified 5% CO_2 incubator at 37˚C for cell attachment, the circular PDMS microchannel was turned upside down, and same amounts of HUVECs were introduced again. After another incubation period of 2 h for complete and stable cell adhesion inside the entire circular microchannel, the chip was immersed in a petridish containing a static media solution, which prevented any possibility of cell detachment or bubble formation during the cell culture.

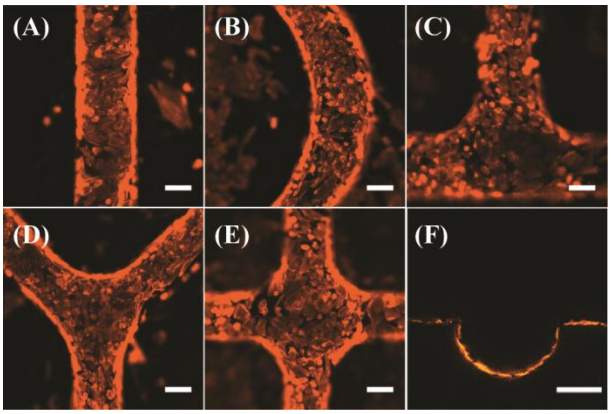

Figure 2. Primary HUVECs were cultured in various shaped half-circular PDMS microchannels for 3 days and stained with phalloidin to image filamentous actins of the cells. (A) straight, (B) curved, (C) T-shape, (D) Y-shape, and (E) cross-shape. (F) The cross-sectional fluorescence image of the cultured cells in the half-circular microchannel. Scale bars: 100 μm

RESULTS AND DISCUSSION

We cultured HUVECs inside the half-circular PDMS microchannels, and investigated the alignment and morphological response of the endothelial cells. Primary HUVECs were seeded and cultured for 3 days in the straight, curved, T-, Y-, and cross patterned microchannels, and then stained with TRITC labeled phalloidin, which specifically binds to filamentous actins of the cells. As shown in Figure 2, the HUVECs were cultured and uniformly spread all over the fibronectin coated half-circular microchannels, and the cells were well aligned along straight (Figure 2(A)), curved (Figure 2(B)), T-shape (Figure 2(C)), Y-shape (Figure 2(D)), and cross-shape (Figure 2(E)) channel patterns, respectively. In particular, the filamentous actins of the cells attached on the junction regions of the T- and Y-shape channels showed stretchability toward the branches of the channels, suggesting that HUVECs can moderate their fine attachment according to the channel routes. The cross-sectional fluorescence image in Figure 2(F) demonstrated that HUVECs are well attached to the half-circular channel.

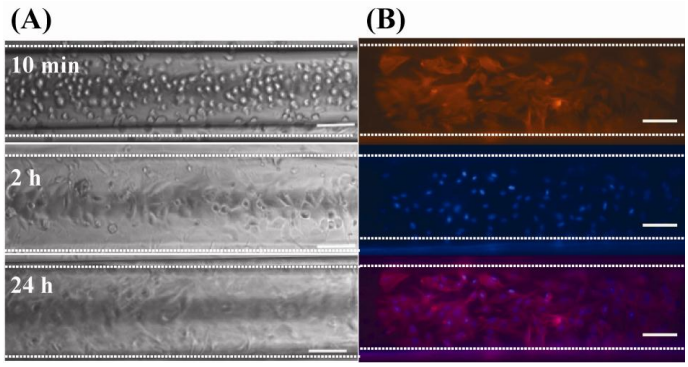

Figure 3. HUVEC culture in the complete circular microchannel. (A) Time dependent cell attachment profiles after introduction into the fibronectin-coated circular PDMS microchannel. (B) Fluorescence staining images of the filamentous actins (top panel), nuclei (middle panel), and a merged image (bottom panel). Scale bars: 100 μm.

We bonded the half-circular microchannels with careful alignment to produce a complete circular PDMS microchannel, and cultured the HUVECs inside the microchannel. To ensure entire cell coverage, we seeded the cells up and down twice in the channel. Phase contrast images showing the progress of cell attachment during 24 h are presented in Figure 3(A). The initial round shape of the cells was changed into an elongated morphology after 2 h and the cells then gradually spread over the channel surface after 24 h. The fluorescent staining for filamentous actins in Figure 3(B) (top panel) revealed filamentous actins aligned along the channel direction, while the nuclei staining (Figure 3(B), middle panel) shows a large population of HUVECs occupied the whole channel, indicating successful primary HUVECs culture in the circular PDMS microchannel. The cell viability was also confirmed by a live and dead cell assay after 3 day culture.

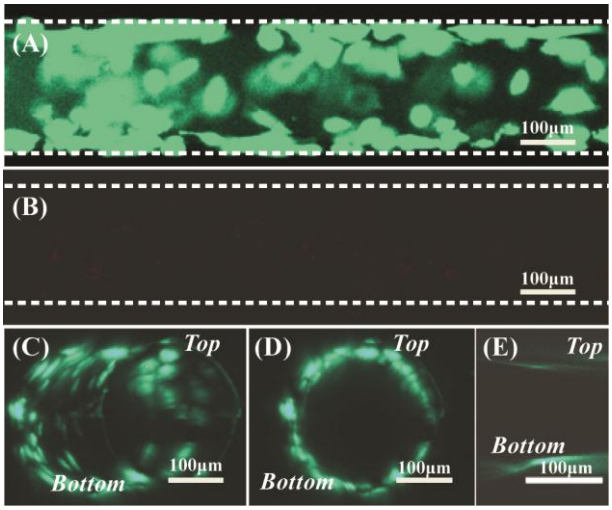

Figure 4. Live and dead cell assay of HUVECs cultured in the circular microchannel for 3 days. (A) Green fluorescence image for the live cells, and (B) red fluorescence image for the dead cells. (C) Tilted and (D) front view of the 3D z-stack image for the live HUVECs that adhered to the entire circular PDMS microchannel. (E) Side view of the top and bottom layer of the HUVECs in the microdevice.

As shown in Figure 4(A) (live cell image) and Figure 4(B) (dead cell image), more than 99% of cells were alive even after 3 days in the circular channel, suggesting a high possibility of providing an artificial blood vessel based bioassay platform. The HUVEC cell coverage on the microchannel was calculated to be about 74 %. To visualize the live HUVEC adhesion in the entire conditions of the circular microchannel, we used a z-stack confocal microscopy evaluation. The tilted and front images of the 3D z-stack (Figure 4(C) and 4(D)) show that the endothelial cells formed a hollow tube structure inside the microchannel. The rotation of the z-stack projection confirmed the circular endothelial cell layer along the microchannels. A side view of the top and bottom layers of the microdevice was also visualized, as shown in Figure 4(E), displaying uniform cell attachment along the microchannel wall.

CONCLUSION

We have developed an efficient fabrication method for generating a nearly perfect circular PDMS microchannel to mimic the 3D endothelial cell layer system. Our novel approach is based on soft lithography combined with a thick photoresist reflow phenomenon. We could tune the diameter of the circular channel as well as the channel routes with ease to form the microvascular structure. Furthermore, we successfully cultured the primary HUVEC inside the circular PDMS microchannel with 74% coverage, and demonstrated cell alignment along the channel direction and cell viability after 3 days. The proposed cell chip for in vivo-like cell culture could provide a more reliable and practical platform than the conventional 2D based cell assays in the fields of drug screening and chemical/biological diagnostics.

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