Effect of channel width on human umbilical vein endothelial cell (HUVEC) culture in microfluidic channels

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EFFECT OF CHANNEL WIDTH ON HUMAN UMBILICAL VEIN ENDOTHELIAL CELL (HUVEC) CULTURE IN MICROFLUIDIC CHANNELS

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ABSTRACT

This report describes the development of endothelial cell (EC) cultivation devices with different channel widths (60 to 360 μm). Crucial features of the devices include even cell distribution along the channel, seeding reproducibility, and compatibility with microscopy and flow application. The main achievement of this work is the design of chips which allow reproducible HUVEC culture in narrow (< 400 μm) channels.

KEYWORDS: microfluidic cell culture, narrow microchannels, endothelial cells

INTRODUCTION

ECs line all blood vessels and play a crucial role in trafficking molecules between the blood stream and tissues. Therefore, they play a fundamental role in the regulation of drug transport from the blood stream to tissues, tumor neovascularization and inflammatory response [1]. Microchannels mimic the in vivo environment of ECs better than well-plates due to matching dimensions. The additional possibility of flow application makes microchannels well suited to endothelial biology studies [3]. However, channels reported in the literature have large widths (often ≥ 400 μm) [3], whereas our interest lies in investigating microvasculature. Small blood vessels are characterized by substantial molecular exchange with blood because of low fluid velocities. The behavior of endothelium is strongly influenced by its local environment [2]. However, seeding EC into channels as small as 60 μm is difficult, with cells tending to distribute unevenly along channels. Moreover, the availability of primary endothelial cells from microvasculature is limited [4], so cell concentrations and amounts used for culture must be significantly reduced compared to those employed in reported microfluidic devices [3]. Therefore, we decided to develop new channel designs which address these issues.

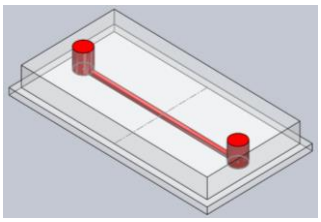
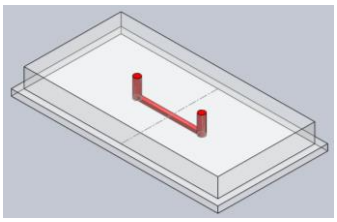
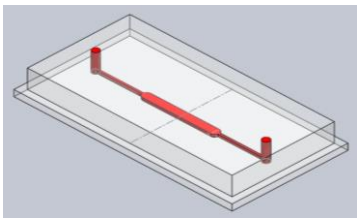
EXPERIMENTAL

The three channel designs tested are summarised in Table 1. PDMS channels were fabricated using masters prepared in SU-8 negative photoresist. They were irreversibly bonded to the sterile microscopic glass slides by exposure to O2 plasma. Microchannels were coated with 1% gelatin (crosslinked with 0.5% glutaraldehyde) as a substitute for extracellular matrix (ECM). The injected volumes of cell suspension were experimentally adjusted to match internal device volumes. Cells were cultivated in an incubator at 37°C and an atmosphere of 95% air and 5% CO2.

RESULTS AND DISCUSSION

Reversibly bonded straight channels of uniform width and 2.5 cm length (Design 1, Table 1) were successfully used previously under static conditions [5], but started to leak upon application of flow. Therefore, new, irreversibly bonded channel designs were tested, which exhibit significant improvement of cell load and distribution in the channels (Designs 2 and 3, Table 1). Design 2 has decreased reservoir diameters (2.5 mm down to 1 mm) and length (25 mm down to 10 mm) compared with Design 1.

Table 1: Layout of three channel designs with parameters tested for cell cultivation

	Design 1	Design 2	Design 3
Sketch			
Cultivation channel length [mm]	25	10	10
Diameter of the reservoirs [mm]	2.5	1	1
Height of the channel [µm]	100	100	100
Tested widths [µm]	360, 120, 100, 80, 60	360, 120, 100, 80, 60	360, 120, 100, 80, 60

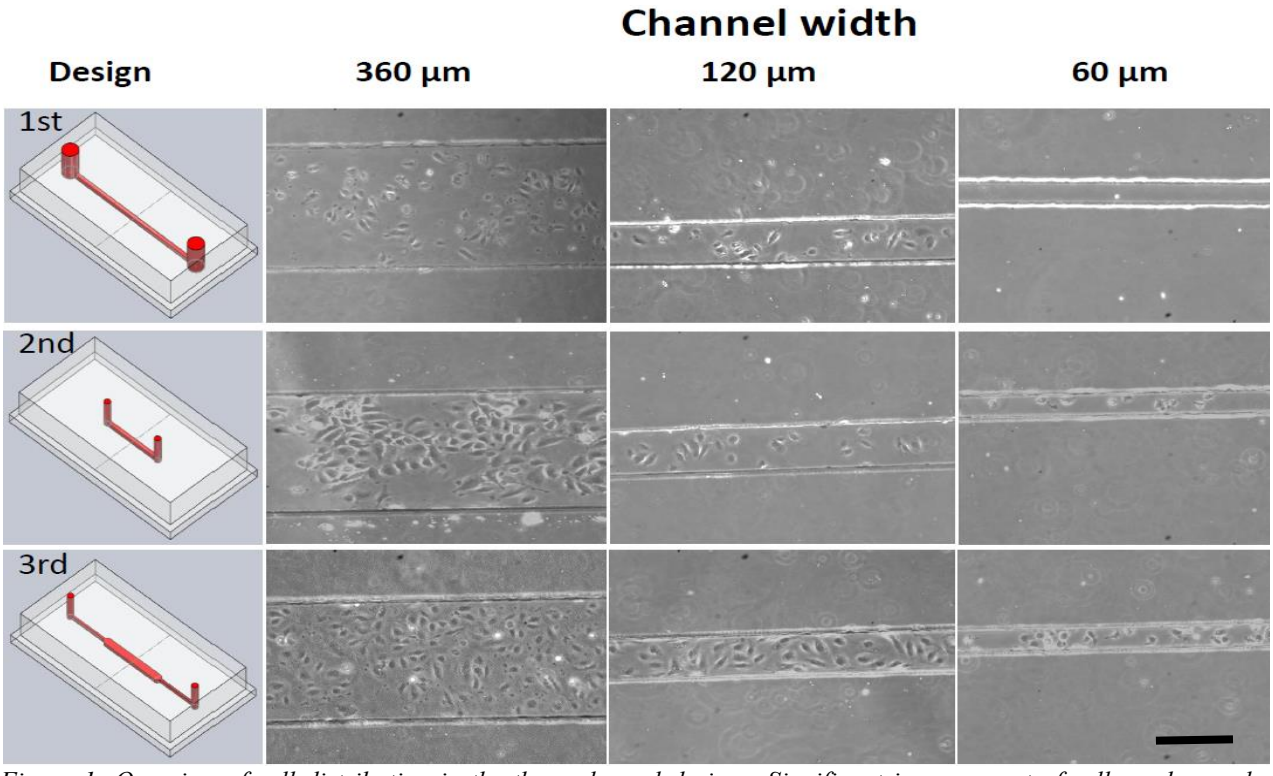


Figure 1: Overview of cell distribution in the three channel designs. Significant improvement of cell number and distribution is visible for Design 3 compared to Designs 1 and 2 for all presented channel widths. Cells were seeded at a concentration of 5000 cells/µL. Cell suspension volumes introduced to the channels were as follows: 8 µL (360 µm wide channels); 6 µL (120 µm wide channels); and 3 µL (60 µm wide channels). All photos were acquired 5 mm from the beginning of the cultivation channel 2h after cell seeding; capture area of the photos is 977 x 590 µm. Magnification: 10x; scale bar: 200 µm

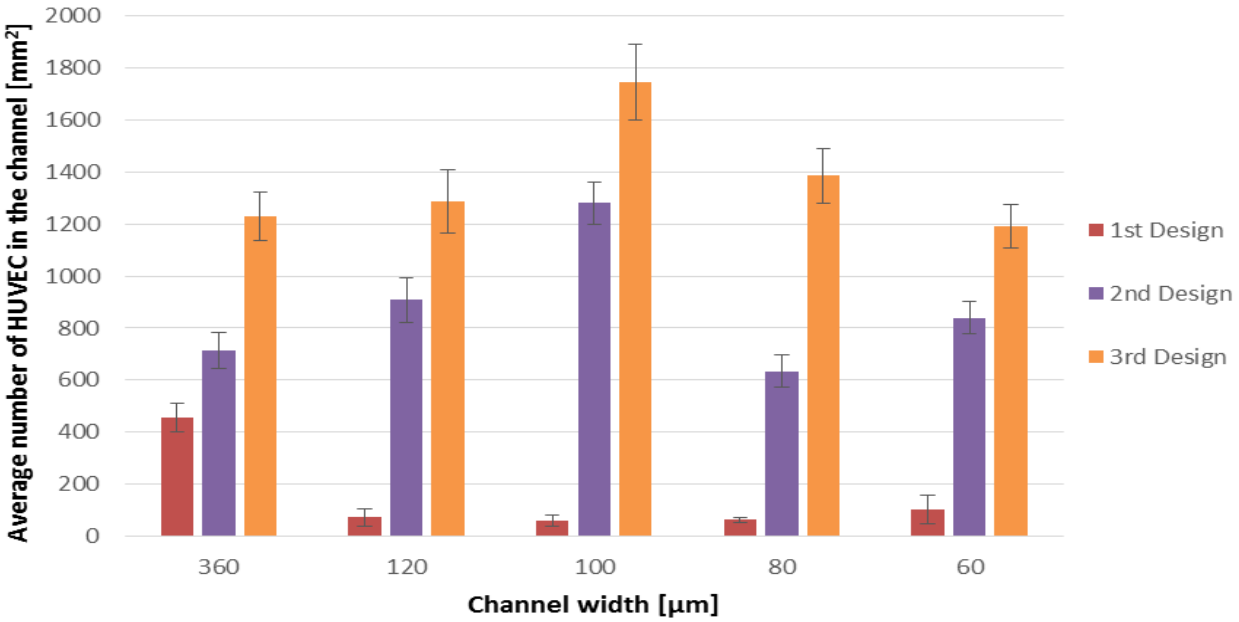


Figure 2: Overview of the number of cells attached in the channels with different widths (Figure 1) 2h after seeding at a concentration of 5000 cells/µL. Design 3 shows the highest number of attached cells for all tested widths. Design 1 is unsuitable for cell cultivation in channels narrower than 360 µm; Design 2 shows satisfactory results for channels down to 100 µm wide; x-axis represents widths of the channels, y-axis represents average number of cells per mm² [n=5].

RESULTS AND DISCUSSION

Design 2 shows satisfactory results in a range of channel widths from 360 µm to 100 µm; however, in narrower channels, the cell load and distribution is insufficient to obtain confluent cell culture (Fig. 1). In Design 3, an inlet and outlet having half the width of the cultivation channel were implemented to overcome this limit. These additional structures slow down fluid flow during cell injection and create backpressure at the outlet from the cultivation channel, promoting retention of cells in this channel. This improves cell adhesion to the ECM significantly compared to other designs (Fig. 2). Cell seeding becomes possible in channels as small as 60 µm wide (Fig. 1).

CONCLUSION

Currently, the glass-PDMS chip is compatible with microscopy, which allows the monitoring of e.g. inflammation processes in different channel widths. Moreover, sensors could be integrated into devices to allow determination of chemical parameters related to endothelial dysfunction.

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