Development of a microfluidic platform for cell cultivation in narrow channels

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Aim

Improvement of cell distribution and optimization of an endothelial cell (EC) cultivation platform in narrow channels (<120 µm).

Introduction

Microchannels are a good mimic for the *in vivo* environment of endothelial cells. However, reported channels have large dimensions [1], whereas our interest lies in the investigation of microvasculature. Seeding EC into channels as small as 25 µm wide is difficult, with cells tending to distribute unevenly along channels; cell layers containing gaps are often the result [2].

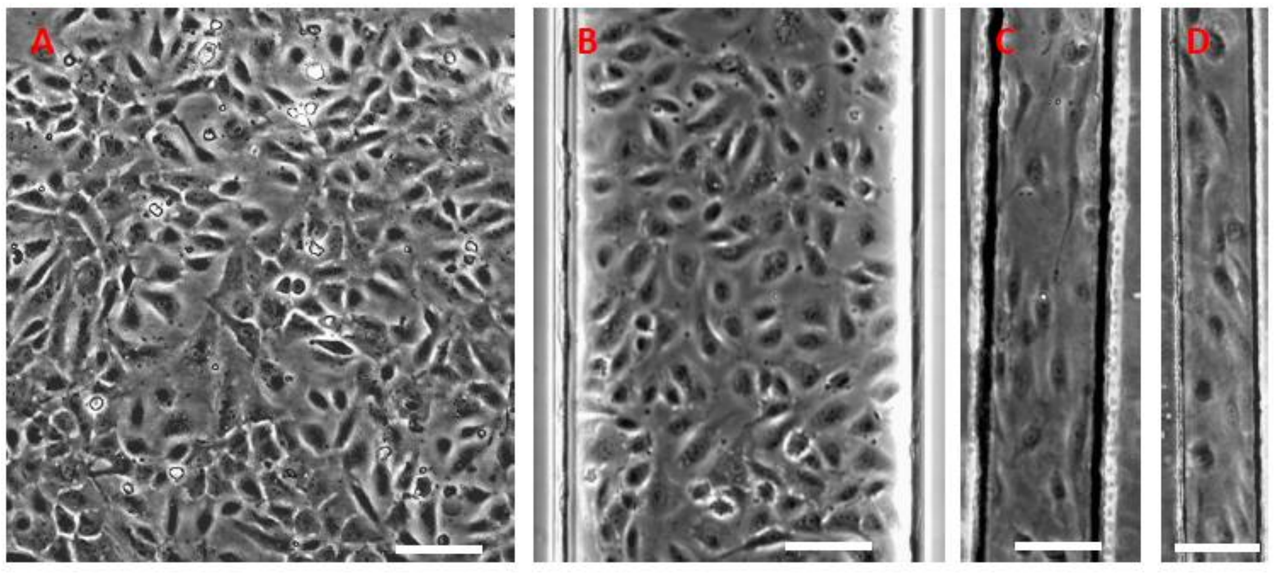
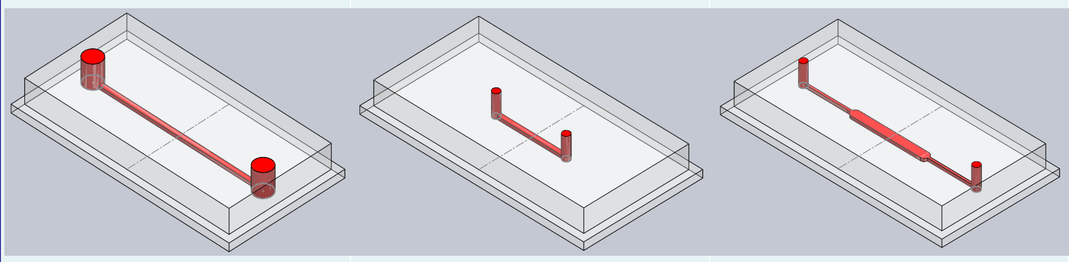


Fig.1 Comparison of HUVEC morphology in the standard well plate (A) and in different microfluidic channels (B,C,D). Results confirmed that HUVEC align themselves according to physical parameters of the cell cultivation platform. The presented microchannels have the following widths: 400 µm (B), 120µm (C) and 80 µm (D). Magnification 20x; all scale bars (lower right): 100 µm.

Material and Methods

Table 1	Design 1 [2]	Design 2	Design 3
Sketch			
Cultivation channel length: [mm]	*25*	*10*	*10*
Diameter of reservoirs: [mm]	*2.5*	*1*	*1*
Height of the channel [µm]	*100*	*100*	*100*
Proposed widths [µm]	*120, 100, 80, 60, 40, 25*	*120, 100, 80, 60, 40, 25*	*120, 100, 80, 60, 40, 25*
Additional parts	*No*	*No*	*Glass capillaries and 3D-printed reservoirs (Fig. 3)*
Materials	*Glass and PDMS*	*Glass and PDMS*	*Glass and PDMS (+PLA reservoirs)*
Type of coating	*1% gelatin + 0.5% glutaraldehyde*	*1% gelatin + 0.5% glutaraldehyde*	*1% gelatin + 0.5% glutaraldehyde*

Cell type	Human umbilical vein endothelial cells (HUVEC)
Cell concentration	5,000 cells/µL
Cell media	RPMI medium supplemented with 10% fetal calf serum
Cultivation conditions	95% air and 5% CO_2
Temperature	37°C

Results

- Successful cultivation of HUVEC for up to 72 h in all three chip designs.
- Progressive improvement of cell distribution along microchannels in chip designs 1 to 3, where Design 3 showed the best cell distribution and seeding reproducibility (Fig. 2).
- This significant improvement in cell distribution is thought to be due to the back pressure introduced to the system by the presence of the narrow exit channel in Design 3.

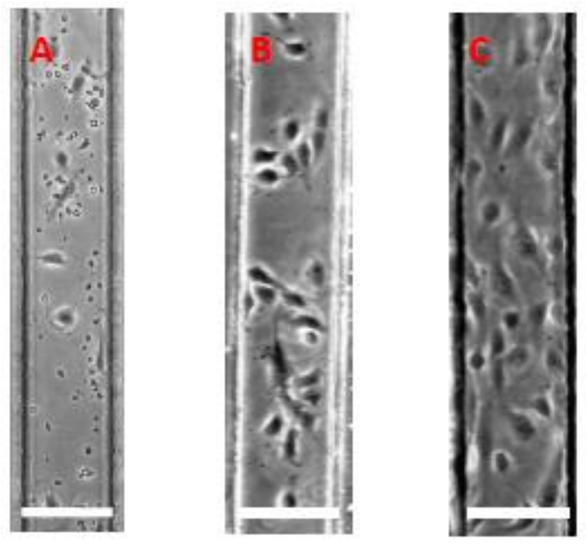


Fig.2 Figure presents comparison of progressive improvement of HUVEC distribution in different channel designs with a width of 120 µm (A<B<C). Photos were taken 2h after cell seeding. Magnification: 20x; all scale bars: 120 µm.

Outlook

- Developed platform will allow cultivation of cells in narrow channels (<120 µm) (Table 1, section: proposed widths), to better mimic *in vivo* conditions.
- Application of 3D-printed reservoirs in polylactic acid (PLA) to facilitate cell loading and attachment to pumps. Introduction of flow will help better mimic *in vivo* conditions.

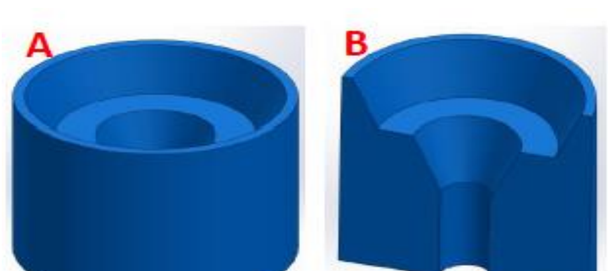


Fig.3 3D-printed reservoir (A) and its cross-section (B). Outer diameter: 4.25 mm, Inner diameter: 0.8 mm, Volume of inner reservoir: 3.5 µL, volume of outer reservoir: 30 µL, Height: 4 mm.

References

[1] Young EWK, Simmons CA, Macro- and microscale fluid flow systems for endothelial cell biology, Lab Chip 2010;10:143-160.
[2] Mulder PPMFA *et al.*, Characterization of HUVEC cultivated in microfluidic channels, Proc. Micro Total Analysis Systems 2005, Transducers Research Foundation, San Diego, U.S.A., 1383-1385 (2005).

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SEVENTH FRAMEWORK PROGRAMME

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LiPhos

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