Building endothelial cell covered microfluidic 3D channels in miniaturized bioreactors

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Enormous efforts have been made to develop circulation systems for physiological nutrient supply and waste removal of *in vitro* cultured tissues. These developments are aiming for *in vitro* generation of organ equivalents such as liver, lymph nodes and lung or even multi-organ systems for substance testing, research on organ regeneration or transplant manufacturing. Initially technical perfusion systems based on membranes, hollow fibers or networks of micro-channels were used for these purposes. However, none of these circulation systems were suitable for long-term tissue culture. Here we demonstrate a closed and self contained circulation system emulating the natural blood perfusion environment of vertebrates at tissue level. The system uses a miniaturized physiological blood circulation with an integrated micro-pump to provide circulation of microliter-volume to support milligrams of tissue. A procedure to colonize the whole channel surface with human microvascular endothelial cells (HMVEC) was developed. These artificial vessels are an important approach for systemic substance testing in multi-organ-chips. They create the conditions for circulation of nutrients through the tissue-chamber and prevent clumping inside the channels. The poster presents data on colonization of the microfluidic 3D channels and cell viability of the endothelial cell layers.

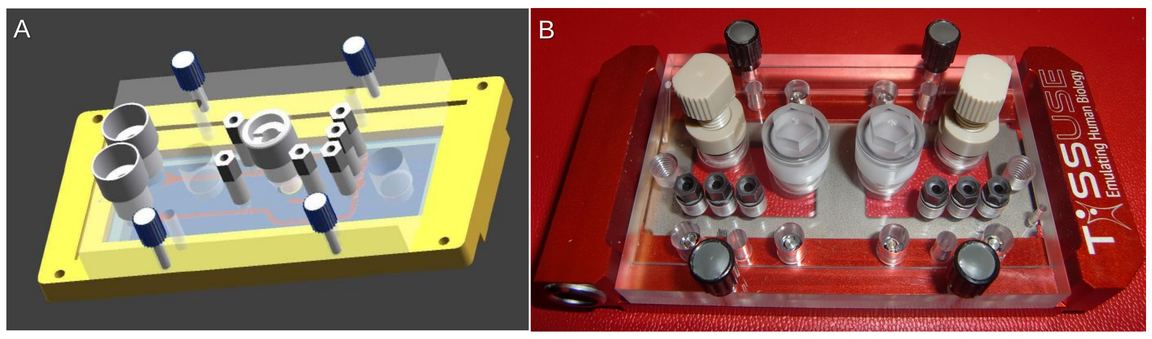
Methods

Microfluidic 3D channels were formed in PDMS by replica molding from master molds and were afterwards closed by bonding to a cover-slip by air plasma treatment. To retain PDMS hydrophilicity, channels were filled with culture medium immediately after sealing. An immortalized human microvascular endothelial cell line (HMEC-1) that stably expresses green fluorescent protein (GFP) was used for preliminary colonization tests. To emulate the natural blood perfusion environment, human dermal microvascular endothelial cells (HDMEC; Promocell) were used. The cells were seeded into the PDMS channels and formed confluent layers after subsequent static cultivation on each channel side. HDMECs were cultured over a period of 14 days in PDMS channels under dynamic flow.

Surface treatments intended to enhance cell adhesion on PDMS surfaces were evaluated by comparing cell morphology after 72 hour incubation.

Results

A miniaturized circulation system has been established over a period of 14 days by fully covering all channels and surfaces of the miniaturized bioreactor with human microvascular endothelial cells (Fig.2; Fig.4G and 4H). A peristaltic micro-pump was used to create culture medium circulation (Fig.1). By injecting 20 million cells/ml into the channels, a homogeneous distribution of cells throughout all channels was achieved (Fig.4A). During subsequent static incubation, cells adhered well to the air plasma treated channel walls (Fig.4B). After adaption to shear stress, HDMECs showed an elongation and alignment parallel to the flow direction. During the whole time they maintained adherence to the channel walls and were positive for CalceinAM viability staining (Fig.4C-G). Three-dimensional images indicate that cells formed confluent monolayers on the top and bottom of the channels (Fig.4H).



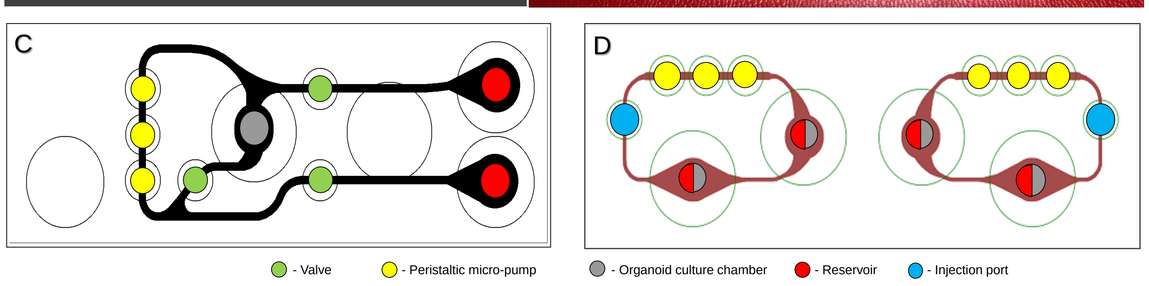


Fig. 1. Microfluidic device for perfused HMVEC culture. (A) Three-dimensional CAD design of the microfluidic device. Three layer device fabrication (top layer: polycarbonate, middle layer: PDMS, bottom layer: glas) placed on a holder supporting microscopic observation. **(B)** Photograph of the microfluidic device. **(C-D)** Schematic sketches of two different microfluidic designs with a channel width of 1000µm (C) and 500µm (D). A peristaltic micro-pump (●) was used to create circulation of culture medium through the channels. Collectors for extra fluid (●) can be used to manage the liquid levels and exchange in the system. Different organoids perfused by the same circulating fluid can be integrated into organoid culture chambers (●). An injection port (●) can be used for i.e. addition of substances into the system.

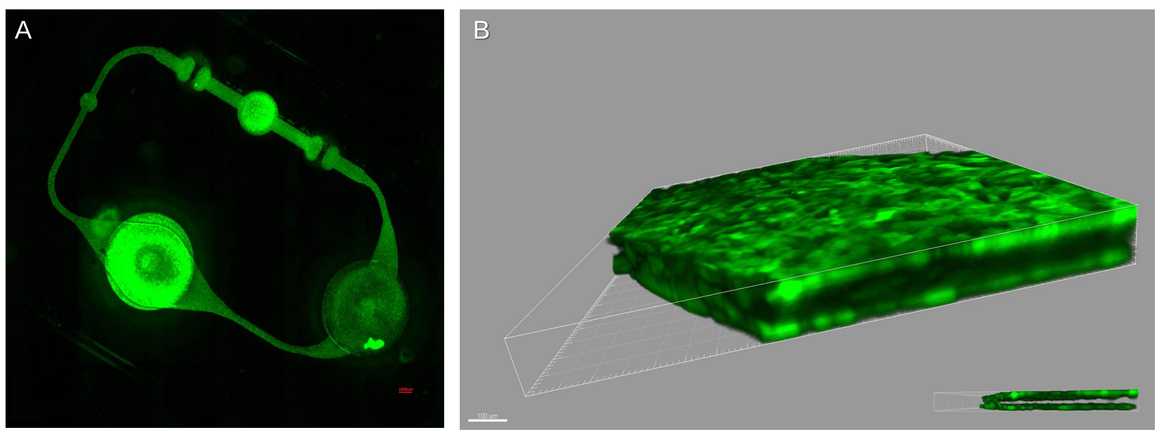


Fig. 2. HMEC-1-GFP lining the channels of the microfluidic device. (A) HMEC-1-GFP (green) form a confluent monolayer at the channel walls. **(B)** Three-dimensional view of one of the HMEC-1-GFP lined channels. HMEC-1-GFP cells cultured on top, bottom, and side faces mimicking a fully-lined blood vessel. The image was taken by two-photon microscopy.

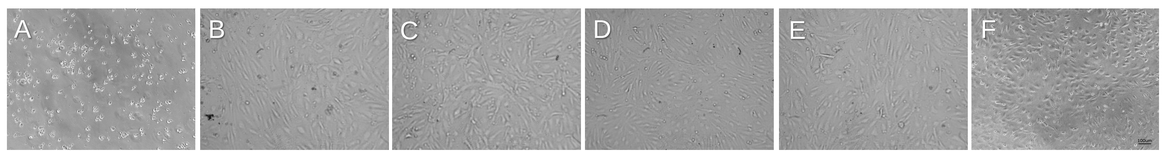


Fig. 3. Representative images of HDMEC on different treated PDMS surfaces after 72 hour incubation. (A) HDMEC did not adhere on untreated PDMS. **(B-F)** HDMEC cultivated on 0.2% gelatin coated (B), 0.02mg/ml collagen IV coated (C), 0.02mg/ml fibronectin coated (D), 0.1mg/ml MaxGel coated (E), and air plasma treated PDMS (F) formed a confluent monolayer.

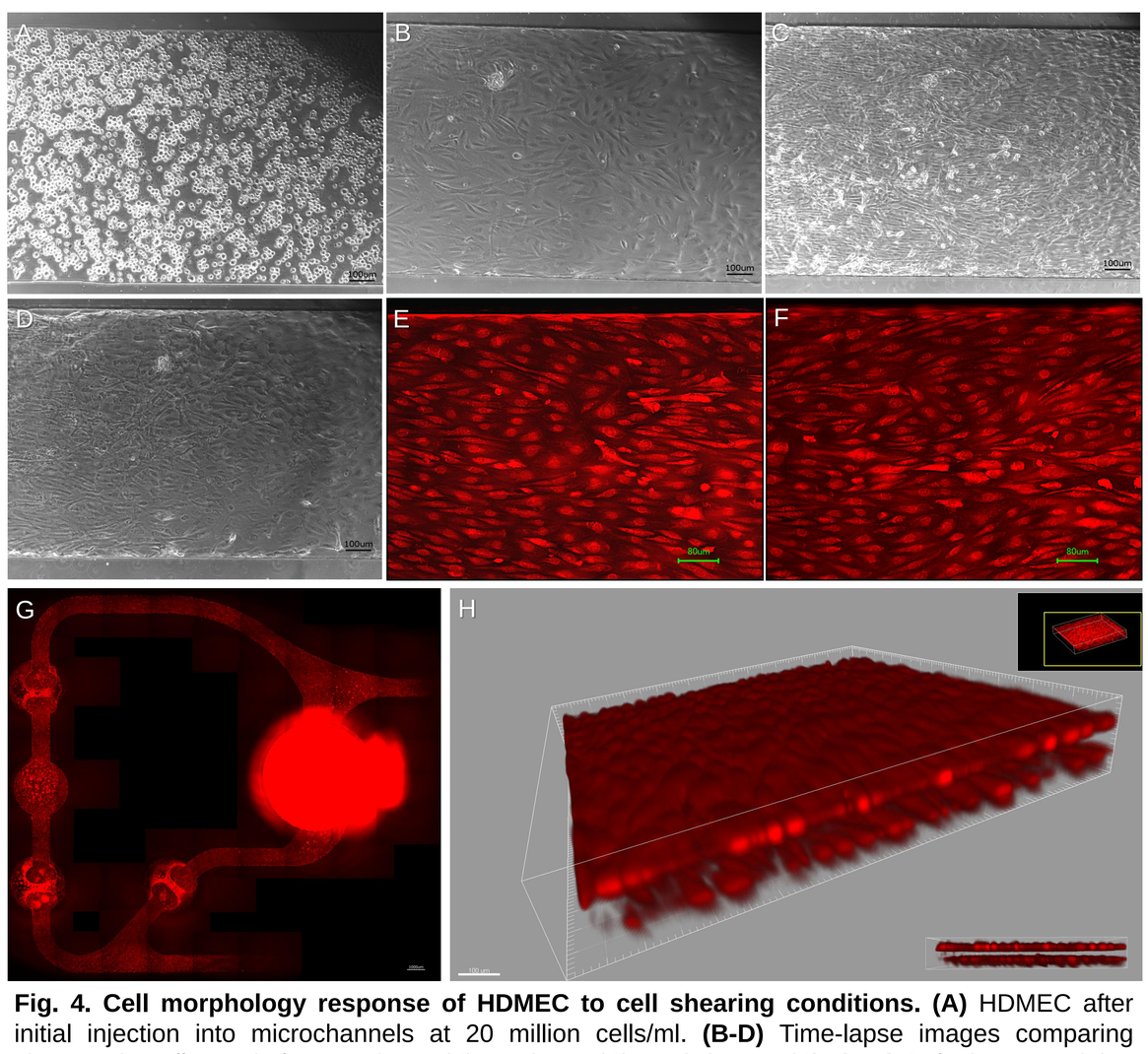


Fig. 4. Cell morphology response of HDMEC to cell shearing conditions. (A) HDMEC after initial injection into microchannels at 20 million cells/ml. **(B-D)** Time-lapse images comparing changes in cell morphology at day 1 (B), at day 7 (C), and day 14 (D). **(E-F)** Calcein AM staining (red) of HDMEC seeded on bottom (E) and top (F) faces of the channels. **(G)** Calcein AM staining showed viability of almost all HDMEC cultivated inside the microfluidic device. **(H)** Three-dimensional image of Calcein AM stained HDMEC lining the channel walls. The image was taken by two-photon microscopy.

Conclusion and Outlook

By designing and fabricating a series of different microfluidic chip designs, colonization with different HMVEC types has been established. Long-term cultivation of elongated and flow-aligned HDMECs inside the chip-based microcirculation was demonstrated over a period of 14 days. For such endothelialized microfluidic devices to be useful for substance testing, it is essential to show long-term viability and function in the presence of physiological flow rates as shown here. These artificial vessels are an important approach for systemic substance testing in multi-organ-chips. The miniaturized circulation system creates the conditions for circulation of nutrients through the organoid culture chamber and prevent clumping inside the channels.

Compared with conventional cell culture techniques, a microfluidic-based cell culture may mimic more accurate *in vivo*-like extracellular conditions, as the culture of cells and organ models in perfused microfluidic systems can improve their oxygen and nutrient supply. This makes it suitable for long-term cultivation and more efficient drug studies. In future, such endothelialized bioreactors might be used for testing vasoactive substances.

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