Emulating the Human Vasculature in a Multi-Organ-Chip Platform

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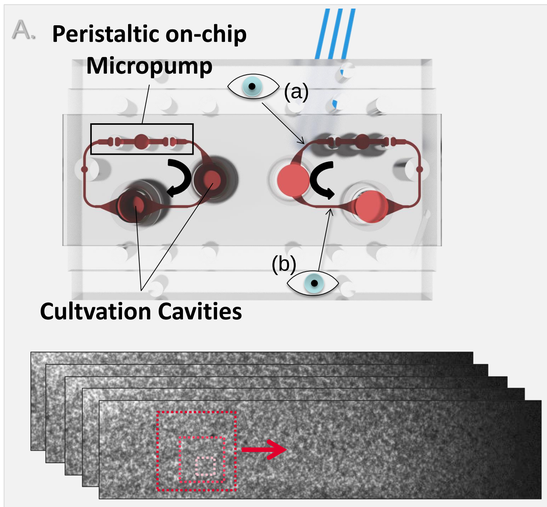
Background

Our multi-organ-chip platform (MOC) contributes to the ongoing development of *in vitro* substance testing systems with the ultimate aim of replacing animal models. The two-organ variant (2OC) comprises independent circuits each containing two separate cultivation cavities for any combination of 3D tissue constructs. The cavities are interconnected by microfluidic channels. The incorporated, on-chip micropumps provide pulsatile circulation at a microliter scale. Each circuit contains only 600 µL of volume, enabling autocrine and paracrine crosstalk between the cultivation cavities through the enriched medium.

Artificial vessels are vitally important in this testing platform. Not only because of their role in supplying tissues but also as the endothelial barrier interacts with medium constituents and regulates their diffusion into subjacent tissue. Attempts to recreate a continuous endothelial monolayer inside the cultivation cavities were conducted.



	(a)	(b)
Mean volumetric flow rate	3.06	3.78
Mean positive shear stress (→)	5.20	2.89
Mean negative shear stress (←)	-4.09	-2.70
Mean absolute shear stress	4.64	2.81

Figure 1: Micro Particle Image Velocimetry (µPIV). (A) Bottom view of the 2OC's independent circuits. Pulsatile perfusion of the system is created by a peristaltic on-chip micropump operated by periodic lowering and lifting of three successively arranged membranes. The volumetric flow rate in the chip can be controlled by the frequency of actuation, pressure and vacuum, which are externally applied to the membranes. In contrast to classical µPIV red blood cells (RBCs) were introduced into a pumping chip instead of polymeric beads. Consecutive images were acquired at accessible spots (a) and (b) with a high-speed CMOS camera (Baumer HXC40) connected to a standard light microscope. Images were analysed using PIVlab (© W. Thielicke). The procedure is even possible if visibility is impaired by cells, such as endothelial cells (ECs), lining the channel walls. Arrows indicate the direction of flow. (B) Example of a µPIV analysis using RBCs. Positive velocities indicate a movement as illustrated by the arrows in (A). The data are evaluated to optimise the shear load on the introduced ECs to then establish a physiological-like pulsatile flow.

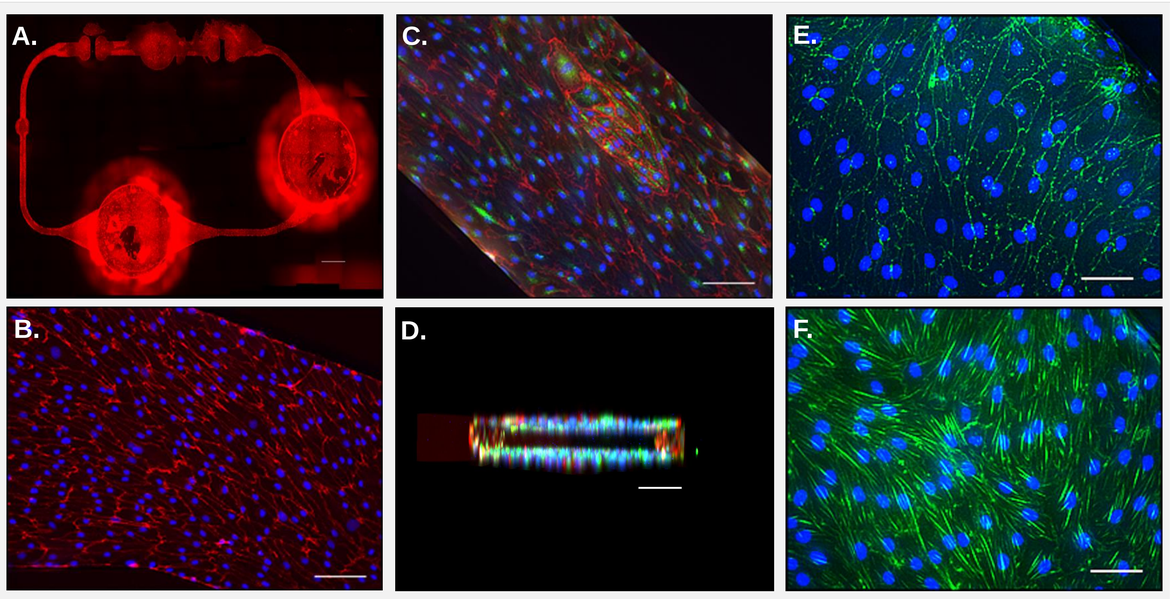


Figure 2: Endothelialisation of the microfluidic channels. (A) Human dermal microvascular ECs (HDMECs) were seeded into the channels of the 2OC. CalceinAM assay demonstrated viability and even distribution in all areas of the circulation after 4 days of pulsatile flow. Scale bar is 2 mm. (B) VE-Cadherin, (C) CD31 (red) and vWF (green) expression at spot (a) indicating proper endothelial cell behaviour. Scale bars are 100 µm. (D) Cross-section of HDMECs inside a microchannel stained for CD31 (red) and vWF (green). HDMECs were able to cover all the walls of the channels, forming a tight layer. Scale bar is 100 µm. (E) The EC's actin filaments under static and (F) dynamic culture conditions. Only the latter exhibits bundles of stress fibres aligning along the direction of flow. Scale bar is 50 µm. Nuclei in images (B) to (F) were counterstained with Hoechst (blue).

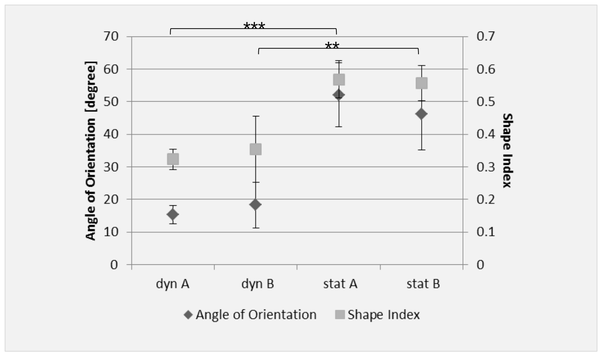


Figure 3: Quantification of alignment and morphology of ECs. Alignment (angle of orientation) and morphology (shape index, SI) of the HDMECs were compared under static and dynamic conditions at spots (a) and (b) after four days. The ECs migrate, proliferate and orientate with the direction of flow. Student's t-test with statistical significance **: $p < 0.01$; ***: $p < 0.001$.

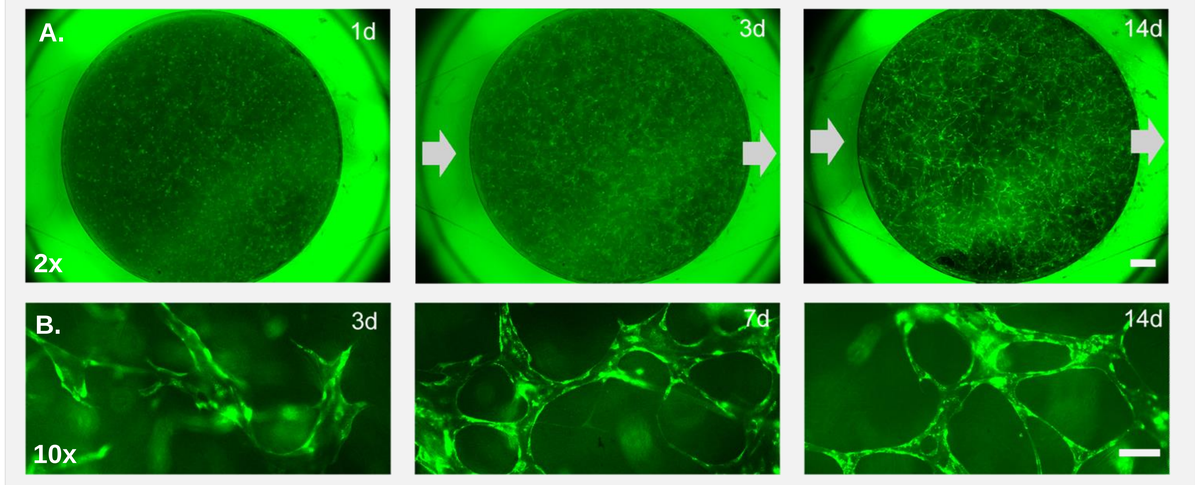


Figure 4: Creating a vascular bed for co-cultivation of 3D organoids. (A) Human umbilical vein ECs (HUVECs) in co-culture with adipose-derived stromal cells (ASCs) inside a fibrin scaffold. HUVECs were transfected with GFP. Despite the dynamic environment and cellular activity the fibrin gel maintained shape and stability over the course of 14 days. Scale bar is 500 µm. (B) Magnified view. Microvessels form within 3 to 7 days. The flow did not interfere with vessel formation. However, the tube-like structures were not perfused. Scale bar is 100µm.

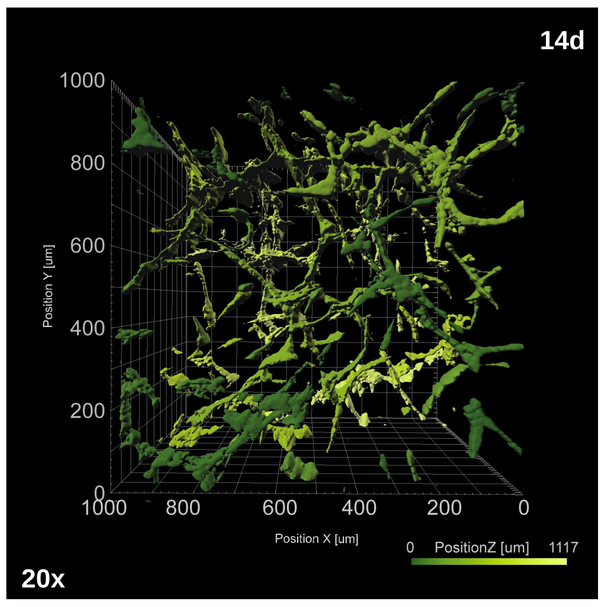


Figure 5: Three-dimensional organisation of the vascular bed. Rendering of a z-stack acquired with a two-photon-microscope. The z-positions of the branches are colour-coded – darker coloured branches being closer to the glass bottom. Branched microvessels were visible in great depth of the fibrin gel, thus, protruding the entire scaffold. The gel was approximately 3.5 mm thick.

Summary

The endothelialisation of the microfluidic channels was a first step to recreate a physiological environment to any biological derivative. The sophisticated 2OC design enabled the cultivation of HDMECs over a period of up to 40 days under near physiological conditions and provided a suitable shear stress environment to the ECs. The production of typical EC markers CD31, vWF and VE-cadherin demonstrated proper cell behaviour.

µPIV measurements were utilised to characterise and optimise the chip's flow dynamics. In contrast to polymeric beads RBCs prohibited the occasional adherence of particles to channel walls, cells, or cell residues considerably.

The integration of HUVECs in a fibrin scaffold created first, not yet perfusable microvessels. The basic structures and features of blood vessels were three-dimensionally recreated and are the basis for future organoid integration. Further, it is envisaged to establish a continuous endothelial barrier from the bulk artificial vessels (Fig. 2) to the smallest, spontaneously emerged microcapillaries (Fig. 5). This is crucial for physiological-like interactions, regulation and homeostasis within an organoid (co-)culture. Moreover, it is a prerequisite for using blood in the chip and to address immunological queries.

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