Development of 3D human cells-based microvessels microfluidic model for replacement of animals in microvascular disease study.

Human**z**ellenbasierten **M**ikrofluidik-**M**ikroblutgefäß**m**odells zum Ersatz von Tierversuchen (**HZ-MMM**)

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It has been recognized that microcirculation plays an important role in pathogenesis of many diseases. Understanding and regulation of microvasculature are urgently needed for developing effective therapeutic strategies. However, suitable models allowing in depth biomedical research of microcirculation are missing. Most of the research is performed with the use of animal models. The main aim of the project is to develop human cells-based microvessel microfluidic model (HZ-MMM). Vessels are grown from microvascular endothelial cells (EC) and accompanying cells in a microfluidic device by the process of angiogenesis. The model is adjusted for applications in basic research and drug development.

In the Seventh Report from the Commission to the Council and the European Parliament on the Statistics of the number of animals used for experimental and other scientific purposes in the member states of the European Union it is stated that the general number of animals used for experimental and other scientific purposes has decreased to just below 11,5 million [1]. On the contrary, the number of animals used for fundamental biological studies has increased by 21% since 2010. The number of animals used for drug research and development has also increased. This statistics necessitates further attempts to establish and integrate alternative models of experimentation into basic and transitional research.

At present, in vivo animal models are the main tools for studying microcirculation. Experimental approaches are mainly based on imaging of prepared tissues on anesthetized animal. This approach assumes induction of diseased conditions followed by observations on animal for several hours and leads inevitably to the sacrifice of the animal after the experiment is completed. Rodents and bigger animal (rabbits, newborn pigs) are used for such kind of research. However, despite recognized limitations of animal kidney disease models [2,3], rats and mice remain the favorite models. Considering multiple limitations of in vivo approach, like effects of anesthesia and tissue preparation surgery, large number of animals required to reach high quality statistical results, difference between human and animal studies, this research leads to unjustified animal suffering. In the frames of 3R strategy [4], the HZ-MMM should replace animals in basic research and reduce the number of animals used at the development stage to the minimal required for pharmacokinetic optimization and toxicity.

In this project, microfluidic chip for controllable growth of microvessels (Kapillar- und Arteriolen Bildung durch den Prozess der Angiogenese, KABA-chip) and microfluidic actuation platform for operation of this chip are developed. Principles of the chip operation and microvessels formation are shown schematically in Fig. 1.

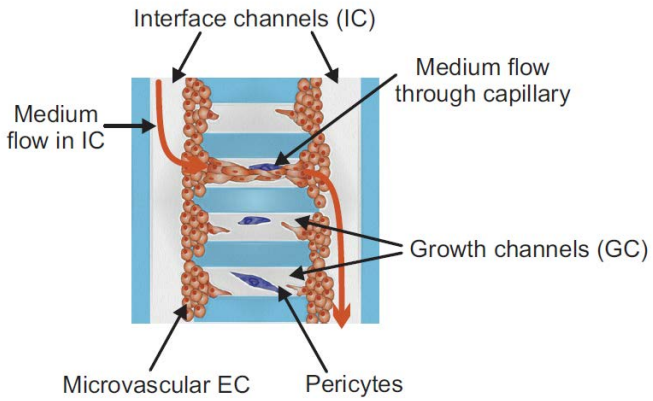

Figure 1. Schematic of the KABA-chip and microvessels angiogenesis in the chip.

The KABA-chip is molded in polydimethylsiloxane (PDMS) and covered by glass slide using plasma bonding [5]. The new approach is implemented for fabrication of the mould by direct laser writing in the photo-resist using two-photon polymerization (2PP) technique [6]. 2PP technique allows 3D fabrication of polymer objects with sub-µm resolution. Since lithographic mask is not needed for this process, fabrication of mould is very flexible allowing fast production of moulds of any desired complexity. Ability of 2PP direct laser writing to create 3D structures with sub-micrometer resolution is crucial in fabrication of the KABA-chip. An example of the chip replicated in PDMS is shown in Fig. 2 (Left).

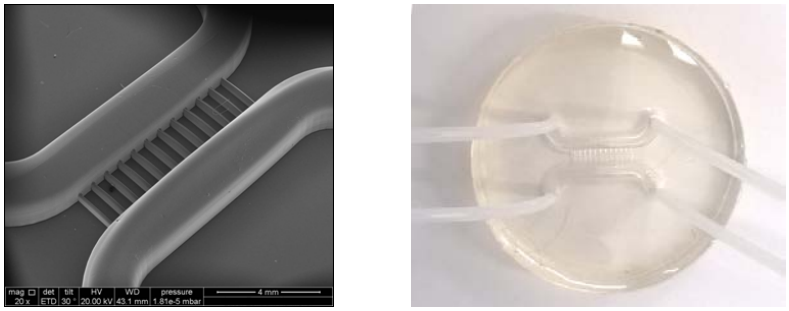

Figure 2. Scanning electron microscope image of the KABA-chip replicated in PDMS (Left) and assembled KABA-chip (Right). This chip contains 12 growth channels of 200x200 µm cross-section and 2 mm long. Cross-section of the medium flow cannels is 1x2 mm.

Angiogenesis in the KABA-chip was achieved by seeding endothelial human cells and fibroblasts into the medium flow channels. The angiogenesis is starting on the fourth day after seeding. An example of the microvessel grown into the thin channel is shown in Fig 3.

In conclusion, formation of blood microvessels in newly developed KABA-chip has been demonstrated. Further objective is to achieve the microvessel penetration through the whole length of the growth cannels and perfusion of the microvessel by liquid in controllable way. The developed model will be applied for studies of microcirculation disorders.

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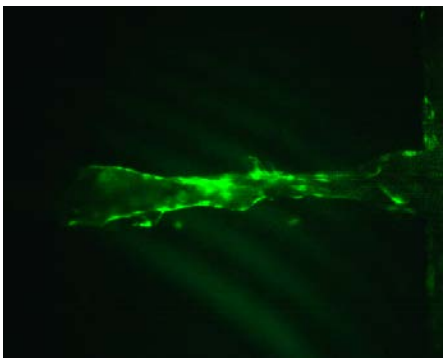

Figure 3. Scanning confocal microscopy image of the fibroblasts assisted angiogenesis in KABA-chip. The image is recorded on the seventh day after seeding.

References

1. http://ec.europa.eu/environment/chemicals/lab_animals/reports_en.htm

2. Becker GJ, Hewitson TD (2013) Animal models of chronic kidney disease: useful but not perfect. *Nephrol Dial Transplant* **28**: 2432-2438.

3. Susztak K, Bitzer M, Meyer TW, Hostetter TH (2008) *Animal models of renal disease. Kidney Int* **73**: 526-528.

4. Russell, W.M.S. and Burch, R.L., *The Principles of Humane Experimental Technique*, Methuen, London, 1959

5. D.C.D. Duffy, J.C.J. McDonald, O.J.O. Schueller, G.M.G. Whitesides. Rapid prototyping of microfluidic systems in poly(dimethylsiloxane). *Anal. Chem.* **70**, 4974–4984 (1998).

6. M. Farsari, B. Chichkov, Two-photon fabrication. *Nat. Photonics* **8**, 450–452 (2009).