On-Chip Two-Photon Polymerization of Vascular Structures

A. Dobos[1,2], P. Gruber[1,2],J. Van Hoorick[3,4], S. Muehleder[2,5], W. Holnthoner[2,5], S. Van Vlierberghe[3,4], A. Ovsianikov[1,2]

[1] TU Wien, Institute of Materials Science and Technology, Getreidemarkt 9, 1060 Vienna, Austria

[2] Austrian Cluster for Tissue Regeneration

[3] Polymer Chemistry and Biomaterials Group, Centre of Macromolecular Chemistry, Ghent University, Krijgslaan 281, S4, 9000 Ghent, Belgium

[4]Brussels Photonics, Department of Applied Physics and Photonics, Flanders Make and Vrije Universiteit Brussel, Brussels, Belgium

[5] Ludwig Boltzmann Institute of Experimental and Clinical Traumatology, Donaueschingenstrasse 13, Vienna, Austria

One of the central challenges in tissue engineering is the lack of vascularization in the created artificial tissue constructs. There are several different approaches which have been examined for the formation of vascular networks, such as three-dimensional (3D) bioprinting, classical UV-photo-polymerization, micropatterning, and decellularization and reseeding of preexisting extracellular matrixes[1]. However, the directed alignment of endothelial cells and the construction of a stable perfusable system within a microfluidic channel present difficulties with the previously mentioned methods. Two-photon polymerization (2PP) is a powerful technique for the fabrication of high resolution 3D structures using a femtosecond near infrared laser focused into a photo-polymerizable resin[2].

The photosensitive material used throughout this study was a combination of thiol and norbornene modified gelatin (i.e. gel-SH[3] and gel-NB[4]). Compared to traditional (meth)acrylate based systems, thiol-ene click chemistry provides advantages such as less swelling/shrinkage of the hydrogel upon polymerization and insensitivity to oxygen inhibition, which is particularly beneficial in a microfluidic application. By using this dual-component system with a biocompatible diazosulfonate two-photon initiator, it was possible to structure a perfusable microchannel system with physiologically relevant diameters within a microfluidic chip (**Figure 1**).

Direct encapsulation of co-cultures of human umbilical vein endothelial cells (HUVECs) and supporting adipose-derived stem cells (ASC/TERT1) could also be obtained via 2PP. This approach provides high initial cell loading and homogeneous distribution of cells within the printed hydrogel. The fabricated channels within the gel act as a guide for the alignment of cells within the matrix, creating a more directed vascular tube formation.

This platform for the fabrication of microvascular networks-on-chip using 2PP can advance the development of novel drug screening platforms, and more complex tissue and disease models.

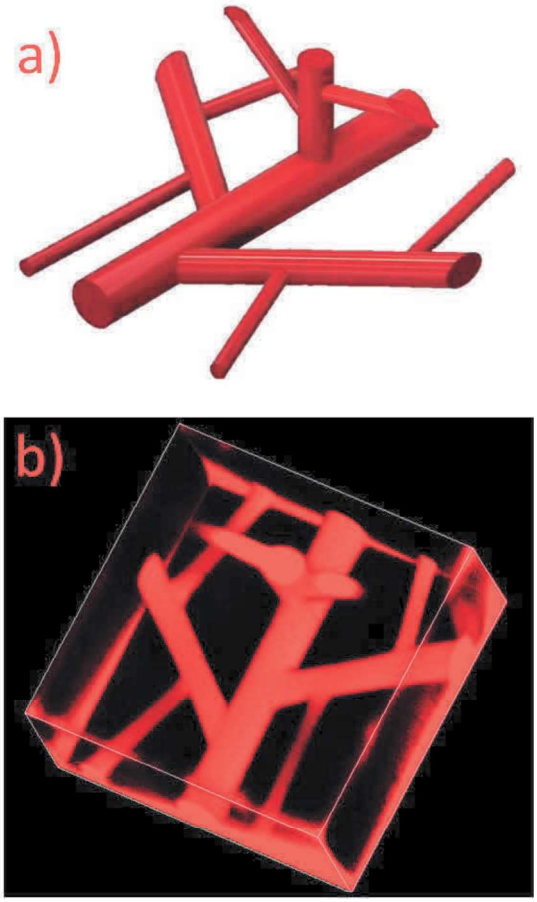

Figure 1. Microvessels-on-Chip. a) CAD image of the vessels b) Two-photon polymerized hydrogel-on chip based on the CAD design

References:

1. Kim, J. J., Hou, L. & Huang, N. F. Vascularization of three-dimensional engineered tissues for regenerative medicine applications. *Acta Biomater.* **41,** 17–26 (2016).
2. Ovsianikov, A. *et al.* Laser printing of cells into 3D scaffolds. *Biofabrication* **2,** 014104 (2010).
3. Van Vlierberghe, S., Schacht, E. & Dubruel, P. Reversible gelatin-based hydrogels: Finetuning of material properties. *Eur. Polym. J.* **47,** 1039–1047 (2011).
4. Van Hoorick, J. *et al.* Highly Reactive Thiol-Norbornene Photo-Click Hydrogels: Towards Improved Processability. *Macromol. Rapid Commun.* (2018).