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Zhang, Rujing; Martínez, Rodrigo Guzmán; Larsen, Niels Bent

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Stereolithography-based 3D printing of hydrogels for long-term cell culture and synthetic vasculature

Rujing Zhang¹, Rodrigo Guzmán¹, Niels B. Larsen*¹

1: DTU Nanotech

*Corresponding author email: nibl@nanotech.dtu.dk

Three-dimensional (3D) soft biomaterial scaffolds for long-term cell culture are critical components in tissue engineering and regenerative medicine. However, it is still challenging to construct such scaffolds with desired structural stability and resolution using soft hydrogel. We have developed a method to fabricate 3D biocompatible hydrogel scaffolds at sub-200 μm resolution using projection-based stereolithography to address the biomedical challenges of stem cell culture and synthetic vasculature. Poly(ethylene glycol) (PEG) hydrogels were 3D printed by spatially controlled, light-induced solidification of an aqueous pre-polymer solution (PEG-diacrylate 700 Da and 5000 Da, lithium acylphosphinate photoinitiator, Quinoline Yellow as absorber) using a modified commercial stereolithography printing system (envisionTec Micro, 405 nm illumination). Optimization of the printing configurations allowed for printing of pyramid-shaped micro-containers for long-term 3D stem cell culture and perfusable micro-channels approaching arteriole dimensions (100 μm cross-section). Human mesenchymal stem cell (hMSC) culture on the pyramidal micro-containers showed that hMSC spheroids formed spontaneously after 24 h incubation, and high cell viability (> 80%) was sustained in the stable cultured spheroids for 7 days of incubation. Compared to the technically delicate state-of-the-art hanging drop methodology used for spheroid formation, our time- and work-efficient approach in 3D printed low cell adhesion hydrogels provides improved control of hMSC spheroid size and shape. As synthetic arteriole and venule analogs, our channel structures could be freely designed and constructed at sub-200 μm resolution in a single automated process (100 μm X 100 μm square channels), a resolution few methods can achieve in soft hydrogels with full design freedom in all three dimensions, compared to conventional methods such as hydrogel bonding and sacrificial molding. The aim of printing micro-channels within bulk hydrogels is to further fabricate 3D microvascular scaffolds for tissue engineering, since vascularization is generally considered as the most important obstacle in the field. On-going cell culture experiments show high compatibility of the printed micro-channel structures to an endothelial cell line (CRL2922) to be employed for endothelialization of the printed vascular network analogs.

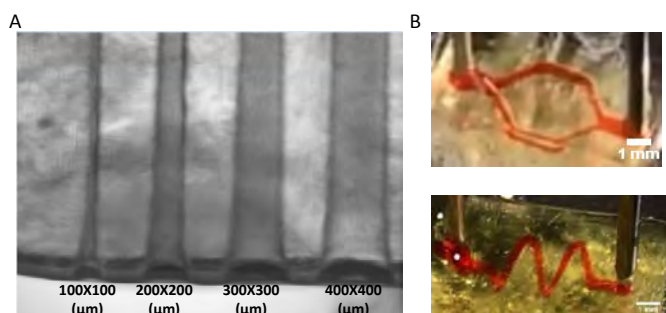


Figure: A) Top view of printed micro-channels by phase contrast microscopy. B) Snapshots for perfusion of a bifurcation channel system (top) and a spiral channel (down).