Stereolithography-based 3D-printing of transparent and biocompatible microfluidics for Organs-on-a-Chip applications

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Introduction

Soft-lithography methods are routinely applied in biomedical research, for instance for the fabrication of microfluidic devices, multichannel bioprinting print heads and Organs-on-a-Chip. While soft-lithography offers excellent resolution, it is a complex process and geometrically limited. Transparent 3D-printing could overcome these challenges by enabling complex 3D-shapes, round features and single step fabrication of closed channel systems. However, to compete 3D-printed microfluidics need to comply with multiple requirements, such as low surface roughness, good optical transparency, highly resolved features and high cytocompatibility. Stereolithography printing has proven to be a suitable method for this purpose, as it offers excellent resolution (down to 30 – 40 µm) and is generally suited to process transparent and biocompatible materials. However, sufficient transparency for high magnification and fluorescence microscopy, closed channel fabrication and long-term cytocompatibility currently remain a challenge.

Materials and methods

In order to fabricate 3D-printed components that fulfil the above-mentioned criteria, we established a novel stereolithography strategy based on a modified Asiga Pico 2^{HD27} printer. We investigated a commercial resin (Asiga PlasClear) and two own PEG-DA formulations. To reduce surface roughness and bulk defects, modifications of the build plate and resin vat were realized. The surface quality, optical transparency and chemical composition of the prints were characterized by confocal microscopy, AFM as well as UV/VIS and Raman spectroscopy. Finally, cytocompatibility of the applied materials was fluorescence microscopically assessed using primary endothelial cells and fibroblast cell lines.

Results and discussion

The confocal microscopy and UV/VIS spectroscopy results revealed a significant advantage of the build plate and resin vat modification compared to a non-modified printer. All prints exhibited low surface roughness of less than 0.3 µm and superior optical transparency of over 90 % in the visible light wavelength range (400 – 800 nm). However, during light microscopy, a periodic structure was visible in all printed parts indicating interference patterns caused by the voxel illumination during DLP. Additional AFM microscopy supported this assumption, as the dimensions of the surface features were of the size of the DLP pixels of 27 µm. Interestingly, the height of these periodic features were found to be dependent on the photoinitiator (BAPO) and -sensitizer (ITX) concentration and varied between 40 and 400 nm. Even more, the presence and concentration of photoinitiator and photosensitizer proved crucial for optimum print resolution and defined channel geometries. For this reason, different combinations and concentrations of the photoinitiator and photosensitizer were systematically investigated regarding minimum feature size and general printability. The commercial resin proved to be easy in use and offered a minimum feature size of 200 µm. Open channel structures could be achieved with a diameter of down to 350 µm. For the

PEG-DA based resins, prints without photosensitizer exhibited low spatial resolution and low channel quality. The addition of low concentrations of photosensitizers led to clearly defined prints with minimum feature sizes of 150 µm outperforming the commercial resin.
Cytocompatibility tests with primary endothelial cells and established fibroblast cell lines and Raman spectroscopy revealed the need for post-fabrication treatments such as prolonged solvent extraction and UV-exposure to remove toxic photoinitiator and photosensitizer residues. Additionally, resin formulation and photoinitiator concentration were found to further impact post-fabrication cytocompatibility. Finally, high magnification phase contrast and fluorescence microscopy (up to 400 x) could be successfully demonstrated with various fluorescent dyes. Still, the commercial resin was shown not to be compatible with DAPI staining, as the cured parts absorbed most of the light with wavelength below 400 nm, which lies within the excitation wavelength band of DAPI. Finally, the printed microfluidic chips were used in Organ-on-a-Chip models containing cells directly cultured on the surface of parts as well as in a hydrogel matrix. Cell culture under perfusion was feasible for various days with cells showing the expected morphology under perfusion.

Summary and outlook

In summary, our work reveals that DLP printing is a versatile and reliable platform for the fabrication of transparent microfluidic devices that entail high feature resolution, optical clarity and cytocompatibility. Even more, we could prove that printer modification and resin optimization can be advisable to fully exploiting its potential. These qualities make DLP printing a promising technology for rapid prototyping of microfluidic components, customized cell culture dishes and Organ-on-a-Chip devices.

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