Fabrication of 3D Scaffolds by Two-Photon Polymerization

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Statement of Purpose: The application of scaffolds for growing tissue substitutes is an important topic in tissue engineering. Numerous methods have been developed and applied for the fabrication of scaffolds. In order to study and control cell migration and cellular interactions within the scaffold, novel technologies capable of producing 3D structures in accordance to predefined design are required. It is therefore not surprising that in recent years active scaffold fabrication methods, having their origin in rapid prototyping, have received increasing attention. These include stereolithography, selective laser sintering, and fused deposition modelling. Fabrication of CAD scaffolds with relatively large pores and spatial resolution ranging from ten to hundreds of micrometers, depending on the method and the used material, is possible with these technologies.
Two-photon polymerization (2PP) is a rapid and straightforward method, capable of producing complex 3D structures in a single step. In this novel approach, the multiphoton absorption of femtosecond laser pulses is used to induce a highly localised chemical reaction leading to a photopolymerization of the material. By moving the laser focus in 3D the trace of modified material is created. In the next step, the unmodified material is removed by an appropriate developer, and the fabricated structure is revealed. Therefore, fabrication of any computer generated 3D structure by "direct laser recording" into the volume of photosensitive material is possible. Recently we have explored suitability of the 2PP technique for the biomedical applications and in particular for the fabrication 3D scaffolds for tissue engineering. The resolution of the 2PP technique can be scaled up and down by choosing appropriate applying different focusing optics and laser powers. Structures with feature sizes, ranging from just below than 100nm up to hundreds of micrometers can now be produced, using this single technique. Therefore, 2PP allows closing the existing gap in structural parameters of scaffolds, produced by different methods.
Methods: In our work we apply near-infrared Ti:sapphire femtosecond laser pulses (120 fs, 80 MHz, 780 nm) for 3D material processing. Polyethylene glycol diacrylate (PEGda) is used for the fabrication of scaffolds. In order to obtain a photopolymerizable formulation photoinitiator is added to the material by mixing. After the 2PP processing the PEGda samples are washed in distilled water, in order to remove the unpolymerized material. The structures are analyzed by optical, fluorescence and scanning electron microscopy.

Results: It is found that PEGda can be structured by the 2PP technique, producing structures with submicrometer features. Figure 1a shows the CAD design of the 3D scaffold composed from layers of hollow cylinders in the hexagonal arrangement. The scaffold represents a section of the tube-like construct. A characteristic detail of this highly porous scaffold is vertical pore orientation allowing easy access from the top for cell deposition.

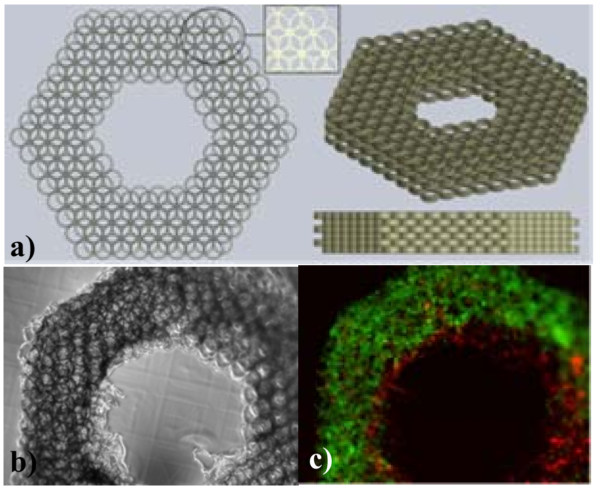

Figure 1. 3D Scaffold: (a) CAD design; (b) optical image of the 2PP-fabricated scaffold and (c) fluorescence image of the scaffold seeded with two different cell types - SMCs (green) and ECs (red).

Finally the scaffolds were seeded with smooth muscle cells (SMC) and endothelial cell (EC) by means of laser-induced forward transfer. It is demonstrated that within the 2PP-produced scaffolds it is possible to precisely arrange the location of the different cell types the entire thickness of the scaffold (see Figure 1c).
Conclusions: A novel approach for the fabrication of the 3D scaffolds from photopolymerizable materials is presented. It is shown that by two-photon polymerization (2PP) structures with submicrometer feature size can be produced from polyethylene glycol diacrylate (PEGda). Furthermore, 2PP has been used to fabricate highly porous 3D scaffolds with vertical pore orientation. Such scaffold configuration is well suited for seeding the scaffolds with laser induced forward transfer and therefore allows to arrange the location of different cell types precisely within the scaffold.