Simple 3D direct laser writing for tissue engineering

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One of the main goals in tissue engineering research is to reproduce in vitro structures, mimicking the extracellular matrix (ECM), able to favorite the stem cell differentiation and the tissue growth. A 3D porous structure suitable to the scope is called scaffold.[1,2] In this contribution, we propose a simple process for scaffold fabrication based on photolithography using one photon polymerization. We used polyethyleneglycol-diacrylate (PEGDa) as photopolymerizable material for the scaffold. The PEGDa solution was prepared using PEGDa (MW 575) at 75% in ethanol. Irgacure 819 was dissolved in the alcoholic part of the solution to promote the polymerization process at a 7 mM concentration.

The main concerns in 3D photolithographic applications is to restrict the polymerization to a well-defined volume in order to achieve the desired resolution. The vertical resolution is often achieved exploiting two photon absorption (2PA) due to the dependency from the square of the light beam intensity. That requires the use of ultrafast-pulsed lasers which are able to concentrate enough energy in a well confined spatio-temporal volume. We present a simple alternative to such 2PA set-up that allows to directly write a photopolymerized pattern using a blue diode laser.

Figure 1 shows the optical setup. We used a diode laser (60 mW) at 448nm wavelength coupled with a multimode optical fiber (600µm core diameter) (OF). The outlet of the OF was set above a microscope objective. The distance between the OF and the objective was 22 cm. The objective was mounted on an x–y movement in order to control the position of the focusing point. The optical power focused on the sample was 60 µW.

The excitation wavelength falls on the tail of the absorption of the photopolymerizable PEGDA solution. As a consequence, the efficiency of the photopolymerization activation is strongly reduced. The efficiency of photopolymerization, as measured by the width of the polymerized linear structures, is represented as a function of the Irgacure concentration in Figure 2. A threshold value for the Irgacure concentration is set at the value of 6 mM below which no polymerization is produced. Figure 3 shows the intensity profile of the excitation light as a function of the depth in the solution for three different focusing optics. The intensity of the light reduces by a factor 5 within a penetration depth equal to the depth of focus (DOF) of the optical system. That sets the limit for the active volume to the minimal waist of the focused beam. The threshold for polymerization is not reached outside that volume where the intensity is not enough to trigger the process.

Figure 4 shows an optical image of a 3D scaffold (alternate woodpile structure) realized with this technique. We achieved a resolution of about 5-7µm for samples of about 1x1x1 mm^3. Evidence of cell differentiation using human Lin- Sca-1+ cardiac progenitor cells has been shown in absence of any concurring biochemical stimulus using woodpile scaffolds fabricated by this technique.[3]

[1] F. Mochi, et al (2016) IET Conference Proceedings, 84 (4.)
[2] P. Prosposito et al. (2017) Materials Science Forum, 879:1519-1523
[3] M.Ciocci et al, (2017) Stem Cell Dev. submitted.

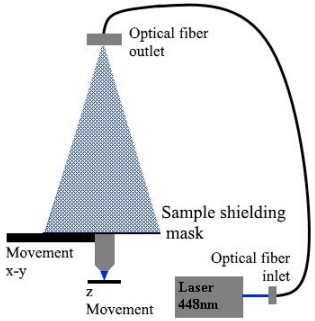

Figure 1. Scheme of the optical setup.

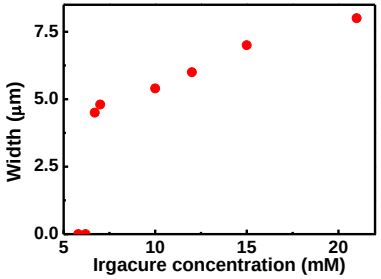

Figure 2. Width of the polymerized linear structures as a function of Irgacure 819 concentration

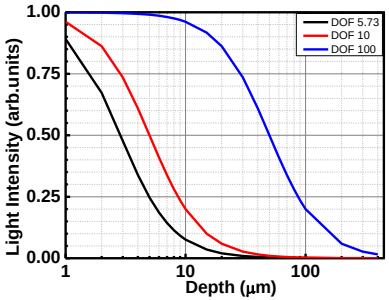

Figure 3. Intensity profile of the excitation light as a function of the depth in the solution for three different focusing optics.

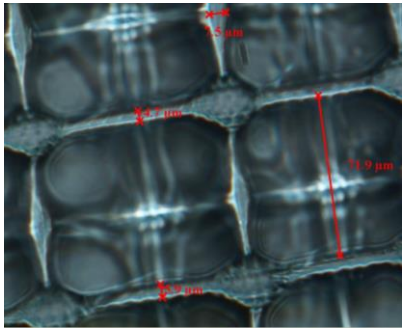

Figure 4. 50x optical micrograph of 1PP-PEGda (MW 575) woodpile structure.