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One Photon 3D Polymerization via Direct Laser Writing

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Abstract. A way to produce 3D scaffold is via laser stereolithography. We propose a method of direct laser writing for micro-stereolithography in which we use as light source a low power blue diode laser with a wavelength of 448nm. The material chosen for scaffold fabrication is a polyethylene glycol diacrylate (PEGDA) solution at concentration of 75% in ethanol. We chose a short PEGDA molecule with a molecular weight of 575 g/mol, in order to obtain a better control over the polymerization. We used Irgacure 819 as photoinitiator to initiate the photopolymerization. The absorption of the Irgacure 819 almost drops to zero at the excitation wavelength, so the efficiency of the photopolymerization is strongly reduced. Since the intensity of the light reduces by a factor 5 within a penetration depth, equal to the depth of focus of the optical system, we achieve a fine control of the vertical and lateral photopolymerization of the solution. The threshold for effective polymerization is not reached outside that region.

Introduction

The quest for transplantable portion of organs and/or for organ substitutes or model in modern medicine and surgery has boosted the development of the field of tissue engineering.[1,2,3] Medicine, biology, chemistry, physics and engineering cooperate in the attempt to produce portions of tissue endowed with specific functionalities, able to restore, maintain, or improve damaged tissues or even whole organs.[4,5] Besides stem cells and bioactive growth factors, tissue engineering requires biocompatible structures (scaffolds) able to favourite the in-vitro growth and organization of cells towards the generation of functional bio-substitutes.[6,7] Since the extra-cellular matrix (ECM) modulates the cell's proliferation, differentiation and architectural organization in the living tissues, it has constantly grown the interest on the techniques suitable to realize three-dimensional bio-scaffolds mimicking the ECM.[8,9] Several techniques can be employed to obtain such structure like phase-separation, electrospinning, 3D-printing.[10,11] In particular, 3D-printing offers the possibility to design bio-structures which efficiently mimic the extracellular matrix by means of a fine control of the morphology and mechanical properties of the scaffold.[12,13] Stereolithographic (SLA) methods use a deflected laser beam or a projected light source to cure and harden exposed areas of photopolymer at the surface of a reservoir of material. Multilayer scaffolds are typically fabricated by lowering a stage and curing successive layers of the construct. The construction of tailored structures with sub-100 nm resolution can be achieved by direct laser writing (DLW) by multi-photon polymerization (MPP) exploiting the two photon absorption.[14] Although it is rather a slow technique and requires expensive and specialized equipment, such a stereolithographic technique offers superior resolution and does not require the need of recoating or layer-by layer fabrication.[15] The efficiency of 2P-absorption depends on the square of the light intensity and it is several orders of magnitude weaker than linear absorption. That determines the extreme spatial resolution of the technique since a sufficient energy density is reached only at the tight focus of the beam while out of the focus region only linear processes would be allowed but the long wavelength is not resonant with any absorption transition. Linear absorption photopolymerization, or one photon polymerization is limited by the effect of the penetration of the light below the layer that is intended to be written (i.e. to layers which were previously written). Therefore, particular care must be put in order to reduce the penetration or to mask the effects.

In this work we propose a simple direct laser writing method based on 1P microstereolithography exploiting the optical characteristics of carefully selected photopolymerizable system (polymer, photoinitiator and absorption limiter).

Experimental

The photosensitive hydrogel solution was composed by polyethylene glycol diacrylate (PEGDA) MW 575 g/mol and the Irgacure 819 (IRG819) solubilized in ethanol. All reagents were purchased by Sigma Aldrich.

The same hydrogel solution was used as in a previous work where microstereolithography technique was used to obtain the scaffold. [12] Differently from the latter, however, we did not need to use an absorber dye to achieve vertical resolution. The solution was composed by 75% of PEGDA and 25% of ethanol (percentage in volume). The concentration of the IRG819 was varied from 125 µM to 32 µM.

A 448nm diode laser was used as light source coupled into a 50µm optical fibre in order to achieve a better homogeneity of the beam. This entails a noticeable reduction in beam intensity which, however, does not prevent from yielding a power level sufficient to trigger the photopolymerization.

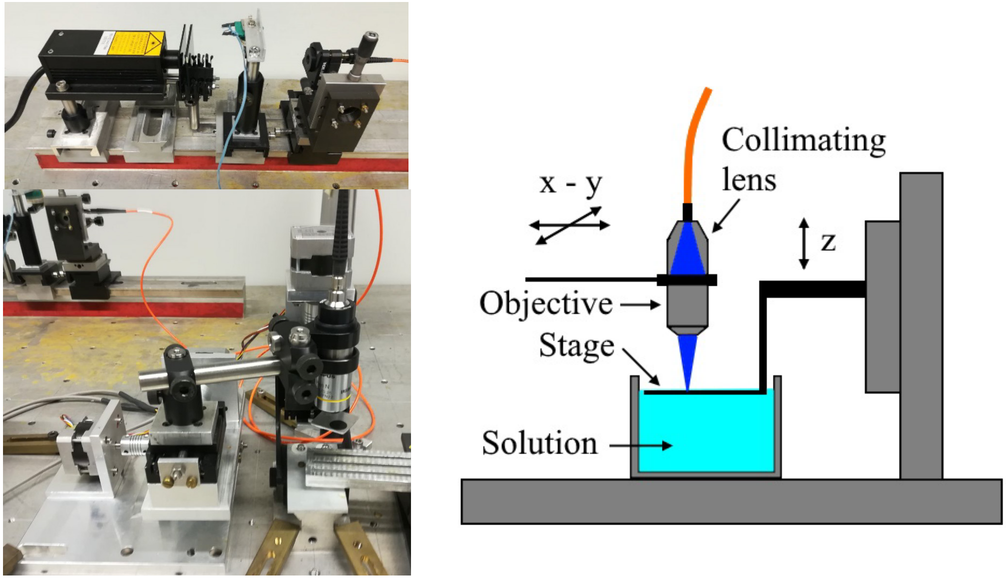

Figure 1. *Direct laser writing system. A sketch of the laser scanning system is shown on the right.*

We collected the light at the exit of the fibre using a collimating lens (f=34.74mm, NA=0.26). A microscope objective (10x NA=0.22) focused the beam on the polymer solution. The fibre exit and the focusing optics were scanned by a micrometre x-y stage to realize a 2D pattern on the solution surface layer. The scanning velocity was about 7,8mm/s. After the completion of the layer writing, the structure obtained is immersed in the solution to make the subsequent layer. The direct laser writing system is shown in fig.1 In the left side of the figure, the optical path from laser source to optical fibre inlet (upper picture) and the 3D movement stage, focusing optics and sample holder (lower picture) are shown in the left side of the figure. A sketch of the photolithography set-up is reported in the right side.

After the laser exposition, the 3D-structures were carefully washed in ethanol to remove the unexposed material. In this work we realized simple wood-pile structures as proof-of-concept for the linear absorption technique with steps of 1.23 µm in the layer plane and steps of 0.31 µm between subsequent layers. A sketch of the woodpile structure is shown in Fig.2

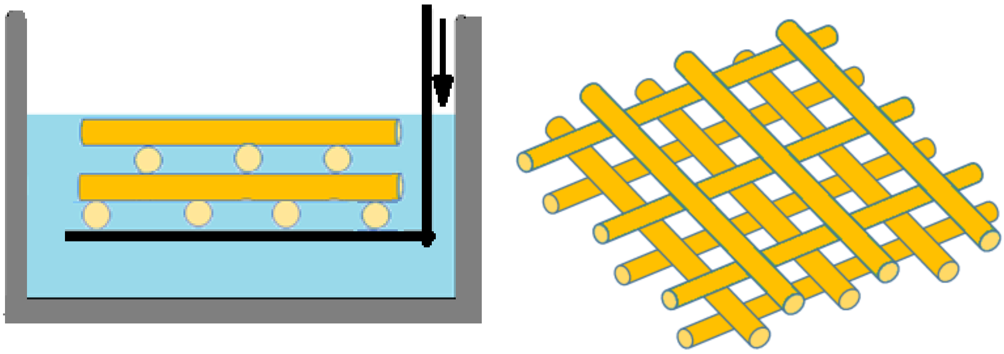

Figure 2. *Sketch of the alternate woodpile structure.*

Photolithographic method

Laser-propagation can be approximated by assuming that the laser beam has an ideal Gaussian intensity profile and such a beam can be focused into the most concentrated spot. A Gaussian beam is a beam of monochromatic electromagnetic radiation whose transverse magnetic and electric field amplitude profiles are given by the Gaussian function; that also implies a Gaussian intensity (irradiance) profile as showed in Eq. 1:

$$E_s = E_0 \exp\left(-\frac{r^2}{w^2}\right) \qquad I(r) = I_0 \exp\left(-\frac{2r^2}{w^2}\right) \tag{Eq.1}$$

r is defined as the distance from the centre (axis) of the beam, and *w* (beam waist) is the radius at which the amplitude is *1/e* of its value on the axis and the intensity is $1/e^2$. Assuming a propagation along the x-axis, we have the relation illustrated in Eq. 2

$$\frac{w_0^2}{w^2(x)}\left(\approx {I(x)}/{I_0}\right)=\left[1+\left(\frac{2x}{DOF}\right)^2\right]^{-1} \qquad DOF = \frac{8\lambda}{\pi}\left(\frac{1}{2NA}\right) \tag{Eq.2}$$

The depth of focus (DOF) is defined as the distance between the values of x where the beam waist *w(x)* is √2 times larger than the minimum w_0 (i.e. at focus). [16] The focal length and the entrance pupil (actually their ratio, i.e. twice the numerical aperture NA) of a lens system determines the DOF of the projected image.

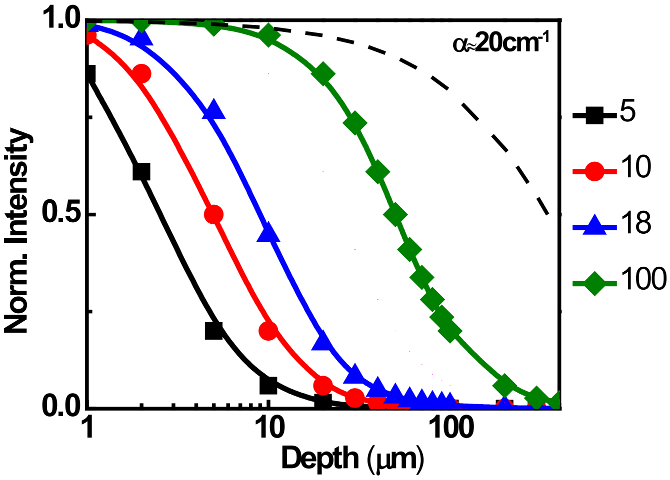

Figure 3. *Normalized intensity of a beam at 450 nm as a function of the penetration depth for different value of depth of focus (full lines). The effect of inclusion of a dye at a typical value of concentration yielding α=20 cm^{-1} is shown (dashed line).*

In order to limit the vertical penetration of the activating light several approaches are possible. One consists in the addition of a biocompatible dye, acting as inner filter, with an optical absorption overlapping that of photoinitiator. In a precedent work [17], curcumin, a natural and biocompatible dye well known for its antioxidant, anti-inflammatory, antimicrobial, anti-carcinogenic and immunomodulatory activities was used to this aim.[18] Alternatively, a careful design of the optical set-up can delimit the volume of effective interaction. The latter implies that the effect of the spatial confinement of light in the focus and the efficiency of the photoinitiator at the wavelength of excitation must be opportunely combined with the inner filter. Fig. 3 shows the normalized intensity of a beam at 450 nm as a function of the penetration depth for different value of DOF with or without an inner filter at a typical value of concentration (α=20 cm^{-1}). It is evident that the contribution of the inner filter is largely overwhelmed by the contribution of an appropriate choice of optics. However the quick decay of the light intensity out of the focus region could be not sufficient to preserve from re-exposure effects if the excitation of the photoinitiator is well over the threshold. Indeed it has been observed an incident power threshold for any photoinitiator concentration.

Fig. 4a shows the variation of the thickness of the produced structures in function of the power of the laser, for different concentration of photoinitiator. The curves are relative to three different concentration of IRG819: 125 μM, 63 μM and 32 μM. The minimum thickness value was 6 μm which was obtained at highest photoinitiator concentration. An increasing power threshold results for decreasing IRG819 concentration, as expected. That is related also to the wavelength of the exciting light with respect to the absorption spectra of the photoinitiator (Fig.4b).

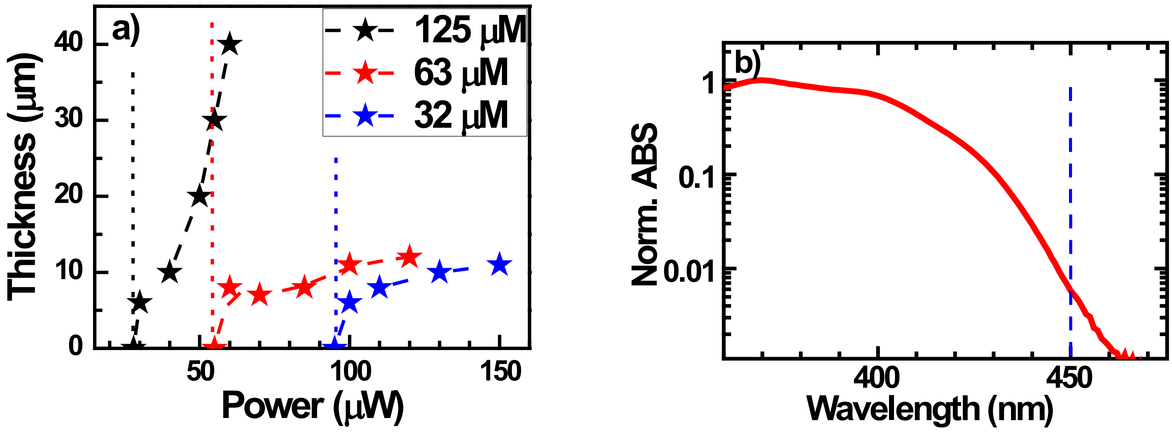

Figure 4. *a) Thickness of the produced structures in function of the power of the laser, for different concentration of photoinitiator; b) Normalized absorption spectra of the IRGACURE 819.*

The absorption spectra of the IRG819 presents a maximum at about 350 nm and drops below 1% of the peak value at 450 nm. As such, it is possible to effectively confine the spatial volume of excitation by exciting the photoinitiator in a non-efficient spectral region.

Therefore we set the power at 100 µW falling on the PEGDA solution with a 32 µM IRG819 and set a laser scan to write an alternate woodpile scaffold with a lateral pitch of 80 µm between structures 6µm thick and vertical step of 15 µm (fig 5).

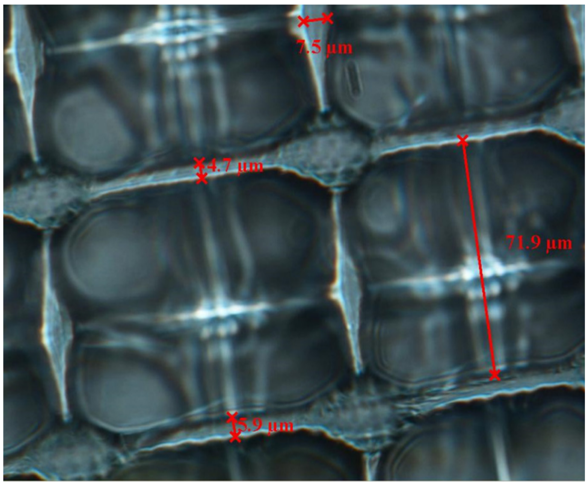

Figure 5. *Optical microscope view of an alternate woodpile structure.*

Conclusions

A simple direct laser writing method based on 1P microstereolithography exploiting the optical characteristics of carefully selected photopolymerizable system (polymer, photoinitiator, optics) has been implemented. Alternate woodpile scaffold were realized showing a lateral pitch of 80 µm and vertical step of 15 µm with a line thickness of 6 µm. This set-up has the has the remarkable merit of simplicity and can be easily implemented for the realization of 3D scaffolds with a design suitable for different uses

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