

# Direct Laser Fabrication of Composite Material 3D Microstructured Scaffolds

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We present direct laser fabrication of three-dimensional (3D) microstructured scaffolds consisting out of a few polymeric materials having different biological properties. Direct laser writing in photo/thermo-sensitive materials using ultra short light pulses of high repetition rate laser provides unmatched flexibility in controllable 3D microstructuring of a variety of biocompatible materials. It enables manufacturing of structures with sizes of up to 1 cm<sup>3</sup> overnight, making it an attractive method to fabricate scaffolds for cell studies and tissue engineering applications. We have manufactured 3D microporous multicomponent polymer scaffolds out of different organic-inorganic substances. Such composite constructions offer several biological functionalities including biostability or biodegradation (depending on the material used) as well as the possibility to micro/nanopattern the surface with various bioactive materials such as proteins, all spatially distributed within a structure with 1 - 10 μm features. The potential of this approach is applicable for cell adhesion, migration, proliferation and differentiation mechanism studies in 3D as well as suggesting the answer for best material and architecture combinations for scaffold needed in tissue engineering and regenerative medicine applications. DOI: 10.2961/jlmn.2014.01.0006

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## 1. Introduction

Three-dimensional (3D) laser micro/nano-structuring of polymers has advanced dramatically during the last decade. The progress was driven by the development of high pulse repetition rate ultrafast lasers, synthesis of novel materials, and emerging new applications [1]. Three-dimensional scaffolds produced by means of nanophotonic lithography [2] of biocompatible polymers [3] is currently one of the most actively developed fields as it is a very attractive approach for live cell studies and its practical applicability in tissue engineering as well as regenerative medicine. Artificially created constructs should mimic extracellular matrix (ECM) which is a complex and dynamic environment maintaining bidirectional cell-ECM interaction [4]. Investigation of stem cell behavior on artificially designed polymeric compartments [5] or constructs as biomedical rigid [6] or movable implants [7] are a few common approaches to mention [8]. Direct laser writing (DLW) has advantageous versatility in engineering custom shaped scaffolds [9] with controlled pore size within the range of 1 – 100 μm and general porosity from 10 to 90% [10]. Such constructs can be produced with up to 1 cm<sup>3</sup> dimensions overnight [11]. Although several other material processing technologies [12, 13] could be employed to fabricate scaffolds of much larger dimensions much more rapidly, however all of them have other drawbacks. For example, layer by layer stereolithographic approach [14], indirect stereolithography [15] and 3D printing [16] by themselves cannot warrant totally free choice of 3D geometry (may need to use supports for overhanging features) and are also limited in resolution. DLW offers a possibility to use various smooth lay-

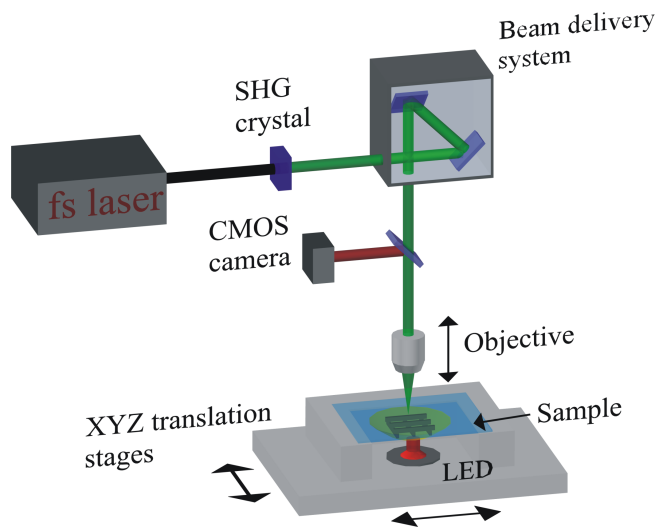
ered [17] or nanostructured dielectric/semiconductor/metallic substrates [18] and allows integration of several functional components into one micro device [19]. Recently, carving of biodegradable acrylated polyethylene glycols (PEGs) [20] or photosensitized gelatin [21] as well as elastomeric polydimethylsiloxane (PDMS) in 3D at microscale [22] was reported. Furthermore, structuring of hydrogels *in vivo* in direct contact with a living organism was demonstrated [23]. Finally, applying femtosecond pulses and tight focusing conditions non-sensitized materials can be nano-structured via controlled avalanche conditions [24]. Avoidance of toxic photosensitizers commonly used in lithography and applying only pure materials broadens the potential of current and possible future bio-applications, especially implementing biodegradable features.

Optically active composite structures consisting of the same acrylate based [25] or hybrid organic-inorganic [26] materials doped with different fluorescent dyes has been already shown. This principle was applied for the creation of two-component composite material scaffolds in 3D at a microscale [27]. Yet it was performed within the dimensions of tens of micrometers and relatively simple geometry. In this paper, we report 2D and true 3D structures (having three-dimensional intrinsic geometry) of mm size and incorporated micro-structures within.

## 2. Experimental setup

We used experimental setup consisting of a Yb:KGW femtosecond laser (Pharos, "Light Conversion Ltd.") operating at the 1030 nm fundamental wavelength. Second harmonics

(515 nm) radiation of 300 fs pulses set at a 200 kHz repetition rate was used for the fabrication. Laser beam was divided into two parts by a beam splitter and directed to different sample positioning setups. In the first one the polymerization trajectory was controlled by XY-galvanometric scanners (hurrySCAN II 10, Scanlab), a sample positioning system Aerotech ALS130-100 (X and Y-axis) and ALS130-50 (Z-axis) linear motion stages ensuring the positioning resolution of 50 nm. The alternative setup consisted of piezoelectric stages (PI P-563) combined with step-motor stages (PI M-605) which extended the working distance to 50 mm in X and Y directions. Fabrication could be observed in real time by using a LED red light for sample illumination and a CMOS camera (Matrix Vision). Custom made 3DPoli software was used to control laser exposure and positioning/translation of the sample.



**Fig. 1:** Femtosecond laser fabrication setup with in situ monitoring of fabrication: fs beam is frequency doubled and guided through galvanometric scanners and tightly focused to the sample by microscope objective. The pre-polymer (monomer) is selectively exposed in combination of beam deflecting scanners and translation stages. The fabrication system works as transmission microscope as LED illuminates the sample and CMOS camera registers the signal.

For all the fabricated samples 20x NA 0.8 (Zeiss) microscope objective was used to focus the laser beam into the resin except when photostructuring PDMS for which 40x NA 0.65 (Nikon) microscope objective was chosen.

### 3. Used materials

The focus on engineering and synthesis of materials could be distinguished as targeted for optimal structuring [28] or desired biological properties [29]. Several polymeric materials were used: hybrid organic-inorganic SZ2080 (IESL-FORTH) andOrmoclear (Micro Resist), acrylated PEG-DA-700 (Sigma Aldrich) and PDMS elastomer (Dow Corning). SZ2080 and PEG-DA-700 were photosensitized by adding 1% wt of photo-initiator 2-benzyl-2-(dimethylamino)-4'-morpholinobutyrophenone (commonly known as IRG). After

fabrication structures out of SZ2080, Ormoclear and PDMS were developed in 4-methyl-2-pentanone (MIBK) for up to 1 hour, depending on the size of the polymer droplet and produced scaffold's pore size and porosity. Structures fabricated out of PEG-DA-700 were immersed in water for up to 20 minutes.

Different combinations of these materials could be applied in designing and realization of various artificial tissues. Some of the main properties of the materials used are as follows: 1. SZ2080 is especially designed for direct laser writing applications, so it is possible to achieve resolutions of up to 100 nm. It has good adhesive properties and cells tend to grow on this material. 2. Ormoclear is a commercially available hybrid organic-inorganic polymer that is similar in biological properties to SZ2080. It shows good cellular adhesion and proliferation. Mechanically, both SZ2080 and Ormoclear are rigid and therefore more suitable for hard tissue (for example, bone) engineering [3]. 3. PDMS is a hydrophobic material that has poor cellular adhesion properties, but could be covered by ECM proteins to increase its surface suitability for tissue engineering. This material is soft and rubber-like, therefore very suitable for soft tissue engineering (for example, blood vessels) [30]. 4. PEG-DA-700 is hard and biodegradable. These properties make it attractive for tissue engineering in which stiffness is of essence.

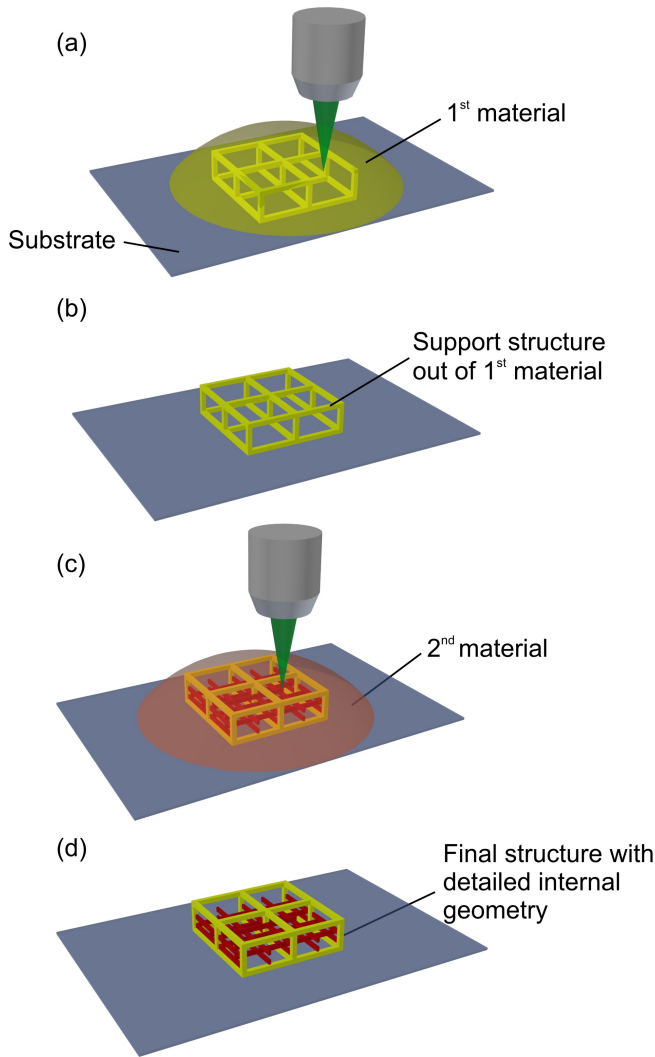
Sample placement in the positioning/translation stages depended on the material used and the size of the droplet needed. Since SZ2080 is gel-like solid the fabrication was performed with the pre-polymer (monomer) facing down without the danger of it dripping. PDMS was also put the same way since it is viscous and only small and thin droplets were needed. However, when fabricating out of Ormoclear or especially very liquid PEG-DA-700 (according to *Sigma-Aldrich*, viscosity of PEG-DA-700 is 0.07 Pa·s as compared to Ormoclear's 2.6-3.4 Pa·s which is given by *Micro Resist*) and when thick droplets were needed (for 3D scaffolds) the sample was placed with pre-polymer facing up to prevent it from dripping.

### 4. Results

Fabrication process of the two-component scaffolds consisted of four steps which are illustrated in Fig. 2. The first structure is fabricated out of one material and then developed. After drying in room temperature the sample is examined using Scanning Electron Microscope (Hitachi TM-1000). Afterwards, another material is poured over the first structure and the sample is again put into the translation stages for fabrication. After aligning the CAD model with the first structure seen in the computer screen a second structure is written inside the first one and again developed. Because of the necessity for several developments, it is crucial to determine the influence of various solvents to the DLW method produced microstructures beforehand so that they would not be damaged.

At first only 2D structures were fabricated to see if the materials stick to each other and are not damaged during fabrication of the second structure or second development. Only then a structure out of second (and sometimes third) material was incorporated into a 3D scaffold. Some of our fabricated sample

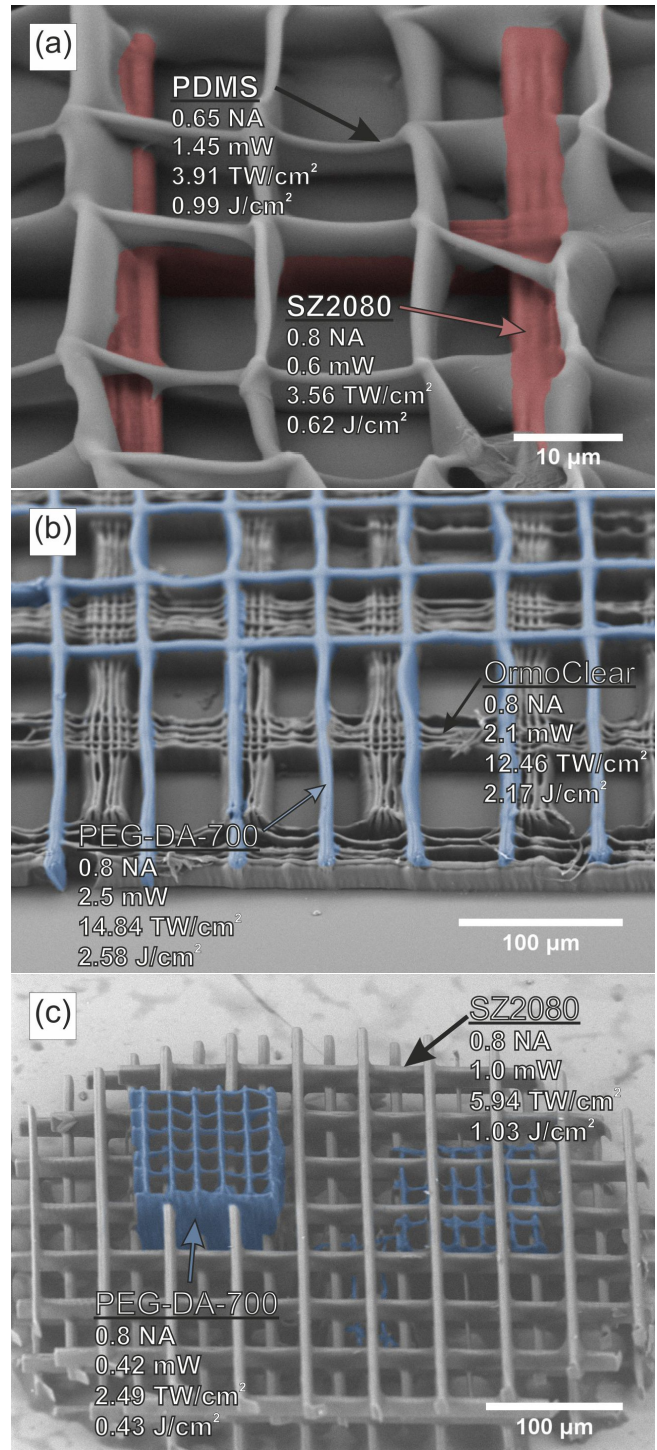
composite structures are shown in Fig. 3 and 4. Fabrication parameters (numerical aperture, average power  $P_{av}$ , peak intensity  $I = \frac{2TE_p}{\tau\pi w^2}$  and fluence  $\Phi = \frac{E_p}{\pi w^2}$ , where  $T$  is objective's transmission,  $E_p$  – pulse energy,  $\tau$  – pulse length,  $w$  – focal spot radius). Closer inspection of the composite structures with SEM showed that they are polymerized together and it is virtually impossible to say where one material ends and another begins. Also, we did not observe any detachment of one material from another during several development stages thus coming to a conclusion, that they stick together well enough for a practical usage.



**Fig. 2:** Direct laser fabrication of a composite scaffold: (a) a support structure with rough geometry giving mechanical strength is fabricated out of one material and is developed (b), then another monomer is poured over the first scaffold and a more detailed geometry is fabricated inside (c). After a second development a composite scaffold made out of two different materials is acquired (d).

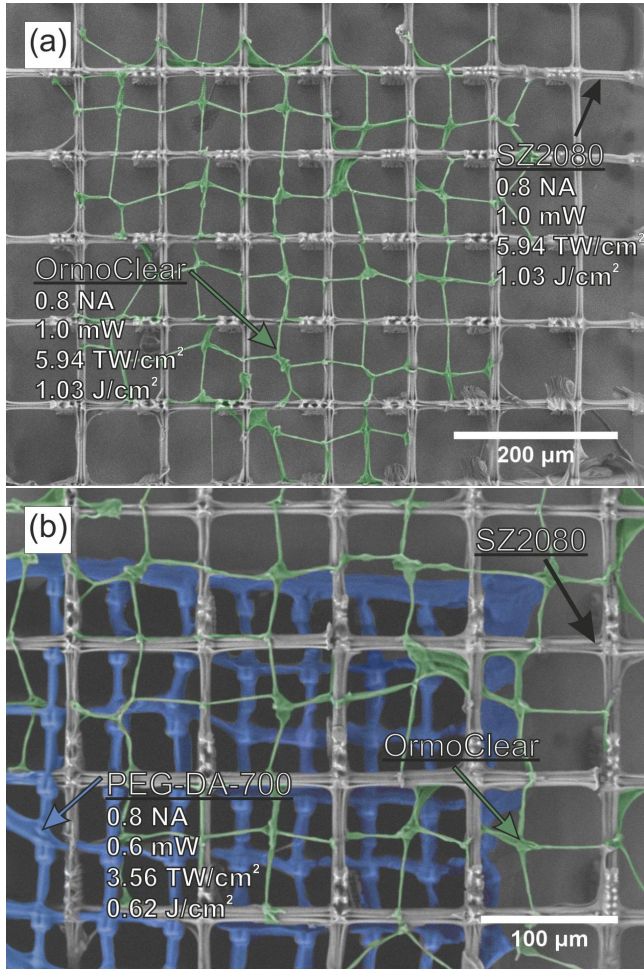
Table 1 summarizes the parameters which were used for the composite scaffolds: materials' combinations, each component material's dimensions, used sample translation velocities and

developer. The largest scaffold with the main part fabricated out of SZ2080 with dimensions of 4000x5000x400  $\mu\text{m}^3$  and an incorporated 2D layer out of PEG-DA-700 with dimensions of 1000x1000  $\mu\text{m}^2$  took about 9 hours to fabricate.





**Fig. 3:** (a) A composite scaffold fabricated out of hybrid organic-inorganic SZ2080 photopolymer (red) and polydimethylsiloxane (PDMS) without using photo-initiators. (b) A composite scaffold fabricated out of hybrid organic-inorganic Ormoclear and PEG-DA-700 photopolymers. (c) 3D composite scaffold fabricated out of SZ2080 and PEG-DA-700. PEG-DA-700 is coloured blue in all pictures.



**Fig. 4:** (a) Composite scaffold fabricated out of SZ2080 and Ormo-clear (green) and (b) the same scaffold with another structure written inside out of PEG-DA-700 (blue).

One of the cases in which a composite scaffold has clear advantages as opposed to one-component scaffold can be seen in Fig. 3 (a) where a 2D scaffold fabricated out of SZ2080 and PDMS is shown. The thin and soft PDMS walls would collapse on their own making it impossible to create scaffold out of PDMS alone with such wall's dimensions as shown in the picture. However, if a rigid grid is fabricated beforehand out of SZ2080 with well chosen spacing then this grid acts as a support structure to which PDMS walls can attach and thus stay standing in between the grid lines. Also, it is important to note, that this scaffold was fabricated out of pure SZ2080 and PDMS without any photo-initiators. Fabrication without photo-initiators increases the scaffold's biocompatibility as photo-initiators are often cytotoxic.

Fabrication of 3D scaffolds proved that DLW does not pose limits to picking the place to which a structure out of second material can be written. In Fig. 3 (c) it is seen that fabrication spot can be chosen freely inside the rough SZ2080 scaffold. Here structures out of PEG-DA-700 were successfully fabricated not only on top of the 3D SZ2080 structure, but also in the middle, as well as on the bottom layer without visible defects.

Also, our experiments demonstrate that we are not restricted to only two different materials for such a composite material (Fig. 4). It can be seen, that even three development stages, needed for such a structure, as well as several consecutive laser exposures did not do observable damage to any part of the previously fabricated structures. Although during polymerization of second and third materials some scattering of the laser light should be present from the previous structures, we consider it negligible as refractive index difference between the materials is small ( $\Delta n < 10\%$ ,  $n$  of used polymers varies between 1.4 to 1.55). The structure fabricated out of Ormoclear turned out irregular because thin lines deformed due to the surface tension of rinse materials in a development process [31]. This could have been prevented by using critical point drier which was

**Table 1:** Material combinations used for composite scaffolds and each step's fabrication parameters.

Materials	Dimensions	Translation velocities	Developer
SZ2080	170x170 $\mu\text{m}^2$	1000 $\mu\text{m/s}$	MIBK
PDMS	195x195 $\mu\text{m}^2$	5 $\mu\text{m/s}$	MIBK
Ormoclear	800x800 $\mu\text{m}^2$	300 $\mu\text{m/s}$	MIBK
PEG-DA-700	800x800 $\mu\text{m}^2$	200 $\mu\text{m/s}$	Water
SZ2080	4000x5000x400 $\mu\text{m}^3$	6000 $\mu\text{m/s}$	MIBK
PEG-DA-700	1000x1000 $\mu\text{m}^2$	200 $\mu\text{m/s}$	Water
SZ2080	1000x1000x100 $\mu\text{m}^3$	500 $\mu\text{m/s}$	MIBK
Ormoclear	1000x1000 $\mu\text{m}^2$	600 $\mu\text{m/s}$	MIBK
PEG-DA-700	400x400 $\mu\text{m}^2$	200 $\mu\text{m/s}$	Water

shown by Maruo et al. [32].

## 5. Conclusions

In summary, it was shown that direct laser writing lithography technique enables one to construct 2D or 3D composite compartments consisting of two or more different polymeric materials. Such niches for controlled cell migration and localization are attractive as they provide several desired biological features precisely deposited within the bulk of the microstructured scaffolds. It opens a way to produce semi-degradable structures which have varying pore size and porosity. The quality of such objects depends on material's mechanical properties and developing conditions. None of the existing alternative technologies can offer such spatial precision, variety of processable materials and fabrication efficiency if counting all together. In the near future, creation of gradient pore size and movable 3D compartments are expected to benefit from the versatility of nanophotonic lithography approach applied in this work.

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