Summary

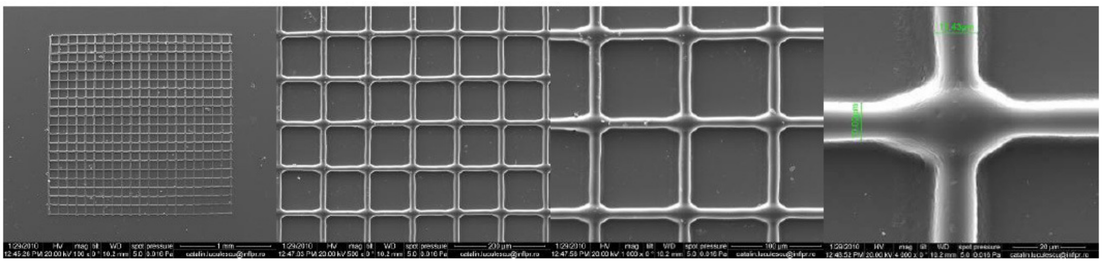
Stage V: Photonic processing and characterization of 3D structures. Processing and characterization of biocompatible polymer thin films

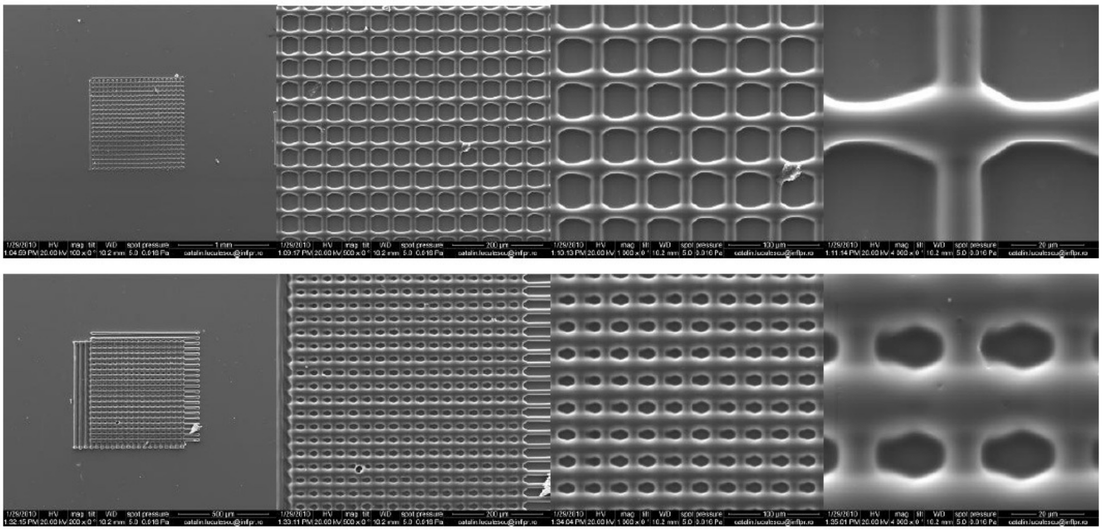
During this last stage of the FOTOPOL project the SIM-1, SIM-2, and SIM-3 monomethacrylates were synthesized; a multifunctional dimethacrylate of oligomer type (UDA-2) was prepared, characterized by the presence of both urethanic and methacrylic groups, separated by a flexible spacer such as poly(ethylene oxide), in order to diversify the profile of hybrid materials resulting from two-photons polymerization.

Two photon polymerization experiments were continued on hybrid monomers, also using the newly synthesized monomers. The used monomers were SIM 1 and SIM 1 in combination with TA1. For the two photons polymerization (2PP) the experimental setup described in the previous reports was employed. The same femtosecond laser, working at a wavelength of 775 nm, having a pulse duration of 200 fsec, and a pulse repetition rate of 2 kHz, was employed.

Following two photons polymerization experiments, and subsequent tests, on the hybrid monomers SIM 1, SIM 1 in combination with TA1, SIM 3 simple or in combination with UDA 2, we can conclude that the obtained polymer structures can be used as scaffolds for advanced studies on the obtaining of skin grafts.

Structures with line spacings of 100, 50, and 30 microns were processed, as well as grid systems having 100, 70, and 50 microns spacings. The laser scanning speed across the sample was varied between 0.2 and 5 mm/s. Some examples of the resulting structures are shown in the pictures below.





SEM images of SIM 1 samples. The laser power was of 2.5 mW and the scanning speed 0.2 mm/s. The line spacing is 100, 50, and 30 microns, respectively.

Following the processing of the SIM 3 : UDA 2 monomer, three dimensional meshes of lines/columns were obtained having the property of being completely detachable from the substrate. These could be easily manipulated without inflicting any damage to them (Figure 1).

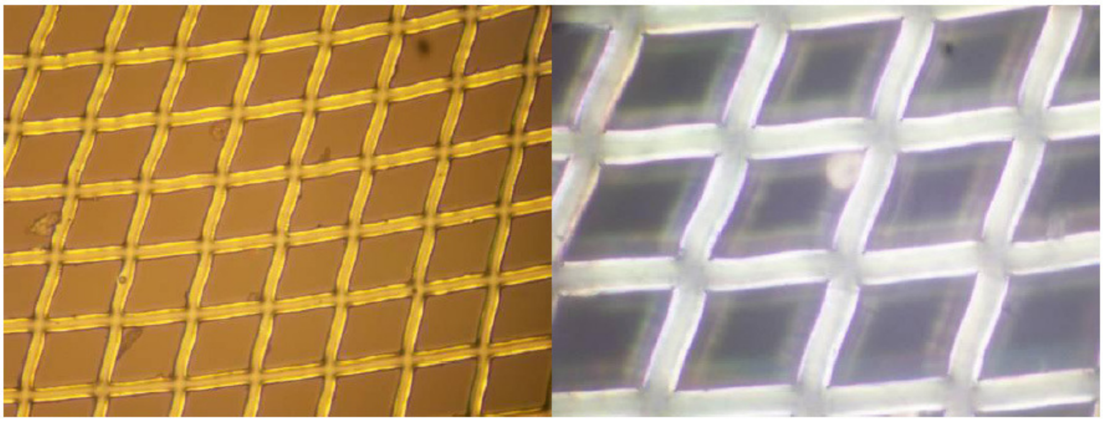
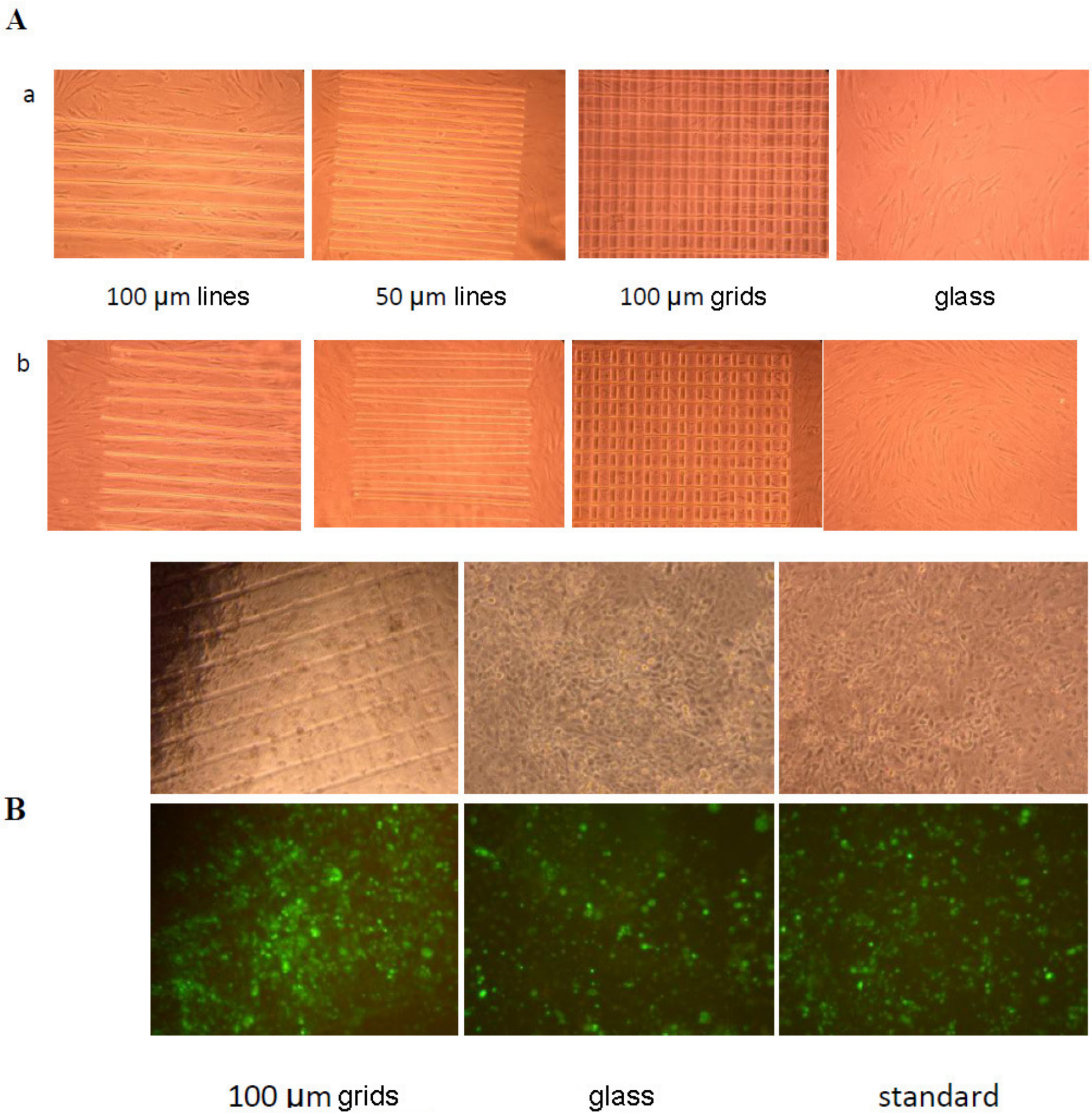


Figure 1. Optical microscopy images of free-standing three dimensional structures of two photons polymerized SIM 3 : UDA 2. The structures are mounted vertically on a support.

The polymerized structures of SIM 1, SIM 1 in combination with TA 1, and SIM 3 simple or in combination with UDA 2, underwent biological tests. We can conclude that the polymer structures can be used for advanced subsequent *in vivo* studies, with the aim of using them as scaffolds for the obtaining of skin grafts.

Studies have shown that the structures obtained in the frame of our project are not toxic for the eukaryotic cell that we worked with. The variation of the deposition parameters and of the structures dimensions influences the cellular adhesion and morphology. Polar cells (fibroblasts) align parallel to the polymer fibers and maintain their intercellular communication capabilities, as well as their proliferation potential. Skin cells tend to organize in star-shaped formations when cultivated in grid structures, anchoring themselves to the corners of the grid squares. Grid structures appear to be the most stable *in vitro* following the action of torsion forces caused by the migration of cells across the scaffolds. Fibroblasts cultivated on the organized polymer surface are ready to accommodate epithelial cells in a superior layer and to make up, together with these, o structure resembling that of the skin.



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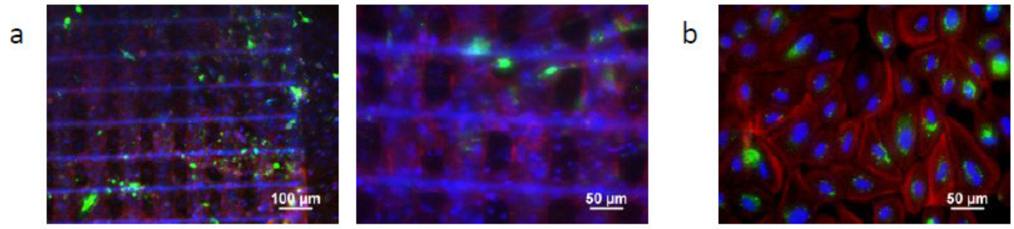

Figure 2. DOK_DiO seeded on HDFs previously cultured on SIM1 : TAcr 1 100µm grids. A – Brightfield images of HDFs grown on SIM1 : TAcr 1 lines and grids (a) or on collagen coated SIM1 : TAcr 1 lines and grids (b). B – Inverted microscopy images of DOK_DiO seeded on top of HDFs previously cultured on SIM1 : TAcr 1 100µm grids. C - Immunofluorescence microscopy images of DOK_DiO seeded on top of HDFs previously cultured on SIM1 : TAcr 1 100µm grids (a) or glass (b).

The MAPLE technology was employed with the aim of obtaining a polymer having medical usage, starting from a medical polymer having a standardized composition and plasticizer content. Following chemical, *i.e.* reducing substances and pH, and physical analyses, *i.e.* absorbance and thermal analysis, it results that the MAPLE processed polymers are suitable for medical usage.