Preliminary study of surface modification of 3D Poly (ε-caprolactone) scaffolds by ultrashort laser irradiation

A Daskalova¹, I Bliznakova¹, E Iordanova², G Yankov², M Grozeva² and B Ostrowska³

¹ Institute of Electronics, Bulgarian Academy of Sciences, 72, Tsarigradsko Chaussee Blvd., 1784 Sofia, Bulgaria
² Institute of Solid State Physics, Bulgarian Academy of Sciences, 72, Tsarigradsko Chaussee Blvd., 1784 Sofia, Bulgaria
³ Biomaterials Group, Materials Design Division, Faculty of Materials Science and Engineering, Warsaw University of Technology, 141 Woloska Str., 02-507 Warsaw, Poland

E-mail: a_daskalova@code.bg

Abstract. Three-dimensional poly (ε-caprolactone) (PCL) scaffolds as suitable biocompatible material for manufacturing tissue replacements are utilized for tissue engineering purposes. The porous structures are fabricated by rapid prototyping method (Bioscaffolder) based on hypodermic dispensing process. The consecution of experiments demonstrated the possibility on creation of surface micro formations, applying different laser fluences, at 1 kHz repetition rate for fixed time of exposure 1 sec at 800 nm central wavelength. The combination of both methods offers possibilities for successful production of 3D matrices with modified surfaces. The obtained results of laser-induced surface modifications of PCL demonstrate the potential of the method to microprocess this kind of material for possible applications in regenerative medicine.

1. Introduction

In tissue engineering, the three-dimensional (3D) scaffolds fabricated from polymers are used to provide structural support and guidance of cells for creation of new tissue generation. The created 3D templates mimic the mechanical and biological properties of native extra cellular matrix. The scaffolds have to possess specific properties for enhancing the cell adhesion and proliferation. Generally, the treatment of bone fractures using 3D scaffolds which are osteoconductive and osteoinductive are able to facilitate the osteogenesis process [1, 2]. Many synthetic and natural polymers successfully are applied as major ingredient for 3D scaffolds creation by different types of methods. One of the main techniques for creation of nanofiber meshes is the electrospinning technique, where the cells tend to adhere mostly on the fibrous surface which is a serious limitation of the method. Many research groups have focused to fabricate 3D structures with smaller diameters abilities to extend their surface area to improve the cell infiltration control [3, 4]. The major limitation of the implementation of PCL scaffolds in regenerative medicine is associated mainly with the problems related to vascularisation of...
the artificial constructs in volume. The additional laser assisted treatment of surface topography by inducing surface roughness could provide enhanced cell infiltration [5].

In this paper, 3D – PCL scaffolds are used for producing artificial structures for hard – tissue replacements. The PCL biomaterial is bioresorbable, non-toxic and suitable for bone and cartilage engineering. The incorporation of such matrices is unsuitable for in vitro cell seeding without additional treatment of the surface which can improve significantly their cytocompatibility [4, 6].

The main aim of the present work is to explore the potential possibility of femtosecond laser – induced surface modifications of 3D – PCL scaffolds for further improving of cell – interface interaction. First important step to reach that purpose was achieved and it was specified that the 3D – PCL structures can be modified by femtosecond laser modification method which was obtained from the SEM morphological analysis of the surfaces.

2. Material and methods

2.1. 3D – PCL scaffolds fabrication

The material used for the fabrication of the 3D templates was Poly (ε-caprolactone) (Mₙ = 45000g/mol) purchased from Sigma - Aldrich. 3D – PCL scaffolds used for these measurements were printed by Bioscaffolder apparatus (SYS & ENG, Germany) based on hypodermic dispensing process. The apparatus is capable of 3D – plotting of different types of polymers for biomedical applications. It is equipped with temperature – controlled stationary table and mobile dispense head [7]. The material was heated to temperature of 56ºC to melt. The samples shape was modelled by Solid Works program.

![Figure 1. Images of the printed 3D – PCL wood pile scaffold: an overview of the sample (a), Dino-Lite digital microscope image of the sample (b), SEM image of non irradiated sample, x200 magnification (c).](image)

The prepared samples have a cubic shape with a woodpile structure composed from 19 layers and 13 fibres. The obtained average fibre diameter is 209 µm and the average distance between the fibres is ~ 148 µm and porosity 40 %.

In our examinations, 3D – PCL scaffolds with dimensions 5 x 5 mm in width and 3 mm in height were printed for femtosecond laser irradiation (see figure 1). The PCL material possesses hydrophobic properties (see figure 2). The obtained contact angle measurement is 90° averaged over 10 measurements).
2.2. Experimental setup
The experimental setup depicted in figure 3, shows a schematic view of the experimental configuration. The femtosecond laser system used in this study is a Spectra – Physics product. The Spitfire Ace amplifier includes an optical stretcher, regenerative amplifier and optical compressor. The amplified femtosecond seed pulses are provided by a separate mode-locked Ti:sapphire laser (Spectra-Physics Mai Tai SP).

![Femtosecond Laser System Diagram](image)

**Figure 3.** Schematic representative of the experimental setup for ultrashort surface modification of 3D – PCL scaffolds.

The required energy for amplifying the seed pulses is supplied by a separate pump laser (Spectra-Physics Empower 45). The Spitfire Ace amplification is provided by a titanium-doped sapphire (Ti:sapphire) crystal. The regenerative Ti:Sapphire amplified laser system emits at 800 nm central wavelength with 35 fs pulse duration and 1 kHz repetition rate. The energy per pulse is 6 mJ. The laser beam is focused onto a sample with a 20 cm focal length lens at normal incidence. The corresponding focussing spot has diameter ~ 200 µm. In order to facilitate the movement of the sample, XY translation stage has been used. A circular variable neutral density filter is used to attenuate the laser beam to desired laser power. The output power and energy are detected by power meter (Ophir Nova II). The applied laser fluences are F = 3.18 J/cm², F = 4.78 J/cm², F = 6.37 J/cm².

3. Results and discussion
The irradiated surfaces of the 3D – PCL scaffolds were sputter coated with gold layer and analyzed using scanning electron microscopy (Microscope JOEL ISM 6390&EDS Oxford INCA). Here, the SEM images after femtosecond laser irradiation with F = 3.18 J/cm², F = 4.78 J/cm², F = 6.37 J/cm² and time of exposure 1 sec for each sample are presented (figure 4). The processing of the sample was performed by scanning the laser beam over the sample surface in X, Y plane. The laser beam was focused on the surface of the PCL samples.
Figure 4. SEM images of 3D–PCL scaffolds irradiated with $F = 3.18 \text{ J/cm}^2$ (a); $F = 4.78 \text{ J/cm}^2$ (b); $F = 6.37 \text{ J/cm}^2$ (c) and time of exposure 1 sec at 800 nm. Each row represents two positions on the sample under same condition.

Figure 4 clearly shows the potential of femtosecond laser for inducing surface modifications of 3D PCL as scaffolds. The negligible heat distribution at the surrounding areas at low laser intensities (figure 4a) were observed. The acquired SEM microscope images reveal that by increasing the laser energy and at constant exposure time (figure 4c) is observed distinct formation of rims created around the laser modified zones which is not that pronounced for lower laser intensities (figure 4a). Figure 5 shows more detailed representation of the laser irradiated area on the laser modified surface of the sample. The three examples of the SEM images on figure 5a, 5b and 5c correspond to x43, x150 and x650 magnifications, respectively. The samples are irradiated with laser fluence $F = 6.37 \text{ J/cm}^2$, exposure time of 1 sec at 800 nm central wavelength. The femtosecond laser interaction with PCL results in clean ablation with minimal heat affected zones when working in low fluence regime. Femtosecond processing regime proceeds with plasma–induced ablation, which leads to rapid formation of plasma and vapor which leads to negligible heat affected zones, absence of collateral damage, very clean ablation. The main reason of this phenomenon is the nature of ultra–short interaction processes where the photon
energy is less than the band gap of the material. The laser photons at 800 nm have 1.55 eV energy while the most polymers have from 4 to 7 eV photon energy. In the case of femtosecond ablation of polymers the near IR photons are nonlinearly absorbed through multiphoton absorption leading to multiphoton ionization, which enables precise microstructuring [8].

The combination of rapid prototyping technique with laser assisted ablation leads to clean ablation craters at the material surface and could be applied for improving the surface properties like roughness and wettability thus enhancing the cell adhesion and differentiation. The obtained experimental results indicated that to increase the level of precision of laser–material microprocessing and obtaining well–defined surface modifications is required precise control over laser beam position on the material surface. In fact, the output laser system parameters such as energy and exposure time enable to expand the laser system capabilities for more detailed studies of the optimal structure for medical applications.

4. Conclusions
In this study, we report first attempts for performing micro modifications on 3D–PCL scaffolds surfaces using femtosecond laser technique. The preliminary results of the present study are utilized to explore the potential of femtosecond laser radiation to create micro topographical features by improving the surface porosity which is a crucial factor for cell–surface interaction. The creation of additional sample modification will assess the mechanical properties of the produced matrices and will provide a path to enhance cell’s infiltration in volume for improved vascularisation. The preliminary SEM investigations confirmed that the obtained laser created modifications on 3D–PCL rapid prototyped samples result in minimum heat affected zones and collateral damage in the low fluence regime. The next step is to perform extended study on the cell cultivation on the fs laser modified surface. Furthermore, investigating the cell adhesion, proliferation and differentiation on 3D laser irradiated PCL scaffolds.

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