Ultra-fast laser modification of poly-lactic acid (PLA) -towards enhanced biocompatibility

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L Angelova[1,4], A Daskalova[1], R Mincheva[2], X Carette[2], A Trifonov[3], E Filipov[1], D Aceti[1] and I Buchvarov[3]

[1]Institute of Electronics, Bulgarian Academy of Sciences, 72, Tsarigradsko Chaussee Blvd., 1784 Sofia, Bulgaria
[2]Laboratory of Polymeric and Composite Materials (LPCM), University of Mons, Place du Parc, 23, B-7000, Mons, Belgium
[3]Faculty of Physics, St. Kliment Ohridski University of Sofia, 5 J. Bourchier Blvd., 1164 Sofia, Bulgaria

E-mail: lily1986@abv.bg

Abstract. In this study, the interaction was investigated of femtosecond laser radiation (pulse duration $\tau = 130$ fs, wavelength $\lambda = 800$ nm) with poly-lactic acid (PLA) 2D scaffolds. Two sets of laser fluences (F) and scanning speed (V) values were applied to PLA matrices – $F = 1.66$ J/cm^2, $V = 3.8$ mm/s, and $F = 0.83$ J/cm^2, $V = 3.8$ mm/s. The PLA samples thickness, roughness, and water contact angle (WCA) were characterized before and after the laser structuring. The fs-induced microstructures were investigated by SEM, EDX, and FTIR analyses. Preliminary cell fibroblast studies were performed. The results obtained clearly show that a precise laser surface structuring could orient the cells matrix ingrowth and thus make PLA bone tissue engineering interbody future application more successful and adaptable to the personal needs of the recipients.

1. Introduction

Bone tissue engineering relies on temporary matrices that mimic the natural cell environment and provide a stable extracellular structure for their natural growth – the body regenerates naturally, as such platforms significantly affect the cellular behavior and, in this way, the overall osseointegration of implants in the body [1]. Functional bone tissue can be developed to replace the damaged one through the application of such "smart osteoimplants". Their optimal composition and structure play a significant role in meeting all cell needs for natural body ingrowth [2, 3]. Bio interfaces on the other hand provide the so needed extracellular matrix qualities for initial adhesion of "osteo-cells" and subsequent integration of permanent bone implants, making the whole process more stable and immune friendly [4]. Such temporal cell matrices must possess the qualities of natural bone, providing stability, strength, resistance, hierarchical porosity, and surface roughness, which are crucial for improving cell adhesion, proliferation, differentiation, and osteointegration [5, 6]. To achieve all these requirements, their additional structuring is obligatory.

Poly-lactic acid (PLA) is a biocompatible and biodegradable polyester of the lactic acid; it is nontoxic for the recipient, as it degrades to by-products natural to the body. This synthetic polymer is

[4] To whom any correspondence should be addressed.

mechanically very stable, which makes it very appropriate for a basic bone graft material [7, 8]. PLA is approved by the US Food and Drug Administration for direct contact with biological fluids applications [9]; it interacts easily with the bone cells and as such a biomaterial can be used not only as a bone scaffold substrate [10-13], but also as contact surface substrate between the natural bone and the external permanent implant [13].

The ultrafast femtosecond laser structuring is a noncontact method, which provides a precise control over the surface roughness, wettability, and porosity of the treated biomaterial. It is an alternative and innovative approach allowing fine-tuning of the treatment process for functionalization of implant surfaces made of a wide range of different types of materials, including transparent biopolymers [14]. The femtosecond (fs) laser processing method can be applied to biomaterials surface functionalization with a high-level of precision without changing the quality composition of the matrix; due to the ultrashort time of interaction with the material and the precise control of ablation patterns, surface microstructuring is obtained without chemical structure alterations of the biomaterial processed [14]. By optimizing the parameters of the applied laser radiation, optimal cell living conditions could be achieved [15]. The study of Lee and co-authors, for example, demonstrates that fs laser processing can be used to increase the cells infiltration into 3D electrospun nanofibrous poly(l-lactide) scaffolds and, at the same time, facilitate the endothelial cell ingrowth [16].

In view of studying the interaction of femtosecond laser radiation with 2D PLA scaffolds, we explored two groups of femtosecond laser microstructured PLA matrices by SEM, EDX, and FTIR and compared them with an untreated control PLA surface. The thickness and roughness of the samples before and after laser structuring were characterized. The wettability change of the treated PLA matrices was also monitored. Preliminary fibroblast studies were performed to evaluate the viability of the cells for a period of seven days. The results obtained clearly show that precise laser surface structuring could be used for the functionalization of PLA-based temporal cell scaffolds.

2. Material and methods

2.1. PLA 2D samples preparation

The PLA samples were prepared using compression molding (Carver 4122 12-12H manual heated press) of the raw amorphous biopolymer compound (PLA 4060D, Nature Works) – the pellets were vacuum dried at 60°C/overnight, then molded according to the following procedure: 3-min contact at 180°C, several degassing cycles, and 2 min at 12 bars. The as prepared PLA 2D samples were cut into 2×2-cm squares and subjected to fs laser structuring.

2.2. Laser treatment

For femtosecond laser micro-processing of the prepared polymer 2D scaffolds, a Ti:sapphire laser was used (Quantronix-Integra-C) system with $\tau = 130$ fs pulse duration at a central wavelength of $\lambda = 800$ nm, $\nu = 0.5$ kHz repetition rate and a scanning speed $V = 3.8$ mm/s. Based on previous studies, two sets of laser fluences (F) were chosen for structuring the PLA matrices – $F = 1.66$ J/cm^2, (**group 1 fsPLA**) and $F = 0.83$ J/cm^2 (**group 2 fsPLA**). The samples were positioned perpendicular to the focused fs laser beam ($f = 10$ cm, $d = 50$ μm) on an XYZ translation stage (figure 1). The precise control of the experiment was performed by the LabView software. Each laser-processed PLA sample of both groups 1 and 2 was further analyzed with respect to the control – an untreated PLA 2D matrix (**group 3 cPLA**).

2.3. PLA samples analyses

The surface morphology and elemental composition of the 2D PLA samples (**group 1÷3**) were evaluated employing scanning electron microscopy (**SEM**) with energy dispersive X-Ray analysis (**EDX**) (TESCAN/LYRA/XMU), after covering the samples with a 10-nm gold (Au) layer.

Possible chemical bonds alterations and phase transformations after the laser treatment were evaluated using a Fourier-transform infrared (**FTIR**) spectrophotometer (IR Affinity-1, Shimadzu, Kyoto, Japan) – working range 4500÷500 cm^{-1} and resolution 4 cm^{-1}.

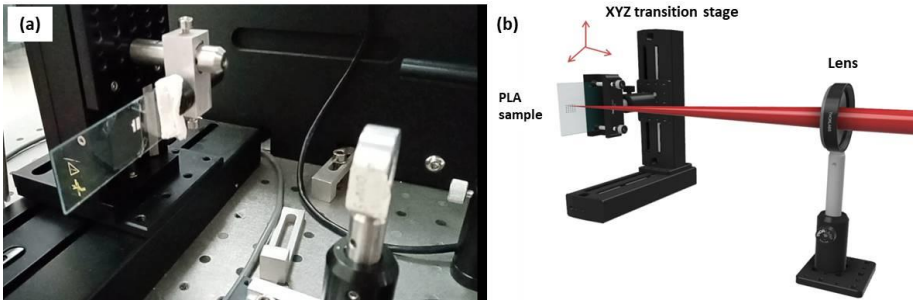

Figure 1. Photograph (a) and scheme (b) of the high-precision XYZ translation stage with the PLA sample positioned perpendicular to the fs laser beam focused by a 10-cm focal distance lens.

The PLA samples **thickness** (coating thickness gauge VA 8042 coating meter) and surface roughness (3D optical profiler, Zeta-20) were characterized before and after the laser structuring. The roughness parameters $\boldsymbol{R_a}$ **and** $\boldsymbol{S_a}$ were measured by means of 2D and 3D roughness analyses. Every value (in μm) was averaged over five thickness/roughness measurements of the fs-treated/control surface areas.

The wettability change of treated PLA matrices was monitored by water contact angle (**WCA**) measurements in air following a contact-angle goniometry method by a homemade installation – 1 μL of dH_2O was deposited by a micropipette (Dlab, ISO9001/13485-0.1-10 μL) fixed above the sample. The ImageJ software equipped with contact angle measurement plug-in was used for WCA evaluation for a period of seven seconds along and perpendicular to the fs grooves with respect to the control sample. Each WCA value was averaged over ten separate measurements.

2.4. Cell viability test

Preliminary cellular experiments were performed on group 1÷3 PLA 2D matrices – the samples from the three groups were first cleaned in 90% ethanol for two hours, then kept in a DMEM culture medium (with 10% FSA and 5% Streptomycin) overnight. Before cell seeding, the PLA samples were taken out of the medium and sterilized by UV light for one hour in a 6-well plate. A fibroblast stem cells stock (cell suspension with culture medium) was prepared at a concentration of 1×10^5 and an appropriate volume of cell stock was added to each well containing a scaffold (the volume was adjusted in accordance with the volume of the 6-well plate). The fibroblast stem cells viability was evaluated on day 1, day 3, and day 7 according to the CrystalViolet staining assay protocol. The stained cells (deep purple nuclei) were visualized by an optical microscope (4× and 10×). The absorbance was measured at 570 nm by an Epoch microplate spectrophotometer (BioTek, USA) against a background control as blank.

3. Results and discussion

As can be seen in the SEM images presented (figure 2 (a)), the fs laser treatment with the selected parameters leads to an ejection of material around the zones of interaction and to formation of surface grooves, thus enhancing the PLA topography micro-roughness in both groups 1 and 2, compared to the smooth surface of the control group 3 (figure3 (a)). Hierarchical porous frameworks can be visualized in the images taken at a higher magnification (5000×). The laser treatment proceeds without the formation of cracks or melted zones at the side of interaction, with no damage of the structure of the biomaterial.

According to the EDX elemental composition [wt. %] measurements (figure 2 (b)), no uncommon elements for PLA are observed after laser treatment. The change in the [C] and [O] concentrations (doubling the O/C ratio) can be assigned to surface oxidation resulting from the fs modification. On the other hand, the positions and shapes of all transmittance peaks in the FTIR spectra of the treated PLA samples (figure 2 (c)) remain at the correct positions with respect to the control PLA 2D matrix, while only the intensity of the characteristic for the PLA peaks (given on figure 2 (c)) is changed relatively to the control group, which does not point to any possible chemical bonds alterations and phase transformations in the structure of the PLA samples.

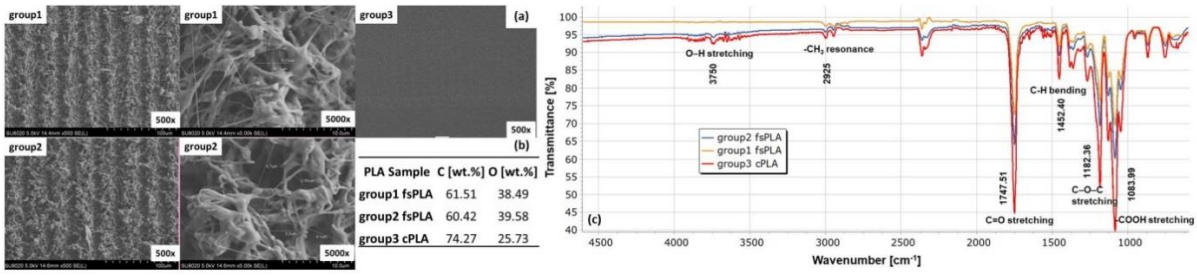

Figure 2. (a) SEM images of group 1÷3 PLA matrices observed at 500× and 5000× magnification; **(b)** EDX elemental composition [wt. %] and **(c)** FTIR transmittance (%) spectra of the corresponding samples.

Although the grooves in group 1 and 2 look very similar on the SEM images provided in figure 2 (a), differences in the width and depth (R_a, S_a respectively) of the fs grooves are clearly observed in the 3D roughness true color composite images given in figure 3 (a) –increasing the fluence applied leads to deeper and narrower grooves and to thinner samples.

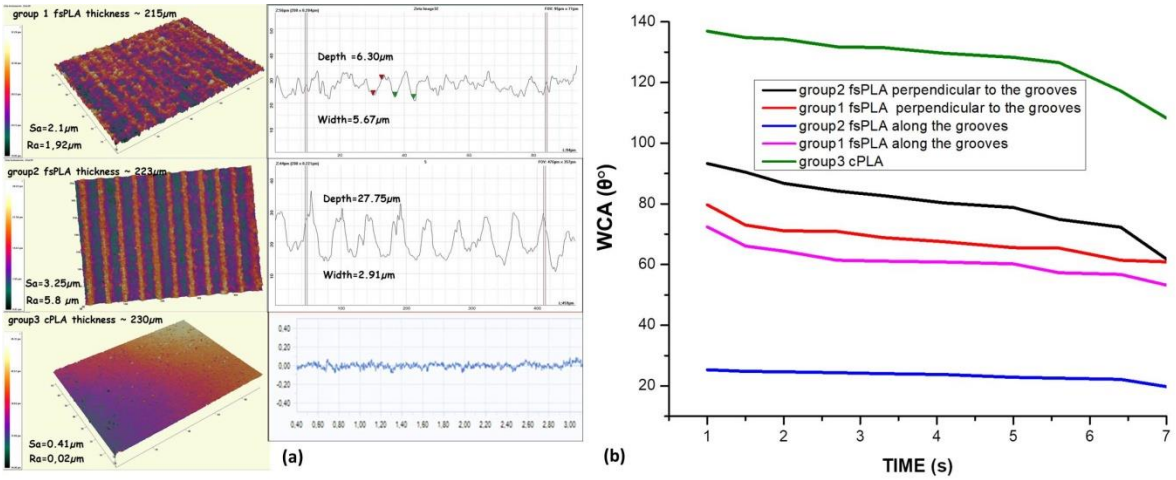

Figure 3. (a) 3D roughness true color composite images of group 1÷3 PLA samples; **(b)** WCA evaluation of group 1 fs and 2 fs PLA matrices performed along and perpendicular to the grooves created by the fs laser processing with respect to group 3 control surface.

A notable change in the wettability of the fs laser functionalized samples is observed in both ways of water drop application with respect to the control – figure 3 (b). Even though in all cases the surface becomes more hydrophilic with time, the control surface stays hydrophobic (WCA changes from 137° at the 1st second of application to 108.3° at 7ths), while the average wettability of the treated samples is enhanced up to 60.9-61.8°. The most striking is the case of dH_2O drop application along the grooves created by the laser on the group 2 fs PLA surface, when the WCA drops from 25.34° to 19.8° for the period of application.

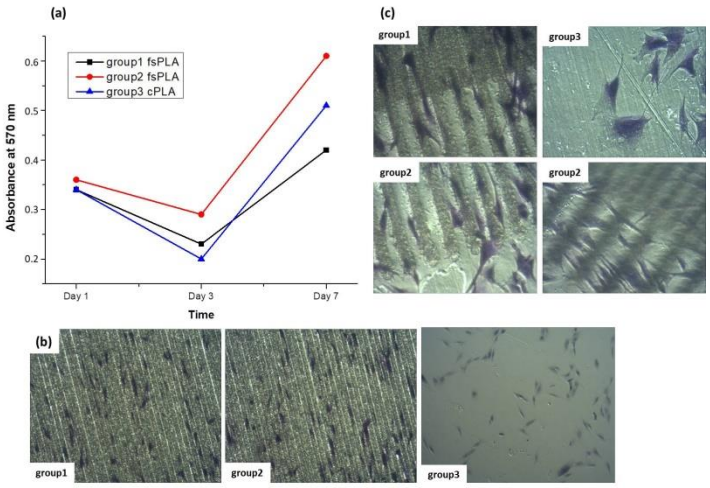

Figure 4. (a) Graph of the fibroblast proliferation; CrystalViolet optical images (nuclei stained in deep purple) of: **(b)** the cell-seeded PLA matrices on day 7 observed at 4× magnification; **(c)** the fibroblast cells "entering" and aligning along the fs laser created grooves on group 1 and 2 fsPLA, compared to group 3 cPLA observed at 10× magnification.

The preliminary cell seeding results demonstrate a notable orientation of the fibroblast cells along the grooves formed by the laser in both groups 1 and 2, as compared to the chaotic spreading of cells cultured on the control PLA group 3 – figure 4 (b-c). In all cases, a minimum in cell proliferation is seen on day 3, while a slightly enhanced fibroblast proliferation is observed in group 2 on day 7 compared to the control PLA samples (figure 4(a)) – the results do not show notable differences between all three PLA groups with respect to the cells viability. The enhanced hydrophilicity of the PLA scaffolds, in combination with the surface micro-roughness and porosity achieved in PLA structures, has been proved to be a key parameter for cells adhesion and proliferation [1].

4. Conclusions

The fs laser pulse surface micro-patterning could enhance the biocompatibility of the studied 2D PLA matrices as it tunes the biological properties of the PLA with respect to surface structuring – roughness, porosity, and wettability with no indication of chemical structure alterations. The data obtained confirm that fs laser processing is a technique suitable for biopolymer surface functionalization as it could direct cells matrix ingrowth; a further precise selection of the applied laser parameters could significantly improve the biomedical applications of PLA-based temporary implants, as optimization of the cell seeding conditions could be achieved, which could make PLA bone tissue engineering interbody future application more successful and adaptable to personal recipient needs.

Acknowledgments

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