Fabrication of Chitosan Scaffolds via Laser Assisted Modification

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Abstract. Surface texturing of a thin film of chitosan is carried out for improving its biocompatibility characteristics for diverse tissue engineering applications. The goal of this study is to develop chitosan-based films for cell culture applications by fs laser-assisted modification. Chitosan has the potential to accelerate the reformation of connective tissues and promote their vascularization. Furthermore, the presence of chitosan benefits wound healing, bone repairs, vascular graft implantation and cell tissue cultures. It also has the desirable properties of being efficiently processed and developed into films and membranes, microparticles and beads, and 3D scaffolds. Diverse ceramic coatings such as CaP or bioactive glass are applied to metallic alloys as coatings, to serve as an interface between the scaffold and the tissue. However, the results were not applicable because of the material's brittle character. An alternative to the approach as mentioned above represents the implementation of chitosan as a bioactive coating to strengthen and accelerate cell proliferation and tissue organization. Most of the surface treatments lead to contamination of the biomaterial. Our approach is to create a model of chitosan microfoam with accurate control over surface morphology to influence cells behavior. Laser modification by pulses in the fs domain provides the ability of modification of thin films of biopolymers unobtainable at longer (nanosecond) pulses.

Keywords: chitosan, laser modification, tissue engineering.

1. INTRODUCTION

Recently, many attempts of seeking for renewable, bio-sourced and biodegradable materials and components creating artificial tissues and organs were made. Many attractive biomaterial candidates such as chitosan, collagen, gelatin, alginate, cellulose, and elastin with positive environmental influence are occurring, capable of replacing the synthetic polymers. The most critical challenge is to obtain bio-based products with properties equivalent to the functional synthetic one (Croisier et. al. 2013; Braghirolli et. al., 2014; Dumonta et. al. (2016). The human body has the ability to regenerate and remodel small damages. Hence, the regenerative strategies like regeneration, cell-based therapy, and tissue engineering developing of self-healing materials and composite implant into the human body are requested. Existing tissue matrixes made of various natural and synthetic products have different characteristics (Hinderer et al. ,2016). The important goal of tissue engineering is to support recovering of damaged tissues under biological microenvironment., in which complex cascade processes such as cell proliferation, differentiation, and intra-cellular matrix synthesis take place. A "golden" platform for tissue engineering has to provide three – dimensional (3D) growing medium mimicking the natural extracellular matrixes (ECM) via pore structure, oxygen diffusion and nutrients delivery (Sivashankari et al., 2016). The ability of the designed matrixes to absorb water (i.e. to possess either hydrophilicity or hydrophobicity), depending on their-application is an important issue (Martins et al., 2017). The ECM characteristics such as topography, composition, adhesion, growth, gene expression, apoptosis, secretion, and motility

are of primary importance and demonstrate strong influence over cell behavior on cell/cell, and cell/material interfaces (Liua et al., (2005). Scaffolds design may follow variety of either chemicals or physical techniques. The conventional methods include solvent – casting, particulate – leaching, phase separation, gas foaming, and freeze drying. Unfortunately, they do not always provide precise pore size, geometry, interconnectivity, and spatial distribution of the pores (Zhu et al., 2013), which are crucial for the scaffold creation. Furthermore, skin layers formed during evaporation. as well as agglomeration of salt particles hinder the pore size control. For instance, for gas foaming, it has been reported that, only 10 – 30% of the pores were interconnected. In addition, the presence of residual organic solvent causes the most significant problems due to the risks cells toxicity and carcinogenicity (Dhandayuthapani et al., 2011).

Femtosecond (fs) laser-assisted surface modification is an attractive approach since it is noncontact and does not require implementation of additional chemicals. The application fields of ultra-fast pulsed lasers have become significantly large. Ultrafast technology has been developed, providing new approaches based on laser-matter interactions, for machining, different materials processing, nonlinear microscopy, and nanotechnology (Stratakis et al., 2009); Boulais et al., 2013). It has been reported that the side effects induced by the interaction of ultra-short pulses with the biological tissues result in reduced cracks formation and heat diffusion, absence of molten zones, and reduced ablation thresholds.

We believe that by applying femtosecond laser pulses to chitosan thin film, control over biomaterial characteristics might be achieved. In the present study, the influence of different number of fs laser pulses with different fluence on the surface topography is examined by means of SEM and FTIR.

2 MATERIAL AND METHODS

Chitosan thin film samples laser treated under various experimental conditions (number of applied pulses, pulse energy, frequency, and pulse duration) were investigated to increase the knowledge on foam- like structure formation phenomena. A chitosan as a primary scaffold material is overspread onto 20 mm x 20 mm glass slides. They were irradiated by femtosecond laser pulses for the examinations.

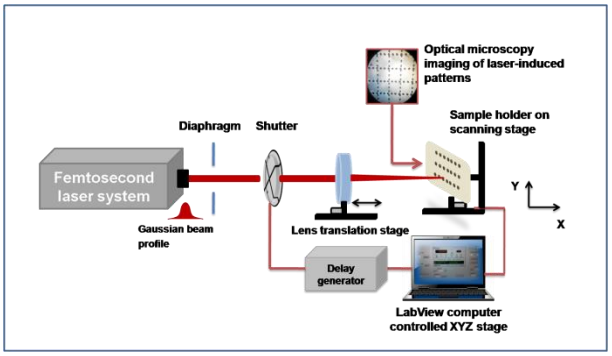


Fig. 1 Overview of the experimental setup for microstructured biopolymer fabrications.

For our experiments, two laser systems, with different pulse durations for comparison of the processing outcome were employed: a) CPA multipass Ti:sapphire laser (Femto-power compact pro) emitting at an 800 nm central wavelength, with a temporal pulse width of 30 fs, at a repetition rate of 1 KHz, and maximum energy of E = 1mJ; b) Quantronix-Integra-C, delivering pulses of 130 fs, at 800 nm. The number of applied laser pulses (N) was controlled by computer-driven fast mechanical shutter, synchronized by controlling software.

The samples were processed by scanning the focused laser radiation over the material surface. The exposures were performed in sets of parallel lines under various experimental conditions such as different distances between the laser modification spots and the rows.

3 RESULTS AND DISCUSSION

The morphology of chitosan thin films induced by 130 fs, different number of laser

pulses (from 1, 2, 5, and 10) and fluencies (F = 0.2; 0.4; 1.04 and 1.24 J/cm^2) are shown-in Figs.2 and 3.

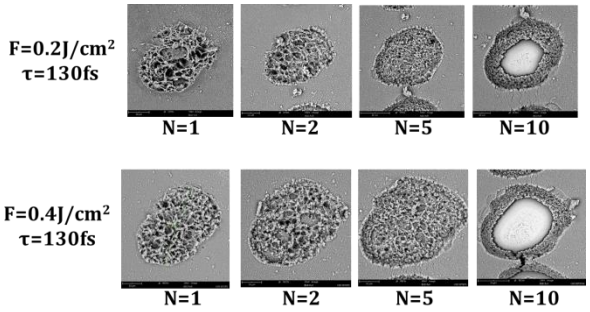


Fig. 2 SEM images of surface morphology of thin chitosan film irradiated by τ = 130 fs and F = 0.2 J/cm^2 and F = 0.4 J/cm^2.

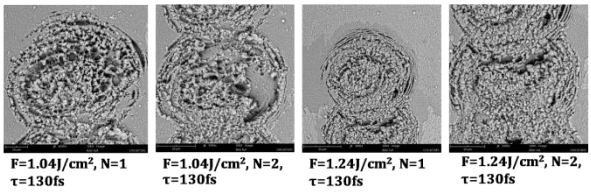


Fig. 3 SEM images of surface morphology of thin chitosan film irradiated by τ = 130 fs and different fluencies F = 1.04 J/cm^2 and F = 1.24 J/cm^2.

The results show clear differences in the achieved morphology depending on the laser pulse parameters (fluence and number of applied pulses). The modification process appears even at a single pulse (N = 1) for all fluence values. Based on SEM image analysis, increasing the number of pulses, leads porosity decrease together with occurrence of sublimation and molten zones. The diameter of the affected zone expands as well. Ablation phenomena, starting from the center of the modified area, increase in magnitude–with the number of incident laser pulses (Fig. 2).

In Fig. 4 several modified zones irradiated by τ = 30 fs, F = 0.02 J/cm^2 and F = 0.2 J/cm^2, N = 1, 2, 5 and 10, and 1 kHz repetition rate, are presented.

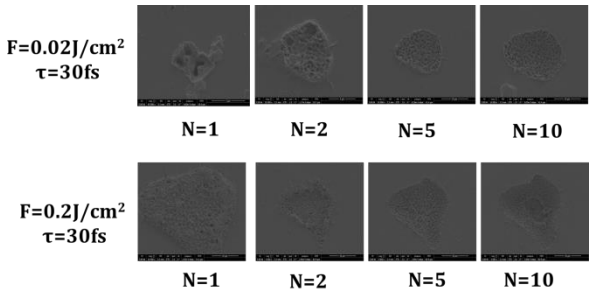
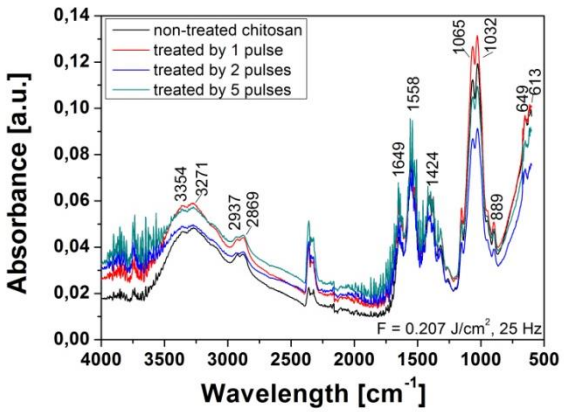


Fig. 4 SEM surface morphology of thin chitosan film irradiated by τ = 30 fs and fluence F = 0.02 J/cm^2 and F = 0.2 J/cm^2.

Similarly to the previous case, a diameter of the structurally modified chitosan surface increases with increasing the number of laser pulses. For N = 1 and 2, well defined foam fibrous structure forms, where the laser ablation takes place. The FTIR spectra of untreated and laser modified chitosan samples are presented in Fig. 5. The measurements are carried out in an absorbance mode in range from 4000 cm^{-1} to 500 cm^{-1}. All results were obtained for chitosan acetate (chitosan samples with a presence of acetic acid as a solvent). The results for irradiated and non-irradiated chitosan thin films show similar spectral characteristics; i.e. the method has no significant influence on the chemical composition of the substrate. The major bands that appear in the spectrum are: 3800 cm^{-1} ÷ 3000 cm^{-1} (stretching of OH groups), stretching of CH link vibration 2900 cm^{-1} ÷ 2800 cm^{-1} (-CH2 at 2919 cm^{-1} and -CH3 at 2878 cm^{-1}).



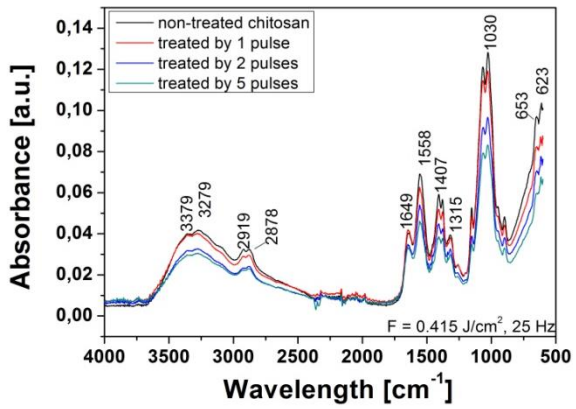


Fig. 5 FTIR spectra of chitosan thin films before and after laser irradiation: (a) F = 0.207 J/cm^2, N = 1,2 and 5, (b) F = 0.415 J/cm^2, N = 1, 2 and 5.

The absorption observed at 1649 cm^{-1} is related to vibration of the carbonyl linkages (C = O) of the amide group. The band found at 1558 cm^{-1} is attributed to NH (NH_2), whereas the small peak at 1649 cm^{-1} characterizes the stretching of C = O group (amide I). Absorbance in the range 1200 cm^{-1} ÷ 1000 cm^{-1} is characteristic of the CO group vibrations that are derived from deacetylation of chitosan. The small-maximum at 889 cm^{-1} corresponds to the structure of chitosan saccharides. The FTIR spectra of the irradiated thin chitosan films for different number of applied pulses (N) and energy fluence (F) are similar with minor variations in the absorption intensities. The FTIR results, obtained here are in a good agreement with previously reported (Silva et al., 2012), proving the successful application of femtosecond laser pulsed method for surface modification of biopolymer thin films.

4 CONCLUSIONS

Surface texturing of thin chitosan films was carried out via controlled laser irradiation in order to enhance their bioactivity. Morphology, texture and photochemical changes of the resulting surfaces were examined using scanning electron microscopy and Fourier transformed infrared spectroscopy. Laser modification results in surfaces consisting of unique microporous structures. The surface morphology depends strongly on the number and the fluence of applied laser pulses. The modification of the chitosan biofilms was observed even at N=1. The FTIR finding proved that the laser treatment does not cause significant chemical structure alteration.

The present study showed the potential of the fs laser-assisted modification to create porous surface structures with high precision, which successfully mimic the extracellular matrix.

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