Morphological characterization of chitosan biopolymer thin films modified via fs irradiation and its potential application as functional surfaces in regenerative medicine

A. Daskalova*[a], I. Bliznakova[a], A. Trifonov[b], I. Buchvarov[b], C. R. Nathala[c,d], W. Husinsky[c]

[a]Institute of Electronics, Bulgarian Academy of Sciences, 72, Tsarigradsko Chaussee blvd., 1784 Sofia, Bulgaria; [b]Department of Physics, Sofia University, "St. Kliment Ohridski", 5, J. Bourchier Blvd., BG-1164 Sofia, Bulgaria; [c]Institute of General Physics, Vienna University of Technology, Wiedner Hauptstr, 8-10/134, A-1040, Wien, Austria; [d]Spectra-Physics Vienna, Fernkorngasse 10, 1100, Wien, Austria

ABSTRACT

The creation of microporous surface modification of chitosan thin films irradiated by ultrashort laser pulses are studied. For this purpose, chitosan substrates were treated by using an amplified Ti:sapphire laser system at 800 nm central wavelength with 30 fs and 150 fs pulse duration and repetition rate 1 kHz and 50 Hz, respectively. Formation of surface modifications for both cases (30 fs and 150 fs) after femtosecond laser irradiation were observed. The threshold values for single-pulse ($N = 1$) and multi-pulse ($N > 1$) modification were evaluated by studying the linear relationship between the squared crater diameter D^2 and the logarithm of the laser fluence (F) for N = 1, 2, 5, 10, 20, 30 and 50 number of laser pulses. The coefficient of incubation ξ, a major parameter in the process of surface modification and ablation of materials also was calculated for multi - pulse fluence threshold estimation by power - law relationship $F_{th}(N) = F_{th}(1)\, N^{\xi-1}$. The surface properties of chitosan based thin films before and after femtosecond laser irradiation were investigated. The aim of this work is to determine the optimal morphological characteristics of the created structures for tailoring of protein adsorption and cell behavior.

Keywords: biopolymers; femtosecond laser microfabrication; chitosan; regenerative medicine

1. INTRODUCTION

Tissue engineering and associated regenerative medicine are an important part of research area allowing development of potential new treatments to many medical conditions. The progress in this particular topic is determined by the participation of researchers from various fields of science, including scientists engaged in cell biology, biomaterials science, characterization of surfaces and interactions with biomaterials. The trend to increase the opportunities for "replacement" and restore almost all types of tissues and organs of the human body, opens up new horizons for the development and application of various methods which leads to - fast and gentle tissue recovery. The main goals of tissue engineering is related to reconstruction, maintenance or improvement of the functions of different types of scaffolds. The basic steps to achieve this objective is directed towards seeding cells on the scaffold surface and within the volume of different types of matrices that mimic the natural cellular environment. The materials used for fabrication of such constructs could be from natural or synthetic origin. From the group of biopolymers can be distinguished the most widespread ones like: starch, elastin, chitosan, collagen, gelatin, alginate, cellulose and chitin, which represent the most attractive candidates for such kind of applications[1-3]. The basic requirements that a layers and matrices from biopolymers should possess are related to their biocompatibility, morphology, mechanical properties, porosity, exchange and opportunity to treat wounds and infections. Also, they must not cause acute and chronic immune reaction of the body. They have to be highly biodegradable and to possess surface with improved cell adhesion, proliferation and differentiation characteristics. Chitosan is a suitable candidate which meets these requirements and can be used as a basis for making chitosan matrices for tissue engineering purposes [1-4].

*albdaskalova@gmail.com

19th International Conference and School on Quantum Electronics: Laser Physics and Applications, edited by Tanja Dreischuh, Sanka Gateva, Albena Daskalova, Alexandros Serafetinides, Proc. of SPIE Vol. 10226, 1022607 · © 2017 SPIE · CCC code: 0277-786X/17/$18 · doi: 10.1117/12.2262679

It can be modified by applying various techniques and methods in order to create 2D and 3D matrices, thin films and porous structures "mushroom", on which are then seeded cell cultures to create cellular matrices closely mimicking the natural one [5,6]. There are various techniques and methods for creating porous structures of many types of polymeric materials such as: solvent casting/salt leaching, freeze-drying, gas foaming, but their main disadvantage is the use of toxic solvents. Moreover, removing the solvent by evaporation, can last from several days to several weeks, the preparation of pores results in irregular shape and insufficient interconnection of the pores inside the structure and volume of the matrix [7].
Previous studies [8] have shown that morphological changes of the biofilms surface, significantly affects the adhesion, growth, proliferation and differentiation of cells. Therefore, the study of surface properties plays a crucial role in biomedicine, because most biological reactions when using this kind of material occur on the border area/environment [9]. The use of the flow of energy the femtosecond laser pulses in represents a unique method for direct surface modification of a major part of various types of materials and allows effective monitoring of the laser - induced changes in the environment. Moreover, femtosecond laser pulses allow creation of 3D structures with high precision and accuracy, causing minimal thermal influences of the environment. One of the main advantages using femtosecond laser ablation is the time for deposit of energy, thus the heat conduction inside the volume is negligible. The plasma recombines before the thermal diffusion occurs and the development and spreading of the formed shockwaves and cavitation is limited in a small volume of material. The combination of locally controlled impact and low threshold fluence could significantly reduce the side effects, which represents a great advantage of the method [9,10].

2. MATERIAL AND METHODS

2.1 Experimental setup

In this work, thin biopolymer films from chitosan are deposited on 20 mm x 20 mm glass slides and subsequently patterned by femtosecond laser irradiation. For this experiment, we use a Ti:sapphire laser system (Quantronix), emitting at 800 nm central wavelength with a Gaussian spatial beam profile, allowing readjustment of the frequency (in our case 50 Hz and 1 kHz), pulse duration (30 fs and 150 fs) and possibility of pulses selection in the range from N = 1 to 100 and above, by inserting a mechanical shutter connected with a delay generator which controls the process via specially designed LabView program.

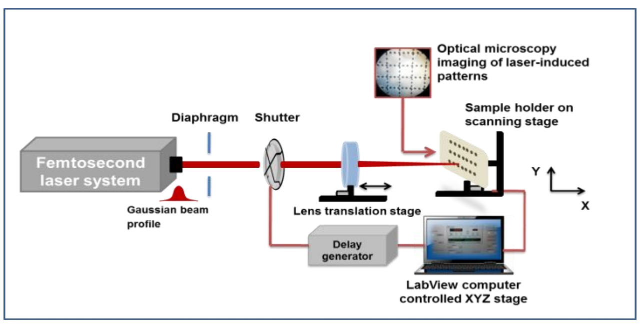

Figure 1. A schematic representation of the experimental setup for ultrashort surface modification of chitosan thin films.

The laser beam was focused on the sample surface by using focal lenses with lengths 10 cm and 20 cm. The focusing lens was placed on a translation stage equipped with micrometer screw for fine adjustment of the focus position on the specimen working surface. The sample was mounted on a XY motorized stage. The use of a motorized table, controlled by specially written program for the aim of the experiment, allows to obtain well controlled geometries, with variation of the sizes (patterns) on the sample surface.

3. RESULTS AND DISCUSSION

3.1 SEM morphological analysis

Figs. 2 and 3 are representing differences in surface modification morphology, after femtosecond laser irradiation, obtained for different laser parameters, respectively. On Fig. 2 are shown three different zones treated by laser pulses with τ=30 fs, pulse energy variation from 0.017 J/cm^2, 0.069 J/cm^2 and 0.349 J/cm^2 and number of applied laser pulses ranging from N=1, 2 and 5 pulses for 1 kHz repetition rate.

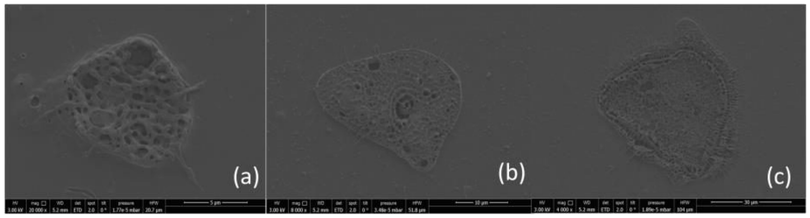

Figure 2. SEM analysis of thin chitosan films modifications: (a) 0.017 J/cm^2, N=2 pulses, 30 fs; (b) 0.069 J/cm^2, N=1 pulse, 30 fs; (c) 0.349 J/cm^2, N=5 pulses, 30 fs; for τ = 30 fs and λ= 800 nm, ν =1 kHz.

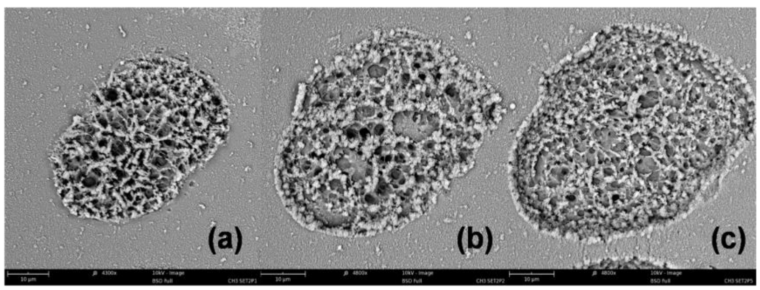

Figure 3. SEM analysis of thin chitosan films modifications: (a) 0.415 J/cm^2, N=1 pulse, τ =150 fs; (b) 0.415 J/cm^2, N=2 pulse, 150 fs; (c) 0.415 J/cm^2, N=5 pulses, τ = 150 fs and λ = 800 nm, ν = 50 Hz.

Similarly, in next figure (Fig. 3) morphological changes of the sample surface for τ = 150 fs, repetition rate 50 Hz, laser fluences ranging 0.207 J/cm^2 and 0.415 J/cm^2 for 1, 2 and 5 number of pulses are obtained. Visible differences were observed in the morphology structure of the two experiments due to difference between laser parameters values. First steps of surface modifications are beginning to appear for both pulse durations (τ = 30 fs and τ = 150 fs) and N = 1 pulse. As a consequence of pulse number increasing, the porosity of the surface structures decreases and zones with molten materials and sublimations are occurring. The results of SEM analysis showed visible differences in the resulting structures between chitosan surface modified by τ = 30 fs and τ = 150 fs. A structure type "grainy" is observed in the case of 150 fs due to the differences in output laser system parameters like pulse energy, pulse duration and repetition rate. An increase of the size of modified zone was observed by applying multiple number of laser pulses for both cases. Structure growth (in the form of foam-like formation), for N = 1 pulse, was monitored as a result of femtosecond laser interaction with the material. By further increasing the number of incident laser pulses, starts to develop laser ablation of the material.

3.2 Estimation of ablation threshold fluence laser accumulation effects

The ablation threshold is an important parameter in the laser microprocessing since it determine the minimal energy necessary to initiate microscopic removal of the material. The dependence of the ablation threshold on the pulse repetition rate and the number of pulses applied to the material was examined in the current study.

The focused laser spot size on the sample surfaces (ω) and the ablation threshold fluences were estimated for each number of applied pulses (N), using the method proposed by Liu[11], starting from the measurement of the craters diameters. The threshold evaluation was determined from the relationship between the incident laser fluence F_0 and the squared diameter D^2 of the formed crater after irradiation with single and multiple pulses eq.1.

$$D^2 = 2w_0 \ln\left(\frac{F_0}{F_{th}}\right) \tag{1}$$

where ω_0 is the size of the spot at the beam waist and F_{th} is assigned for the threshold fluence. A Linear fit is applied to the obtained data according to the relation $D^2 \sim \ln(F)$.

This method is advantageous since it doesn't require a characterization of the laser beam. The craters diameters were measured from the obtained SEM images using ImageJ program. The influence of the pulse laser energy on the size of the craters diameters and its variations for 30 fs (Fig. 4a) and 150 fs (Fig. 4b) laser pulses are presented in Fig. 4. The obtained results show increasing in the crater diameter of the modification with increasing in the number of laser pulses, in both cases. The main reasons could be explained by the so – called "pseudo – Gaussian - shape" of the laser beam or other effects like thermal diffusion into the material volume[12].

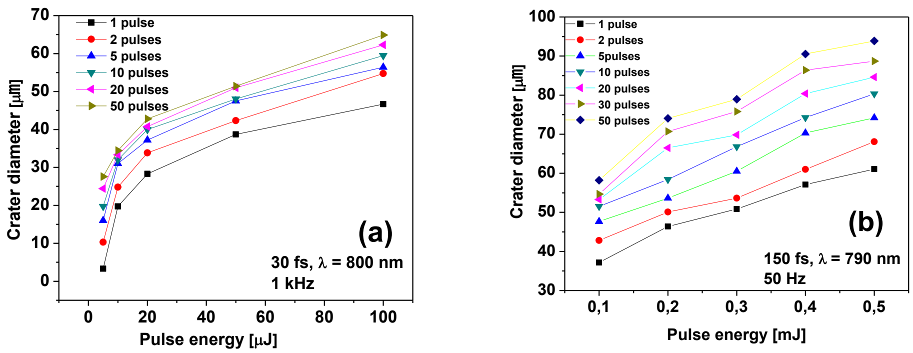

Figure 4. The squared crater diameter D^2 of the modified surface of chitosan for 30 fs (a) and 150 fs (b) versus pulse energy.

The single - shot (N = 1) and multi - shot (N > 1) fluences threshold values for 30 fs (Fig. 5) and 150 fs (Fig. 6) were evaluated, respectively by studying the linear relationship between the square of the crater diameter D^2 and the logarithm of the laser fluence F for each laser pulses. The estimated ablation threshold for 30 fs is F_{th} (N = 1) = 0.034 J/cm^2 and for 150 fs the laser fluence is F_{th} (N = 1) = 0.170 J/cm^2.

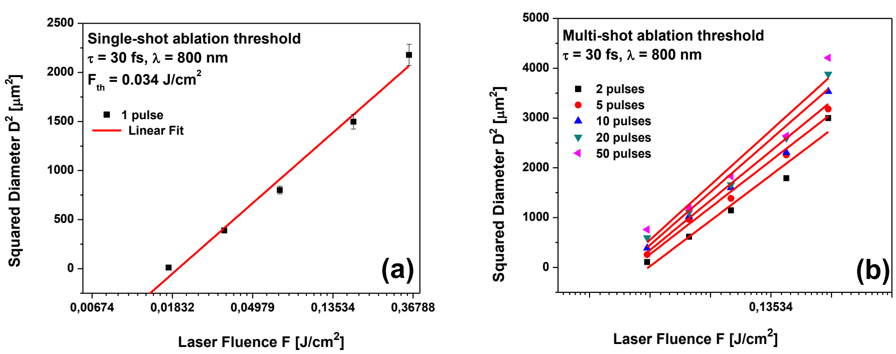

Figure 5. Diameter squared of the regions of modification versus the applied fluence. The single (a) and multiple (b) shot ablation threshold estimations of chitosan thin transparent films at λ = 800 nm and 30 fs laser pulses and 1 kHz repetition rate.

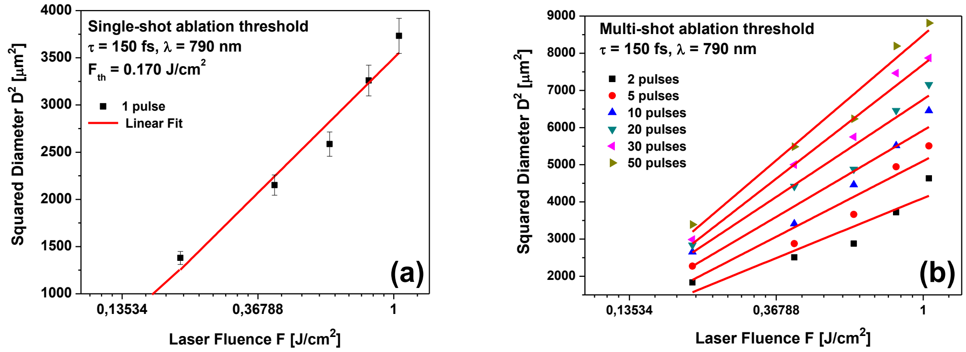

Figure 6. Diameter squared of the regions of modification versus the applied fluence. The single (a) and multiple (b) shot ablation threshold estimations of chitosan thin transparent films at λ = 800 nm and 150 fs laser pulses and 50 Hz repetition rate.

The multi – shot threshold experiments for 30 fs and 150 fs were conducted (see Fig. 4b and Fig. 5b) in order to provide greater clarity of the relations between the singe – and multi – shot ablation thresholds. The assessment of such dependences are determined by analyzing the diameter of ablation craters D generated with multiple laser incident pulses N = 2, 5, 10, 15, 20, 30 and 50, respectively. Laser ablation above the ablation threshold is called "gentle". In this case the efficiency decreases, however it allows very precise material removal. In our case the slope of the curves for both laser pulses (30 fs and 150 fs) is sharper with increasing of the incident number of pulses which is could be explained by the transition from gentle to the thermal ablation regime.

It was monitored for the multi-pulse irradiation condition at (τ=30 and 150fs) a decrease of the ablation threshold. Even at low-repetition-rate of the laser it was deteremined that the ablation threshold (F_{th}) of the material could be reduced after irradiation with a sequence of multiple laser pulses.

The coefficient of incubation ξ, a major parameter in the process of surface modification and ablation of materials also was calculated for multi - shot fluence threshold by power - law relationship form $F_{th}(N) = F_{th}(1)\, N^{\xi-1}$, where N is the number of applied laser pulses. The value of the incubation coefficient have to fulfill the limits: $0 < \xi \leq 1$. When $\xi = 1$, this indicates that no incubation process develops and the ablation threshold does not depend from the number of the applied laser pulses. If the parameter ξ is larger than 1, than the sample surface is hardened by laser irradiation.

Table 1. Estimated values of single and multi – shot ablation thresholds for 30fs and 150fs at 800nm for chitosan thin films.

$\mathbf{F_{th}}$ (N=1) = 0.0132 J/cm^2	$\mathbf{F_{th}}$ (N=2) = 0.0097 J/cm^2	$\mathbf{F_{th}}$ (N=5) = 0.0074 J/cm^2	$\mathbf{F_{th}}$ (N=10) = 0.0071 J/cm^2	$\mathbf{F_{th}}$ (N=20) = 0.0065 J/cm^2	$\mathbf{F_{th}}$ (N=50) = 0.0056 J/cm^2
$\boldsymbol{\tau}$ = 30 fs	ξ = 0.566	ξ = 0.643	ξ = 0.733	ξ = 0,767	ξ = 0,781
$\mathbf{F_{th}}$ (N=1) = 0.17 J/cm^2	$\mathbf{F_{th}}$ (N=2) = 0.154 J/cm^2	$\mathbf{F_{th}}$ (N=5) = 0.132 J/cm^2	$\mathbf{F_{th}}$ (N=10) = 0.116 J/cm^2	$\mathbf{F_{th}}$ (N=20) = 0.105 J/cm^2	$\mathbf{F_{th}}$ (N=30) = 0.098 J/cm^2
$\boldsymbol{\tau}$ = 150 fs	ξ = 0,857	ξ = 0,842	ξ = 0,834	ξ = 0,839	ξ = 0,838

In our experimental results an incubation process was observed in both cases (30 fs and 150 fs). By increasing the number of applied laser pulses, saturation process starts to develop and differences in the values of the incubation coefficient have been observed for 150 fs case. One of the possible reasons could be related to the variations of the pulse repetition rate (1 kHz for 30 fs and 50 Hz for 150 fs) and differences in the applied laser pulse duration.

4. CONCLUSION

In this paper, the femtosecond laser modification of thin films of biopolymer chitosan was studied. The morphological changes on the surface modification of chitosan were observed for 30 fs and 150 fs. The single and multi – pulse values of ablation thresholds for N = 1, 2, 5, 10, 20, 30 and 50 pulses for 30 fs (1 kHz) and 150 fs (50 Hz) at 800 nm wavelength were evaluated. It was experimentally confirmed that the ablation threshold of the biopolymer material depends from the pulse number (N) impinging on the same area. It was monitored a decrease of the ablation threshold by irradiation with a sequence of laser pulses, which could be used for application where higher precision is required. The fundamental parameter for surface modification, like incubation coefficient, was determined for low and high repetition rate processing. The saturation effect of incubation coefficient is observed for 150 fs which could be explained by the differences in pulse duration and repetition rate.

ACKNOWLEDGEMENTS

This work was financially supported by the Bulgarian National Science Fund (NSF) under projects # DMU 03/15/2011-2015, # DNTS Austria/01/1/2013-2015 and EU project COST MP1301 NEWGEN.

REFERENCES

[1] Dhandayuthapani, B., Yoshida, Y., Maekawa, T. and Kumar, D. S., „Polymeric Scaffolds in Tissue Engineering Application: A Review," Hind. Publ. Corp. Intern. J. Pol. Sci. 2011 (2011).
[2] Croisier, F., Jérôme, C., „Chitosan-based biomaterials for tissue engineering," European Polymer J. 49, 780–792 (2013).
[3] Sionkowska, A., „Current research on the blends of natural and synthetic polymers as new biomaterials: Review," Pr. Pol. Sci. 36, 1254– 1276 (2011).
[4] Kim, I., Seo, S., Moon, H., Yoo, M., Park, I., Kim, B., Cho, C., „Chitosan and its derivatives for tissue engineering applications," Biotech. Adv. 26, 1–21 (2008).
[5] Hsieh, W., Liau, J. and Li, Y., „Characterization and Cell Culture of a Grafted Chitosan Scaffold for Tissue Engineering," Intern. J. Pol. Sci. 2015 (2015).
[6] Li, H., Gao, X., Wang, Y., Zhang, X., Tong, Z., „Comparison of chitosan/starch composite film properties before and after cross-linking," Intern. J. Biol. Macr. 52, 275– 279 (2013).
[7] Ji, C., Annabi, N., Khademhosseini, A., Dehghani, F., „Fabrication of porous chitosan scaffolds for soft tissue engineering using dense gas CO_2," Acta Biomat. 7, 1653–1664 (2011).
[8] Daskalova, A., Nathala, C. S.R., Bliznakova, I., Stoyanova, E., Zhelyazkova, A., Ganz, T., Lueftenegger, S., Husinsky, W., „Controlling the porosity of collagen, gelatin and elastin biomaterials by ultrashort laser pulses," Appl. Surf. Sci. 292, 367– 377 (2014).
[9] Sionkowska, A., Kaczmarek, H., Wisniewski, M., Skopinska, J., Lazare, S., Tokarev, V., „The influence of UV irradiation on the surface of chitosan films," Surf. Sci. 600, 3775–3779 (2006).
[10] Daskalova, A., Nathala, C., Bliznakova, I., Slavov, D., Husinsky, W., „Estimation of ultrashort laser irradiation effect over thin transparent biopolymer films morphology," Proc. SPIE 9447, (2015).
[11] Liu, J. M.,"Simple technique for measurements of pulsed Gaussian-beam spot sizes," Opt. Lett., 7, 196, (1982).
[12] Nimmrichter, J., Kautek, W. and Schreiner, M., [Lasers in Conservation of Artworks], Springer, Springer Proceedings in Physics 116, 372 (2005).