Ultra-short laser pulse modification of chitosan/silver nanoparticles (AgNPs) thin films for potential antimicrobial applications

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Abstract. The last several years witnessed increasingly rapid advances in applying biopolymers in tissue engineering for the purposes of regenerative medicine. The growing demand for preparing materials with desired physical, biological and mechanical properties requires active investigations in the field of tissue engineering. Among the wide variety of biopolymers, chitosan proved to be an outstanding material due to its properties, such as biocompatibility, biodegradability and a wide range of available fabrication technologies. The present work is a case study of an extensive research on the functionalization of thin biopolymer films via laser patterning. The aim of the current study is to investigate the optical properties of biopolymer films on the example of chitosan and chitosan with silver nanoparticles additives. As laser sources are used a nano- and a femto-second laser system emitting the wavelengths of 355 nm and 800 nm, respectively. The compositions produced are investigated by spectral analyses using spectrometers and an optical microscope. Furthermore, the morphology of the samples is monitored by SEM analyses. The results obtained demonstrate the potential of the method employed for obtaining diverse porous modifications depending on the laser parameters. Adding silver nanoparticles will drastically increase the thin chitosan films’ antimicrobial properties, thus enhancing the biocompatibility properties of the 2D matrices created.

1. Introduction
In the search for alternatives to the conventional treatment strategies for the repair or replacement of missing or malfunctioning human tissues and organs, promising solutions have been explored through tissue engineering approaches. Tissue engineering offers solutions for improved functionality and biocompatibility of material implants inside the biological environment.

In the last few decades, a range of biomaterials, such as different polymers and bioceramics, have gradually established themselves as alternatives to standard materials (metals and metal sleepers) for permanent replacement and temporary stabilization of orthopedic implants, providing better adaptability, less side effects and faster recovery. At present, the efforts are mainly focused on the
development of synthesized polymeric materials that are as close as possible to the chemistry, structure and function of the natural tissue. The choice of biomaterial is one of the most important steps in the process of manufacturing implants for regenerative medicine. Among the variety of biopolymers chitosan has been shown to be an excellent material due to its distinctive properties, such as biocompatibility, biodegradability and a wide range of fabrication technologies [1-3]. The chitosan can be processed into films and porous structures for applications in regenerative medicine, drug delivery, wound bandaging, blood vessels, and nerve conduits [4-7]. Furthermore, the chitosan is being considered as a potential candidate for bio-dental applications due to its antimicrobial properties and ability to blend with other materials [8]. Studies have been carried out with the use of biopolymers, such as chitosan, gelatin and hyaluronic acid, as precursors for tissue regeneration in bone and cartilage engineering. It is known that chitosan itself has antimicrobial activity due to its cationic properties causing a membrane-disrupting effect. There are theories suggesting that the increased antimicrobial activity of Ag-incorporated nanoparticles in chitosan arise from the high degree of infiltration of the silver component with a resulting high bactericidal effect. Recently, scientists have begun to synthesize chitosan-based nanocomposites, as they demonstrate various advantages, such as antibacterial activity, biocompatibility, higher stability and better cell response. These properties have increased the interest in chitosan nanocomposites that could provide a novel way of developing new functional materials for the purposes of regenerative medicine.

A further step in improving the properties of a biomaterial can be achieved by modification. Over the years, various physical and chemical patterning approaches have been employed for surface structuring, including photolithography, electron-beam lithography, electrochemical deposition, selective growth of carbon nanotubes and plasma treatment [9, 10]. In recent years, nano- and micro-patterned surfaces have attracted an increasing attention among the scientific communities of physics, chemistry, medicine and biology [11-13]. It has been shown that the surface chemistry and topography of biomaterial substrates not only affect strongly the cell morphology [14], but also influences the cell behavior, such as adhesion, migration, orientation, guidance, differentiation, proliferation, as well as gene expression and protein synthesis [15]. Understanding the bacteria-surface interaction mechanism is essential for the design of biomaterial surfaces with improved properties.

The morphological changes of the surfaces of biofilms and 3D polymeric scaffolds affect the cells growth and adhesion and their distribution in a desired direction. In this respect, lasers provide significant advantages thanks to the precise control of the laser power, e.g., avoiding heating and melting and the consequent blockage of the porous structure, as well as controlling cell attachment, proliferation, and differentiation. Laser modification can be used to shape materials both laterally and in depth, hence allowing the manufacture of structures with complex geometries, including 3D shapes or structures with varying wall shapes and etch depths [16].

Bearing in mind the complexity of any process involved in the preparation of bio-implants, such as synthesis, micro- and nano-structuring, cell culture, in-vivo, in-vitro assays, in-depth studies are required on the bio-environment–biomaterial interaction mechanism. Furthermore, optimization of the morphology, topography and physico-chemical composition of the contact surface should be carried out.

The aim of the current study is to investigate the optical properties of biopolymer film specimens of chitosan and chitosan with additives of silver nanoparticles. The chitosan/silver nanoparticles (AgNPs) samples are first laboratory synthesized, then modified by femtosecond and nanosecond laser irradiation. The last step is performing analyses in order to investigate their optical properties.

The article is organized as follows – the Introduction section is followed by Section 2 presenting the experimental setup and configuration. In Section 3 results and discussion are provided. Concluding remarks are given in Section 4.
2. Experimental configuration and settings

2.1. Setup configuration
The laser processing is provided by two laser systems – femto- and nano-second lasers. The femtosecond laser system consists of several units – oscillator, amplifier, pumping laser and optical parametric amplifier. The Ti:Sapphire regenerative amplifier has a pulse width of ~35 fs and a pulse energy of ~6 mJ and operates at a 1-kHz repetition rate and 800 nm as the main wavelength. The femtosecond laser is further equipped with a high-precision planar XY stage, a power-meter and optical and opto-mechanical elements. The nanosecond laser system consists of a Nd:YAG laser generating high-power radiation at 1064 nm, 532 nm, 355 nm and 266 nm at a repetition rate of 10 Hz and a pulse duration of 15 ns. Photographs of the femtosecond laser system setup are shown on Figure 1 as an example.

The optical properties are analyzed by a B-150D–BRPL optical microscope (Optica, Italy) and a spectrometer system consisting of a Cary 1E UV-VIS spectrophotometer, an Ocean Optics DH2000 light source and an Ocean Optics HR4000 optical spectrometer. The spectra are obtained with the spectrophotometer operating in a transmission mode. The scanning electron microscopy (SEM) images are recorded by using a JEOL JSM 5510 scanning electron microscope.

Figure 1. Experimental setup of the femtosecond laser system. Image a) is an overview of the system, image b) shows the optical path elements guiding the laser beam to the sample. Image c) demonstrates the position of the sample on a precise planar stage and the laser beam direction.

2.2. Material
The biopolymer films studied are divided into two groups. The first one is a specimen of chitosan. The chitosan used is of medium molecular weight, Sigma-Aldrich® (Munich, Germany). The chitosan powder is dissolved in a 0.5-mol/L CH3COOH solution and dH2O and heated to 50 °C under continuous stirring for 2.5 h. The second one is chitosan with additives of silver nanoparticles. A solution of silver nanoparticles AgNps–10% Np (v/v) is added to the prepared solution. Suspensions of chitosan-silver blends are prepared with the desired weight ratio of 90% chitosan and 10% silver nanoparticles in 0.5-M acetic acid. The biopolymer films are deposited onto laboratory glass plates and further dried in air. The dimensions of the samples are of 2 cm×2 cm size with 2-mm thickness (Figure 2). Each sample is irradiated and modified by a laser source with controlled sets of laser parameters and then analyzed.

Figure 2. Photo image of chitosan/AgNPs samples.

3. Results and Discussion
The polymer samples are modified under a variety of laser conditions, changing the laser fluence, number of pulses and wavelengths. The laser processing measurements included and discussed in this study are performed by a femtosecond (fs) laser at the wavelength of 800 nm and a nanosecond (ns) laser at 355 nm. The number of laser pulses applied is varied from 1 to 5, which is found to be the
optimal condition in this respect [17]. The optimal applied laser fluence in order to avoid ablation or reaching the substrate bottom is found to be 1.2 J/cm\(^2\) for the fs laser and 3.75 J/cm\(^2\) for the ns laser.

Figure 3 shows optical microscope images. The samples on Figure 3 a) and b) exhibit grid structures of laser spots. Each sample is treated by a single laser pulse (per laser spot) of fs and ns laser for a) and b) respectively. The laser fluence for sample a) is \(F = 1\) J/cm\(^2\) and for sample b) \(F = 3.75\) J/cm\(^2\). The pattern of the laser-irradiated areas and the shape of the laser spots are clearly seen. The laser spot size for the case of the fs laser at wavelength 800 nm is 100 \(\mu\)m and 200 \(\mu\)m for the case of ns laser at wavelength 355 nm.

Figure 4 presents the results of the optical density measurements. The working conditions are as follows – the applied number of pulses is \(N = 1\) and \(2\) at wavelength \(\lambda = 355\) nm and laser fluence \(F = 3.75\) J/cm\(^2\). The distance between the laser spots is varied. In one case, the spots are adjacent, and in the other, the laser spots overlap. The bottom curve in the graphic in Figure 4 shows the result from an untreated chitosan/AgNPs sample. The top four lines represent measurements of treated samples. The differences in the conditions for these samples concern the number of pulses and the distance (adjacent or overlapping) between the laser spots. It is seen that the optical density changes approximately fivefold after laser irradiation. The other observation is that the samples are very sensitive to changing the distance between the laser spots, i.e., whether they are overlap or are adjacent. The results indicate clearly that by controlling precisely the laser patterning one could easily choose the desired optical density within a certain range.

Further, the wettability contact angle (WCA) was also measured. A difference was observed of the WCA values in the treated \((\theta = 101.2^\circ)\) and the untreated zones \((\theta = 80^\circ)\). Thus, we were able to alter the surface properties from hydrophilic to hydrophobic, which is closely related to potential antimicrobial application of the designed thin film.
The morphology of the samples was monitored by scanning electron microscopy (SEM). Figure 5 presents SEM images of a representative chitosan/AgNPs sample irradiated by a single laser pulse with fluence $F = 3.75$ J/cm$^2$ at a wavelength of 355 nm. One can clearly see (in the background) a periodic structure of well-defined elliptic shapes with fine circular shapes inside. This pattern is observed in all samples. To the best of our knowledge, such morphological patterning has not so far been reported in the literature. Thus, further detailed and extensive research will be dedicated to explaining this phenomenon.

![Figure 5](image)

**Figure 5.** SEM images of chitosan/silver AgNPs sample irradiated with laser fluence $F = 3.75$ J/cm$^2$ and one laser pulse, at different magnification.

### 4. Conclusions

The results obtained illustrate the potential of the method of laser irradiation of chitosan/silver AgNPs to obtain various porous modifications depending on the laser parameters applied. The preliminary findings demonstrate that a suitable control of the laser parameters enables one to achieve changes in the optical properties of the biopolymer thin films investigated. Furthermore, adding AgNPs will drastically increase the antimicrobial properties of the thin chitosan films and thus enhance the biocompatibility properties of the 2D matrix created.

As future next steps, we plan to continue investigating the properties of chitosan/AgNPs films with respect to (i) their antibacterial activity following established protocols and procedures; (ii) the periodic patterning observed by the SEM images.

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