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Surface Properties of Femtosecond Laser Irradiated Collagen Films

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The interactions between femtosecond laser and collagen films have been investigated. Collagen was obtained in our laboratory from tail tendons of young albino rats. Thin collagen films were obtained by casting of collagen solution onto glass plates. The changes on film surfaces after femtosecond laser irradiation were examined using scanning electron microscopy. The laser irradiation caused an expansion of materials above the surface of collagen films. The obtained results are considered in terms of possible ablation mechanisms and compared with recent results obtained with nanosecond laser pulses.

Keywords: collagen film; femtosecond laser; surface structure

INTRODUCTION

The femtosecond laser-biomaterial interaction has recently attracted a great deal of attention in various scientific fields [1–3]. Lasers and biomaterials are also implicated in the fields of biomimetics and bioelectronics. The biological components possess several properties relevant to pulsed laser ablation. Firstly, the composition and morphology

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which influence on the optical properties of material and that determine the internal volumetric energy distribution. Secondly, the above mentioned composition and morphology are also involved in energy transport and mediate the thermomechanical response of biomaterial to pulsed laser heating and phase transformation [4].

Collagen is the primary structural material of vertebrates and is the most abundant mammalian protein (about 20–30% total body proteins). It is present in tissues of primarily mechanical function [5].

Collagen has outstanding molecular architecture and properties and it is a suitable material for laser-matter interaction research [6,7]. Collagen in the human body accounts for about 25% of all proteins and it is the main component of connective tissue, occurring in skin, tendons, cornea, bone and membranes [6–8]. It is a strongly hydrophilic protein that explains the ability of collagen materials to bind a large amount of water in its internal structure [8–12].

In our recent papers we presented a new laser processing of the studied biopolymer film surfaces, collagen, collagen/PVP blend and chitosan. A single KrF laser pulse of sufficient energy density for ablation excites the skin depth of the film $(\sim\!15\,\mu\text{m})$ yielding a new microfoam layer [13–19] having some promising biomimetic properties that could be used in cell culturing.

The aim of this work was to study the interaction between femtosecond laser and collagen films and explain the responsible mechanisms. The obtained results will be considered in terms of possible ablation mechanisms [13] and compared with recent results obtained with nanosecond laser pulses [14].

MATERIALS AND METHODS

Collagen (type I) was obtained in our laboratory from the tail tendons of young albino rats. After washing in distilled water, tendons were dissolved in 0.4 M acetic acid solution. Non-dissolved parts of tissue were separated by centrifugation at $7500\,\mathrm{rpm}$. Thin biopolymer films, of $\sim\!35\,\mu\mathrm{m}$ thickness, were obtained by casting the prepared aqueous solution onto glass plates and drying in air at room temperature overnight.

The changes on film surfaces after femtosecond laser irradiation laser (Thales, Alpha 1000, 1 kHz, 800 nm, 200 fs, used pulses 1 to 10) were examined using scanning electron microscopy (SEM Microscope Hitachi S-2500 at ISERM U577). For comparison we used UV radiation from krypton-fluoride (KrF) excimer laser (Lambda Physik, LPX 220i) emitting the wavelength of 248 nm and pulse duration of 25 ns.

RESULTS AND DISCUSSION

Figure 1 show the image recorded for collagen films by means of SEM microscopy after 1 pulse of femtosecond laser with energy 100 mW. The use of single pulse of femtosecond laser radiation causes visible change into the surface structure of collagen. The SEM images of the non-irradiated surface of the examined films have no irregularities and no obvious damages (outside the irradiated spot). The laser irradiation of the specimens caused an expansion of materials above their initial surface with well-pronounced symptoms of its melting.

More significant surface modification appears after two pulses of laser radiation with the same energy 100 mW (Fig. 2). Along with increasing number of pulses, the changes on the irradiated surfaces become more drastic. Further increase of numbers of pulses (at the same energy of one pulse) leads to the surface ruptures of the examined films (Fig. 3) as a result of significant pressure effects generated by incident laser pulse or pulses.

With increasing energy of the pulse the effect of film damage occurs at the same number of the incident pulses. The Figure 4 shows the changes at the surface of collagen films after one pulse of laser radiation with energy 200 mW.

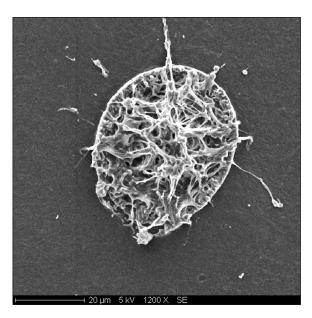


FIGURE 1 Collagen film surface after femtosecond laser treatment (laser energy 100 mW, 1 pulse).

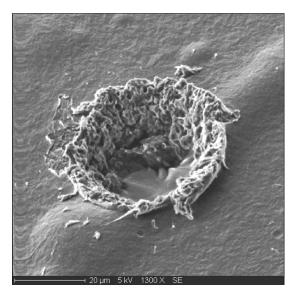


FIGURE 2 Collagen film surface after femtosecond laser treatment (laser energy $100\,\mathrm{mW},\,2$ pulses).

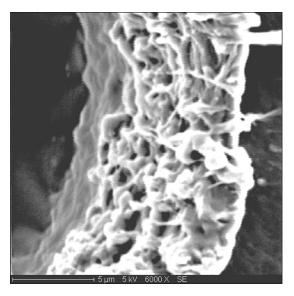


FIGURE 3 Collagen film surface after femtosecond laser treatment (laser energy $100\,\mathrm{mW},\,5$ pulses).

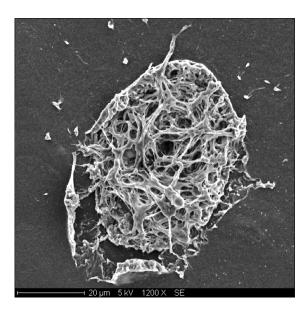
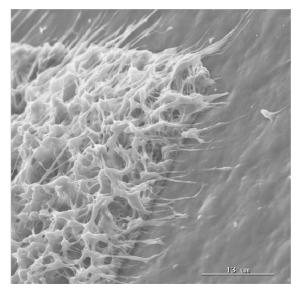


FIGURE 4 Collagen film surface after femtosecond laser treatment (laser energy 200 mW, 1 pulse).



 \pmb{FIGURE} 5 Collagen film surface after nanosecond laser treatment $(F=1.7\,J/cm^2,\,1~pulse).$

The material ejection under the influence of incident laser pulses seems to be slightly more efficient in case of two pulses than for one pulse. The "micro-foam" structure one can observe at the irradiated surface. "Micro-foam" structures obtained for different numbers of pulses seem to be different.

In Figure 5, for comparison, SEM image of collagen films after nanosecond excimer laser (KrF) one pulse with fluence $F=1.7\,\mathrm{J/cm^2}$ is shown.

The structure of micro-foam seems to be similar for both, nanosecond and femtosecond laser treatment. However, the interaction between UV excimer laser and collagen film should be considered from photomechanical and also photochemical mechanism point of view. Interaction between femtosecond laser and collagen film should be rather considered in thermal mechanism point of view.

CONCLUSIONS

Collagen films exposed to femtosecond laser irradiation undergo the surface "foaming" giving rise to the formation of the "micro-foam" structure with well-pronounced symptoms of its melting.

The obtained results indicate that the interaction between the collagen film and UV laser radiation discussed in our previous work [14–19], might be considered in both, in the photomechanical regime and also from the photochemical mechanism point of view. Interaction between femtosecond laser and collagen film should be considered in thermal mechanism point of view.

The microfoam structure has got interesting biomimetic properties. The collagen microfoam can be considered for cell implantation purpose and as drug carrier, burn or wound cover dressings or substrate for tissue engineering. Moreover it seams that surface foaming by laser ablation is a promising approach since it is a "green process" which does not use nor reject any chemical reagent potentially contaminating for the environment.

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