Formation of composite material based on proteins, chitosan and nanotubes by nanosecond laser pulses

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Abstract. Aquous dispersions with albumin, collagen, chitosan and single-walled carbon nanotubes (SWCNTs) in different combinations were studied. It was found, that an increase in the fluence above threshold leads to a significant increasing in the total absorption of radiation, moreover the main part of the energy was taken up by the SWCNTs. Thus, the thermal effect on proteins and chitosan decreased. This was confirmed by the nonlinear absorption coefficients, which were determined for the studied aqueous dispersions. Low threshold values of fluence for albumin 0.006 J/cm\textsuperscript{2} and collagen 0.009 J/cm\textsuperscript{2} are due to the onset of the denaturation process. This parameter increased up to ~0.05 J/cm\textsuperscript{2} after addition of SWCNTs that was caused by the formation of microbubbles due to the water evaporation from the heating of nanotubes. The reverse situation occurs in samples with chitosan (0.2 J/cm\textsuperscript{2}) in which there was no denaturation. Adding SWCNTs leads to a decrease in the threshold fluence threshold to 0.15 J/cm\textsuperscript{2}, which can be explained by the rapid heat transfer from nanotubes compared with chitosan. It was established, that optimal energy density range from 1 to 50 J/cm\textsuperscript{2} for the formation of a composite material using laser pulses with duration 16 ns.

1. Introduction
The layered laser printing of a multilayer composite material for the creation of cellular and tissue engineered structures with an internal scaffold that increases its strength is an urgent task [1]. First of all, it is necessary to develop ink whose composition provides biodegradation, photocrosslinking and sufficient strength of the formed structure for the bioengineering of human organs and tissues. Therefore, one of the promising approaches for solving this problem is the use of proteins that perform many functions and are the most numerous organic substances in the human body, have high biocompatibility and biodegradability [2, 3], in addition they are almost transparent in the visible range [4]. According to previous studies, carbon nanotubes are well suited to form a scaffold that increases its strength [5]. For these reasons, an aqueous dispersion medium with single-walled carbon nanotubes (SWCNTs) and proteins can be used as the ink. The formation of the composite material is carried out using the technology of the sol-gel process by means of laser radiation. The composite created in this way is able to ensure the germination of blood vessels through its structure in the process of biodegradation after implantation in human body [6]. Moreover, the tensile strength and hardness of such a composite material in the presence of a structured SWCNTs scaffold surpasses similar characteristics of the porous human bone tissue [5].
The paper investigated pure aqueous dispersions of albumin, collagen and chitosan, as well as the same dispersions with SWCNTs. Albumin, collagen and chitosan were weighed on scales and mixed with distilled water at concentrations of 25%, 1%, and 2%, respectively. The resulting dispersion was mixed in ultrasonic bath and on magnetic stirrer. SWCNTs at a concentration of 0.001% was also intermixed with distilled water and mixed in ultrasonic homogenizer. Then, a homogeneous aqueous solution of SWCNTs was added to the aqueous dispersions of albumin, collagen and chitosan and was alternately treated in ultrasonic bath and with magnetic stirrer.

2. Nonlinear optical measurement
The optical characteristics were studied using the experimental scheme shown in the figure 1. The laser radiation source was a neodymium Nd:YAG laser with a pulse duration of 16 ns. The experiments were carried out at wavelengths of 0.532 µm and 1.064 µm, the energy regulation was performed by a set of neutral filters, and its smooth variation - by the Glan-Taylor prism, because the beam generated by the laser had a linear polarization. The incident fluence is calculated from the input detector data, which registered the radiation reflected from the beam splitter. The registration of the beam transmitted through the sample was carried out with the help of the output detector. To achieve the fluence, which led to the appearance of nonlinear effects, laser radiation focusing was used, the focal length of the lens was 10 cm.

![Figure 1. Experimental scheme.](image)

Optical parameters characterizing the interaction of nanosecond laser radiation with dispersions of albumin, collagen, chitosan and SWCNTs in different combinations at a wavelength of 0.532 µm were determined. To determine the nonlinear properties of these materials, open aperture Z-scan curves and the dependence of the output energy on the input energy for a fixed location of studied sample were obtained.

2.1. Z-scan and fixed location experiments of proteins
With Z-scan experiments, the incident energy remained unchanged, and the sample moved along the focus of the lens. Input-output curves were obtained during the fixed sample location experiments. The sample was placed in the focus of the lens, and the energy was varied by Glan-Taylor prism rotation. The output energy axis is presented in a logarithmic scale.

Figures 2-3 show experimental data for dispersions containing proteins and SWCNTs under the influence of single laser pulses.
Figure 2. Experimental data for BSA and BSA+SWCNTs dispersions (0.532 μm): a) – Z-scan, b) – Input-output curves.

Figure 3. Experimental data for collagen and collagen+SWCNTs dispersions (0.532 μm): a) – Z-scan, b) – Input-output curves.

Low values of the threshold fluence were obtained for albumin 0.006 J/cm² and collagen 0.009 J/cm², which was related to the start of the denaturation process. The addition of SWCNTs increased the values of this parameter to ~ 0.05 J/cm² due to the active formation of microbubbles while water evaporation as a result of nanotubes heating.

2.2. Z-scan and fixed location experiments of chitosan
Dispersed media with chitosan (figure 4) had a threshold fluence of 0.2 J/cm² due to the absence of denaturation. Therefore, when the SWCNTs were added, the opposite effect was observed, namely this parameter decreases to 0.15 J/cm², which may indicate a more rapid heat transfer from nanotubes compared to chitosan.
2.3. Optical linear and nonlinear absorption studies

However, an increase in the fluence up to 75 J/cm$^2$ led to the splashing of the sample due to the formation of very large bubbles that bursted. Therefore, the controlled formation of composite material with this composition can be made in the range from 1 to 50 J/cm$^2$ with a pulse duration of 16 ns at wavelength of 0.532 μm. Nevertheless, the formation of microbubbles in the sol-gel process makes it possible to ensure the microporosity of the tissue-engineered structure with the internal scaffold.

It was established that an increase in the fluence above the threshold led to significant increase in attenuation of radiation, and the part of radiation absorbed by the SWCNTs began to increase sharply. This was confirmed by the coefficients of nonlinear absorption, which were determined for the following aqueous dispersions: albumin 4 cm/GW, collagen 5.9 cm/GW and chitosan 16 cm/GW, after adding SWCNTs 345 cm/GW, 67 cm/GW and 516 cm/GW, respectively.

The linear absorption coefficient was measured in the fluence range below the threshold fluence, each of the samples was placed in a quartz cuvette with an optical path length of 2 mm. For dispersions of albumin, collagen and chitosan the coefficients were 1.92 cm$^{-1}$, 2.16 cm$^{-1}$ and 2.9 cm$^{-1}$, respectively, and after the addition of SWCNTs, the values increased to 2.7 cm$^{-1}$, 2.94 cm$^{-1}$ and 3.88 cm$^{-1}$. These data are in good agreement with the optical spectra obtained with a spectrophotometer (figure 5-6).

**Figure 4.** Experimental data for chitosan and chitosan+SWCNTs dispersions (0.532 μm): a) – Z-scan, b) – Input-output curves.

**Figure 5.** Optical spectra of: a) – BSA and BSA+SWCNTs, b) – Collagen and collagen+SWCNTs.
Even a low percentage of any components, except albumin, led to a strong decrease in the transmission of the sample, which determined the penetration depth of laser radiation into the dispersion. Due to the growth of nonlinear absorption, radiation was almost completely absorbed in 0.5 mm thick layer.

2.4. Nonlinear optical properties of albumin at wavelength of 0.532 and 1.064 μm
Threshold fluence values were also obtained for aqueous dispersions of pure albumin and albumin with nanotubes at wavelength of 1.064 μm. For the calculation, the results of Z-scan and fixed sample location experiments, shown in the figure 7, were used.

According to the graphs, characterizing the nonlinear properties of the samples, it was clearly seen that pure albumin poorly absorbed laser radiation at wavelength of 1.064 μm. This was confirmed by the optical spectrum and the calculated value of the threshold fluence of 7.85 J/cm². When SWCNTs were added to the dispersion, the same effect was observed as with chitosan dispersions at wavelength of 0.532 μm, and the threshold fluence decreased to 0.19 J/cm². These values confirmed that the formation of composite material with SWCNTs can be performed in the range from 1 to 50 J/cm² and at wavelength of 1.064 μm as well as at 0.532 μm.

![Figure 6. Optical spectra of chitosan and chitosan+SWCNTs.](image)

![Figure 7. Experimental data for BSA and BSA+SWCNTs dispersions (1.064 μm): a) – Z-scan, b) – Input-output curves.](image)
3. Formation of composite material

However, it is not enough to use single pulses with low frequency for laser printing of three-dimensional structures. Therefore, it was proposed to use a pulsed nanosecond laser with a wavelength of 1.064 μm and a beam radius of 20 μm, allowing irradiation of materials at frequency of 30 kHz (figure 8). The ytterbium fiber laser was connected to the positioning system by means of an optical fiber. The system provided beam positioning in the X/Y plane. The collimator located in the positioner generated laser radiation. The lens focused the laser beam on the sample. The Z axis sensor determined the distance from the lens to the sample. The distance sensor, the positioner and the lens were placed in scanning head, which was placed on the rails that moved the scanner along the Z axis. The control unit was connected to the computer and provided feedback and control of the entire system.

To determine the fluence of the pulses, the power of the incident radiation was determined at frequency of 1.6 kHz and the laser power in 10% (figure 9). In this experiment, the irradiation durations were 1, 2, 3, 4 and 5 seconds, respectively. The average radiation power was ~350 mW. Thus, the fluence of incident radiation was ~35 J/cm². The obtained value was within the calculated fluence range, therefore, irradiation of the investigated dispersions was carried out at the indicated laser parameters. The formation of solid composites occurred over 1200-1500 laser passages. By varying the frequency of the incident radiation or laser power, the values of the energy density can be varied and, thus, the rate of the three-dimensional structures formation can be changed. Also, with different laser settings, the change of the print area, i.e. radius of the irradiated region, can be performed.

![Figure 8. Experimental scheme for laser printing of 3D composites.](image)

![Figure 9. Power of incident laser radiation.](image)

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4. Conclusion

The use of nanosecond laser pulses with the above parameters makes it possible to form a composite material with less thermal impact on proteins and chitosan, because the largest part is absorbed by nanotubes during the formation of scaffold, which contributes to the increase in strength. Laser printing of multilayered three-dimensional cellular and tissue structures with structured internal carbon
nanoscaffold with protein and chitosan molecules can be performed at irradiation frequency of 30 kHz for 160-400 passes, depending on the thickness of the layer. Subsequently, this composite material can be used for implantation in the damaged area of the cardiovascular system.

Acknowledgments
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5. References
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