Tissue layers three-dimensional structure formation by nanosecond laser pulses

P.N. Vasilevsky[1], A.Yu. Gerasimenko[1,2], M.S. Savelyev[1,2], S.A. Tereschenko[1]

[1]National Research University of Electronic Technology, Zelenograd, Russia
[2]I.M. Sechenov First Moscow State Medical University, Moscow, Russia

Contact: savelyev@bms.zone

Introduction

Carbon nanotubes (CNTs) attract the attencion of researchers due to theit electronic, optical, thermal and mechanical properties [1]. Thus, there is a tendency to increase the applicability of CNTs for the nanomaterials creation for various purpose, including medical ones.
This work is devoted to the creation of three-dimensional tissue-engineered structures based on albumin and collagen proteins with an inner scaffold made of CNTs. Such structures are well suited as implants for the cardiovascular system due to their high biocompatibility, ability to biodegradation [2] and ensuring the germination of blood vessels net.
Therefore, it is obvious that it is necessary to obtain detailed information on the structural changes that occur during laser radiation influence. Under the influence of this kind of high-intensity radiation on the local area of protein dispersion with CNTs, phase transformations with the formation of a three-dimensional tissue-engineered structure take place. And in the case of using laser beams of nanosecond duration, nonlinear effects arise. That is why it is necessary to conduct Z-scan and fixed sample location experiments to determine the dependence of the total absorption coefficient on the intensity, which is important to consider during the formation of the tissue-engineered structure [3].
As a result of this work, nonlinear properties of protein aqueous dispersions with CNTs were determined using a method based on the radiation transfer equation [4]. The beam radius values were measured depending on the location of sample relative to the focus of the lens and a comparison of these data with the theoretical ones was made. In this way, the values of the input light fluence which fell on the local region of the sample during the formation of the tissue-engineered structure were determined.

Materials and Methods

Aqueous dispersions of bovine serum albumin (BSA) with a concentration of 25% and bovine collagen (BC) with a concentration of 2% and similar dispersions with single-walled carbon nanotubes (SWCNTs) were investigated in the study.
To prepare the dispersion with CNTs, they were premixed in a homogenizer with water with a mass concentration of 0.03% until a homogeneous solution was obtained. This solution was then mixed with the protein water dispersion and processed in an ultrasonic bath for 40 minutes.

Results

As shown in fig. 1, as the input energy density increases, the BSA dispersion with CNTs exhibits strong nonlinear effects (fig. 1b). This is reflected in the sharp weakening of the normalized transmission and, correspondingly, in the increase of the laser radiation absorption. An absorptivity increase is also observed for the dispersion without CNTs, but it is insignificant (fig. 1a). The theoretical curves were plotted from the results of optical parameters calculations.

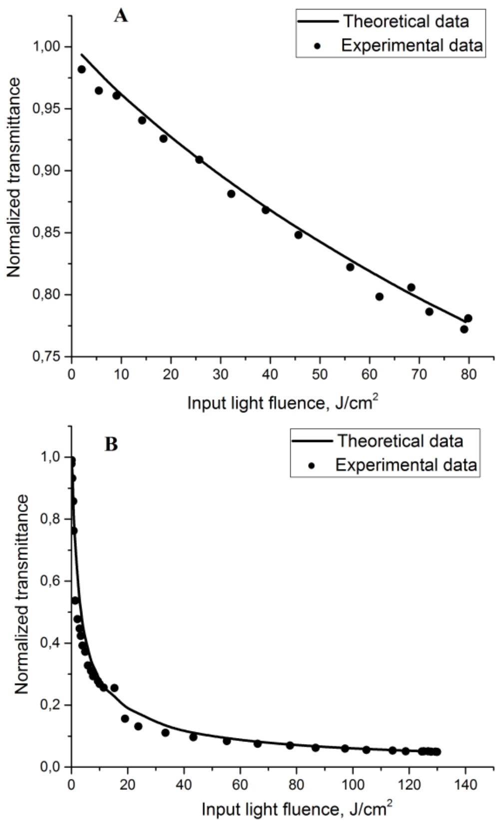

Figure 1: Normalized transmittance dependence on input light fluence for: A – BSA, B – BSA+SWCNT

Fig. 2 shows the normalized transmittance for collagen dispersions without nanotubes (fig. 2a) and with nanotubes (fig. 2b). To better trace the behavior of the sample at low values of the input light fluence, the dependence data are presented on a logarithmic scale. Collagen dispersion also show an increase of the laser radiation absorption when CNTs were added, but it is weaker than for BSA dispersions. The nonlinear properties of protein dispersions with single-walled carbon nanotubes are also confirmed by the calculated calculations.

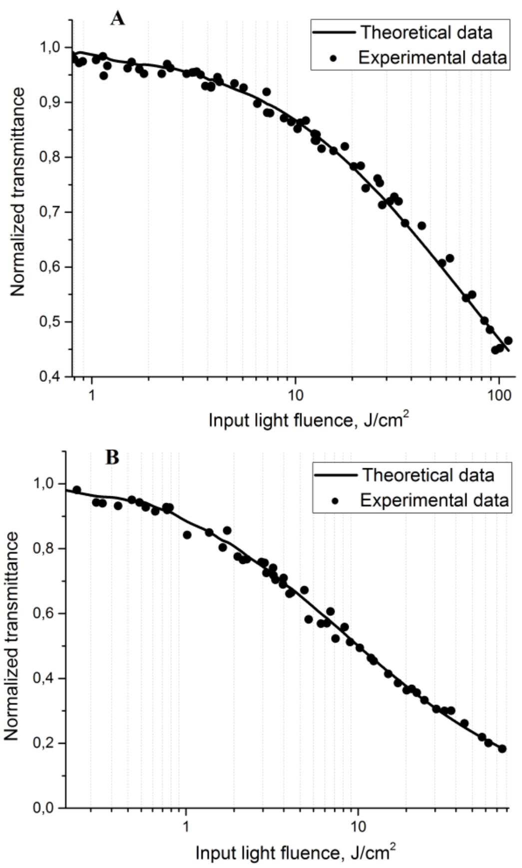

Figure 2: Normalized transmittance dependence on input light fluence for: A – BC, B – BC+SWCNT

Calculations were made using a method based on the radiation transfer equation. When carbon nanotubes were added, the value of the linear absorption coefficient increased however, this growth was insignificant. Albumin and collagen water dispersions without CNTs showed values of the linear absorption coefficient $\alpha = 1.92\ cm^{-1}$ and $\alpha = 2.16\ cm^{-1}$ respectively, and the same dispersions with carbon nanotubes showed $\alpha = 2.7\ cm^{-1}$ and $\alpha = 2.94\ cm^{-1}$.

Unlike linear absorption, the value of the nonlinear absorption coefficient increased significantly with the addition of carbon nanotubes. For dispersions without CNTs, the nonlinear absorption coefficient β was 4 cm/GW and 5.9 cm/GW for albumin and collagen respectively, and for similar dispersions with CNTs β = 345 cm/GW and β = 66.9 cm/GW.

Also, for the suspensions studied, the threshold light fluence values, at which nonlinear effects begin to occur were calculated for studied dispersions. For pure aqueous dispersions of albumin and collagen, this values were 6 mJ/cm^2 and 8.5 mJ/cm^2 respectively, and for dispersions with nanotubes they were 51 mJ/cm^2 and 25.5 mJ/cm^2.

The calculated values of the waist radius for all the experiments were 23±3 μm. Knowing the value of the radius of the beam in the neck, the beam radius values at any point can be calculated as:

$$\omega(z) = \omega_0 \sqrt{1 + \left(\frac{\lambda z}{\pi \omega_0^2}\right)^2} \qquad (1)$$

where ω_0 – laser beam waist, $\omega(z)$ – laser beam radius at any point of the optical axis Z.

To verify the correspondence of the used laser beam to the Gaussian beam, the beam radius depended on the distance from the focus of the lens was studied. To do this, the CCD camera was placed on a mechanized ruler and similarly to the Z-scan experiment was moved relatively to the focus of the lens. As a result, experimental values of the beam radius at different points were obtained, which correlate well with the theoretical values (fig. 3).

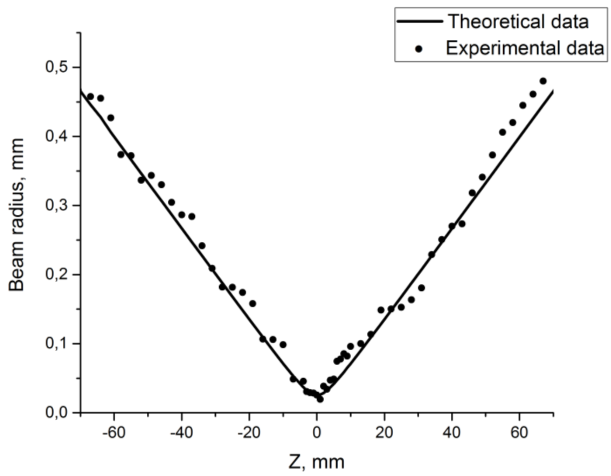

Figure 3: Beam radius dependence on optical axis Z displacement

Conclusions

An increase of the nonlinear absorption coefficient with the addition of CNTs suggests that it is the nanotubes that absorb most of the laser radiation as its light fluence increases, and thus reduce the effect of the laser on the proteins. Absorbing laser radiation, nanotubes heat up and transfer heat to the medium, which leads to the creation of a solid composite. Thus, proteins are less damaged in the formation of three-dimensional structures by laser printing methods.

References

[1] S. Iijima. Helical microtubules of graphitic carbon, Nature 354: 56–58, 1997.

[2] A. Parag, J.B. Hall, C.B. McLeland, M.A. Dobrovolskaia, S.E. McNeil. Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy, Advanced drug delivery reviews 61: 428–437, 2009.

[3] Balu M. White-light continuum Z-scan technique for nonlinear materials characterization, Optics Express 12(16): 3820–3826, 2004.

[4] Tereshchenko S.A., Podgaetsky V.M. Determining the parameters of the laser radiation intensity limiter based on a time-dependent equation of radiative transfer in a nonlinear medium, Quantum electron. 41(1): 26–29, 2011.

Acknowledgements

This work was provided by the Ministry of Education and Science of the Russian Federation (Agreement 14.578.21.0234 RFMEFI57817X0234).