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Deformation response of biological phantoms and cartilaginous tissue at laser exposure

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Regeneration of cartilaginous tissue and its shape change at laser exposure can be used as a basis for prospective medical operations, improving patient's quality of life. The most important criterion of such operations success is a cell survival after laser exposure, therefore reduction of exposure duration and power is an important task at such methods development. Nanoparticles are actively used in medicine, and one of their intended usages is photothermal effect enhancement at laser exposure to biological tissue. However, articular tissue is quite resistant to foreign agents penetration, therefore the study of nanoparticles penetration capability and their impregnation effect is the priority task for achieving the desired medical effect. Optical coherence tomography (OCT) of gel phantoms and cartilaginous tissue of a joint, impregnated with nanoparticles, at laser exposure with erbium fiber laser with length wave of 1.56 μm is performed in this study. Articular cartilaginous tissue sections of three types (intact, with laser damage and after low laser exposure) were impregnated with nanoparticles of Fe_3O_4 for further study using OCT elastography. Increase of deformations, caused by heating of phantoms and tissue, impregnated with nanoparticles, is observed. OCT elastography data indicate the dependence of tissue deformation on previous tissue exposure history. The work substantiates increase of photothermal impact of laser exposure to tissue deformation at various nanoparticles introduction.

Keywords: nanoparticles, OCT elastography, biological tissue laser modification, biophotonics.

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Introduction

Non-destructive laser induced modification of biological tissue is an actively developing multidisciplinary endeavor with a task to return the initial tissue properties or give the new ones. The separate endeavor in it is the impact on avascular tissues, such as cartilaginous tissue.

Cartilaginous tissue is pervasive in a human body and required for its mobility and support. Therefore any its pathology adversely affects the human life quality. Thus, the articular tissue is subject to significant stress and can be injured or worn. As per statement of „Bone and Joint Initiative“ more than half of all seniors' chronic diseases in USA is attributed to joint conditions [1]. Besides, cartilaginous tissue can also be distorted due to congenital pathology or injury, thus resulting in aesthetic and physiological problems [2].

It was observed, that pulse-periodic nonablating laser exposure to hyaline cartilage results in formation of submicronic pores, thus contributing to histotrophic nutrition and tissue regeneration [3]. While exposure with certain pulse recurrence frequency and radiation intensity to areas with inner stress can stabilize the new shape of cartilaginous tissue and implant [4,5]. However, for successful application in medicine this exposure requires minimization of injuring impact on tissues and structures of cartilaginous tissue, that can result in its lysis later.

Introduction of nanoparticles with photothermal effect to the tissue will allow to use moderate irradiation modes and localize the laser exposure area for above mentioned medical operations. Moreover, such nanoparticles can act as markers of cartilaginous tissue damage, since it was proved earlier, that healthy cartilaginous tissue is resistant to penetration of any particles [6], while nanoparticles settle in places of various damage or injuries [7,8].

Studies of cartilaginous tissue with nanoparticles of Fe_3O_4 magnetite were performed earlier, and it was observed that cartilaginous hyaline tissue impregnation with nanoparticles solutions with concentration of 10 mg/ml increases heating rate at pulse-periodic laser exposure with erbium fiber laser. While studies of nanoparticles of metal-oxide bronzes (Na_xTiO_2, K_xMoO_3, K_xWO_3, H_xMoO_3, where $0.1 < x < 0.3$) revealed, that tissue impregnation with nanoparticles of $Na_{0.2}TiO_2$ increases heating temperature by 15%, while with nanoparticles of $K_{0.1}MoO_3$ — by 30%, the remaining nanoparticles do not bring any additional photothermal effect [10]. It was decided to perform the further works using nanoparticles of Fe_3O_4 magnetite and $Na_{0.2}TiO_2$ metal-oxide bronzes, since after grinding they possess magnetic properties, by means of which their penetration capability can be improved by directional magnetic field application [11,12].

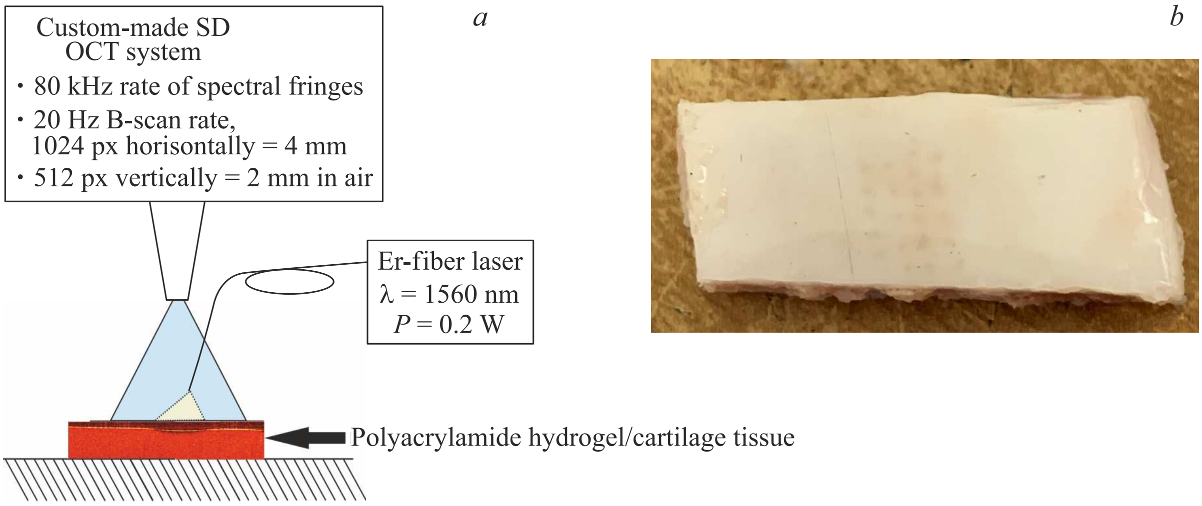

Figure 1. (*a*) Scheme of experimental apparatus, (*b*) sample of pre-treated cartilaginous tissue.

However, control via thermometric method does not guarantee the lack of biological tissue overheating or damaging, since, first of all, cartilage has structural heterogeneities with various heating intensity. Secondly, the maximum temperature at laser heating with erbium fiber laser is often presented not on tissue surface due to water redistribution processes in near surface layer, resulting in error at temperature control on biological tissue surface. Qualitative evaluation of tissue damage and changes, made by laser exposure, is possible, for instance, through issue deformation evaluation. Therefore the purpose of our study was in evaluation of photothermal effect in cartilaginous tissues, impregnated with solutions of various nanoparticles (magnetite and metal-oxide bronzes), in terms of deformation response.

We chose the elastography method, using optical coherence tomography (OCT), for control of heat distribution and change of deformation response of cartilaginous tissue to laser exposure at impregnation with nanoparticles. This method allows to perfectly evaluate and visualize the tissue elastic properties and deformation processes at a depth of about 1−2 mm [13,14].

Nanoparticles of Fe_3O_4 magnetite, that were synthesized in the Biophotonics laboratory of the Institute of photonic technologies of FSRC „Crystallography and Photonics" of RAS using the method of $FeCl_2$ and $FeCl_3$ salts water solution deposition with base introduction under inert gas atmosphere at room temperature [7], and nanoparticles of $Na_{0.2}TiO_2$ titanium oxide metal-oxide bronze, made by self-propagating high-temperature synthesis method [15], were used in the work. Preliminary, before the impregnation, the solutions of nanoparticles with the same concentration were prepared.

When studying through OCT elastography, initially, for reducing the impact of tissues structures anisotropy and prevention from valuable biological material use during experiments, the phantoms — (PAAG), simulating the temperature properties and homogeneous structure of cartilaginous tissue [16], with solutions of nanoparticles of $Na_{0.2}TiO_2$, Fe_3O_4 and PAAG reference sample without nanoparticles were used as samples.

This work does not include any studies with animals use as objects. Cross sections of pig hyaline cartilage, taken from slaughter house, were used as biological object (fig. 1, *b*). Samples were prepared using special cutters, allowing to obtain samples with the same size and weight. The samples were kept in physiological solution for four days maximum at temperature of 2°C. Preliminary, before impregnation with nanoparticles solutions, the cross sections of cartilaginous tissue were subject to laser exposure in two modes: damaging mode for simulation of serious cartilage injury — laser exposure was performed with high power, resulting in areas of dehydrated matrix in cartilaginous tissue (in fig. 1, *b* — areas with changed optical characteristics of darker color); moderate mode — for simulation of increased pore formation area [3]. Also, some samples were left intact and used for reference. Thus, the study was performed on four sample types: intact tissue without nanoparticles, intact tissue after impregnation with nanoparticles solution, tissue after damaging laser exposure and impregnation with nanoparticles solution, tissue after moderate laser exposure and impregnation with nanoparticles solution.

In all cases the irradiation was performed using erbium fiber laser with wave length of 1.56 μm in pulse-periodic mode. For cartilaginous tissue the experiment was performed with nanoparticles concentration of 1 mg/ml.

OCT elastography apparatus, created in the Institute of Applied Physics of the Russian Academy of Sciences (Nizhny Novgorod) by a group under the guidance of V.Yu. Zaitsev, with visualization area of 4 mm width-

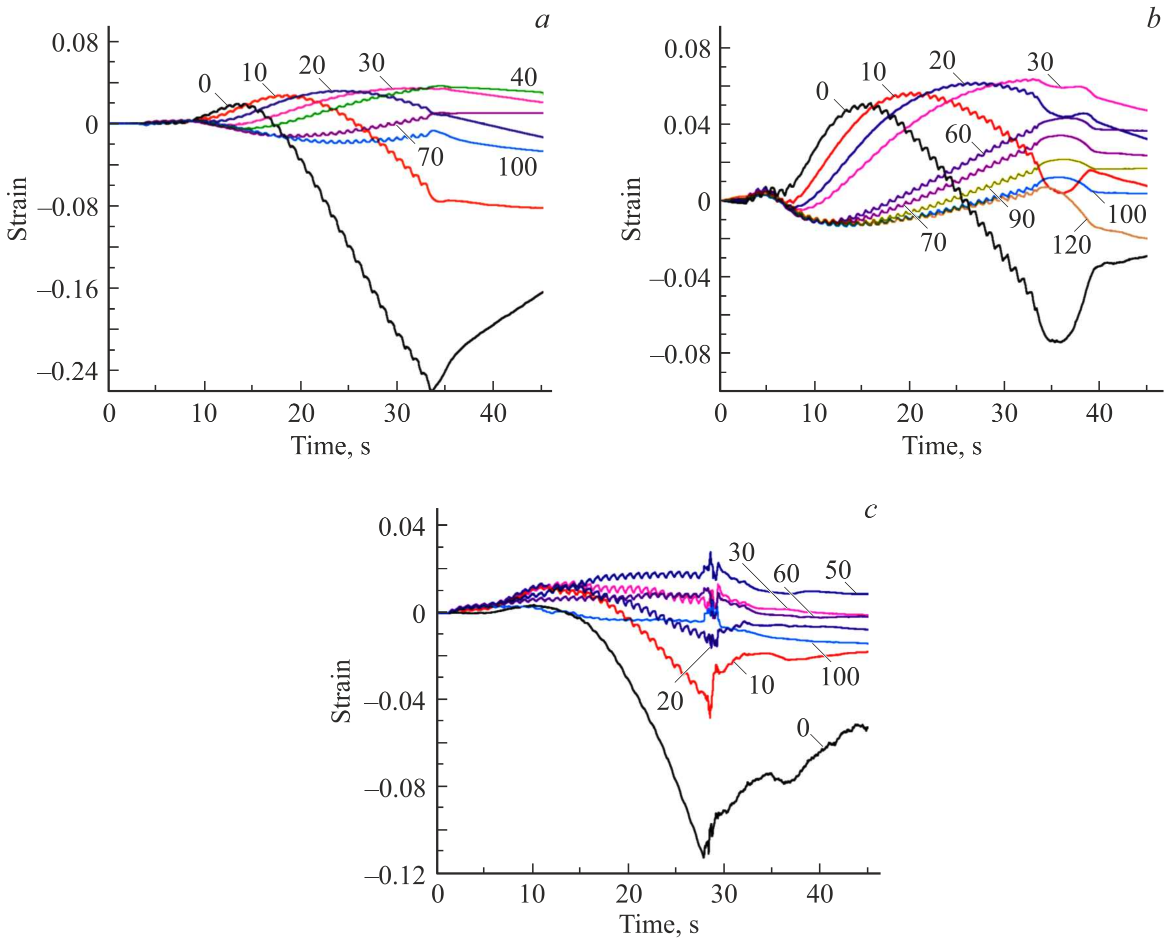

Figure 2. Diagrams of inner deformations of cartilaginous tissue with a depth for (*a*) PAAG without nanoparticles, (*b*) PAAG with nanoparticles of $Na_{0.2}TiO_2$, (*c*) PAAG with nanoparticles of magnetite.

wide and 2 mm depth-wise, allowing to obtain the inner deformations dependence on time, was used in the work.

Results

Patterns of inner deformations of PAAG without nanoparticles and with nanoparticles and diagrams of distribution of inner stresses and deformations over the depth along laser beam axis were observed as the result (fig. 2).

Analysis of observed diagrams showed increase of deformation propagation depth for samples with nanoparticles of sodium titan metal-oxide bronzes, as well as increase of tensile deformations by 1.5 times compared to PAAG without nanoparticles. Polyacrylamide hydrogels with magnetite nanoparticles with concentration of 10 mg/ml at samples OCT elastography yielded „gating" and deformation pattern distortion, as well as frequent ruptures of PAAG surface.

Time dependencies of interframe deformation at laser exposure were obtained when studying samples of cartilaginous tissue, impregnated with magnetite nanoparticles with concentration of 1 mg/ml, using OCT elastography method. The following is demonstrated as a result.

– Intact samples, not impregnated with nanoparticles solutions, response to laser pulse with sharp deformations peak with fast relaxation.

– Intact tissue, impregnated with magnetite nanoparticles, has slightly bigger deformation response in terms of amplitude compared to the tissue without nanoparticles, and their relaxation rates are the same.

– Relaxation slowing by more than twofold is observed for tissue after moderate and damaging laser exposure and impregnation with nanoparticles.

Analysis of tissue deformations at the first laser pulse (fig. 3) revealed the fact, that there is almost no difference between deformation propagation in intact tissue and tissue after impregnation, thus confirming the complication of nanoparticles penetration into fresh/intact cartilaginous tissue. Growth of inner deformations and their localization in the place of laser exposure are obvious for tissue after serious laser injury, thus indicating the nanoparticles penetration into the tissue depth and impact on cartilaginous tissue absorbing capacity in the places of nanoparticles accumulation, that amplifies biological tissue response to laser exposure.

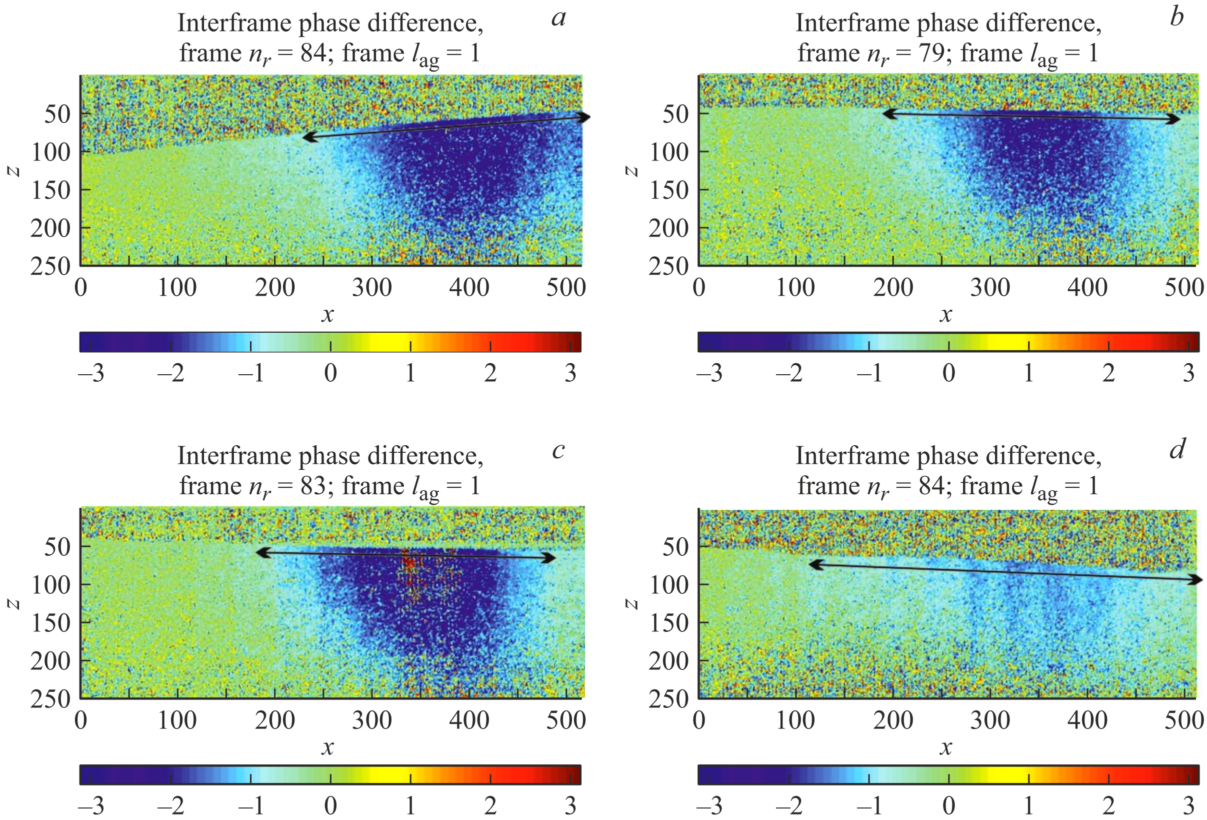

Figure 3. Deformation pattern at the first laser pulse for (*a*) intact tissue, (*b*) intact tissue, impregnated with magnetite nanoparticles, (*c*) tissue after laser exposure and impregnation with magnetite nanoparticles, (*d*) tissue after moderate laser exposure and impregnation with magnetite nanoparticles. Black arrows indicate the approximate width of volume cross section, in which the deformation changes are observed.

OCT pattern for cartilaginous tissue after low laser exposure demonstrates the obvious decrease of the tissue inner deformations and increase of exposure area on the side section (fig. 3, *d*) at laser pulse compared to deformation pattern for intact tissue.

Discussion

Although the overall deformation amplitude for PAAG with magnetite nanoparticles is less than for PAAG without nanoparticles and impregnated with nanoparticles of $Na_{0.2}TiO_2$, the comparison of deformation peaks from individual laser pulses, especially at small depth, revealed the reverse dependence — amplitude of individual peaks is higher than for PAAG with magnetite. This is the important feature, since for pores formation at laser exposure, that can result in regeneration start later, the sign variation of deformations (compression/tension) with a certain frequency, that create sign-variable thermoelectromotive force fields, is important [17]. Thus, it may be concluded that, despite these types of nanoparticles (Fe_3O_4 magnetite and $Na_{0.2}TiO_2$ metal-oxide bronze) make the same influence on temperature growth rate at laser exposure with erbium fiber laser with wave length of $\lambda = 1.56\,\mu$m, the deformation response of the tissue is different.

It should be noted, that in experiments with cartilaginous tissue, due to its anisotropy, as well as partial dehydration in air, the deformation pattern shift is not always linear in the dynamic pattern of OCT, resulting in complicated calculations during deformations evaluation.

Deformation patterns at laser exposure to cartilaginous intact tissue — without nanoparticles and tissue, impregnated with nanoparticles, are almost the same, thus confirming the previously made conclusion, that cartilaginous tissue is resistant to penetration of foreign nanoscale objects.

Simulation of heavy cartilage injuries using laser treatment of cartilage results in appearance of dehydration areas and matrix damaging, which, in their turn, first of all, create paths for nanoparticles passing to the tissue depth, and, secondly, create more elastic areas with different values of Young's modulus, thus preventing from fast relaxation of deformations from laser pulse and resulting in stress accumulation in the area of laser exposure. The moderate laser exposure, on the contrary, creates the additional porous system, resulting in deformation area increase at laser pulse and complying with the conclusion on easier penetration of nanoparticles into the areas with increased structure porosity, that is also confirmed with relaxation rate decrease.

Conclusion

It was demonstrated, that nanoparticles of Fe_3O_4 magnetite and $Na_{0.2}TiO_2$ metal-oxide bronze, exhibiting the same photothermal effect in cartilaginous tissue and increasing its heating by 15%, have different influence on tissue deformation response at laser exposure with erbium fiber laser.

Analysis of the performed OCT elastography using polyacrylamide phantoms showed the increase of overall deformation as a result of introduction of nanoparticles of sodium titan metal-oxide bronzes and increase of deformation amplitude from the single laser pulse at introduction of magnetite nanoparticles compared to PAAG without nanoparticles. Since the experiments with OCT elastography were preliminary performed using PAAG, this allowed to observe the pattern distortion at high magnetite concentration and correct it at experiments using biological tissue, by reducing the concentration to the optimum value of 1 mg/ml.

Different photothermal effect of cartilaginous tissue heating appears due to different penetration of nanoparticles into the cartilaginous tissue with different structure. It was demonstrated, that biological tissue, damaged with laser exposure, is subject to nanoparticles penetration and inner deformation increase under laser exposure. Low laser exposure can redistribute the tissues density, thus decreasing the amplitude and increasing the deformation area. It was confirmed, that, since intact tissue almost completely prevents from nanoparticles penetration, the patterns of OCT deformations for intact tissue and tissue after impregnation with nanoparticles solution are almost the same.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] *United States Bone and Joint Initiative* [Electronic source]. URL: https://usbji.org/about
[2] R. Mladina, E. Čujić, M. Šubarić, K. Vuković. Am. J. Otolaryngology, **29** (2), 75–82 (2008). DOI: 10.1016/j.amjoto.2007.02.002
[3] E. Sobol, O. Baum, A. Shekhter, S. Wachsmann-Hogiu, A. Shnirelman, Y. Alexandrovskaya, I. Sadovskyy, V. Vinokur. J. Biomed. Opt., **22** (9), 091515 (2017). DOI: 10.1117/1.JBO.22.9.091515
[4] O.I. Baum, Yu.M. Soshnikova, E.N. Sobol, A.Y. Korneychuk, M.V. Obrezkova, V.M. Svistushkin, O.K. Timofeeva, V.V. Lunin. Las. Surg. Med., **43** (6), 511–515 (2011). DOI: 10.1002/lsm.21077
[5] O.I. Baum, Y.M. Alexandrovskaya, V.M. Svistushkin, S.V. Starostina, E.N. Sobol, Laser Phys. Lett., **16** (3), 035603 (2019). DOI: 10.1088/1612-202X/aafd21
[6] O.I. Baum, V.V. Golubev, A.I. Omel'chenko, E.N. Sobol', A.B. Shekhter. V materialah III-Evrazijskogo kongressa po medicinskoj fizike (M., 2010). 3, 222–224. (in Russian)
[7] Yu.M. Soshnikova, S.G. Roman, N.A. Chebotareva, O.I. Baum, M.V. Obrezkova, R.B. Gillis, S.E. Harding, E.N. Sobol, V.V. Lunin. J. Nanopart. Res., **15**, 2092 (2013). DOI: 10.1007/s11051-013-2092-5
[8] Yu.M. Soshnikova, A.I. Omelchenko, A.B. Shekhter, E.N. Sobol. Nanobiomaterials in Hard Tissue Engineering (William Andrew Publishing, Norwich, NY, 2016). ch. 15. pp. 443–472. DOI: 10.1016/B978-0-323-42862-0.00015-8
[9] E.M. Kasianenko, A.I. Omelchenko, E.N. Sobol. Bull. Russ. Acad. Sci. Phys., **80**, 463–466 (2016). DOI: 10.3103/S1062873816040171
[10] E.M. Kas'yanenko, A.I. Omel'chenko. Uchenye zapiski fizicheskogo fakul'teta Moskovskogo universiteta, **2**, 1920302–1920302 (2019). (in Russian)
[11] V.Yu. Afon'kin, K.G. Dobrecov, A.K. Kirichenko, V.P. Ladygina, A.V. Sipkin. Sibirskoe medicinskoe obozrenie, **50** (2), (2008). (in Russian)
[12] A.I. Omel'chenko, E.N. Sobol'. Perspektivnye materialy, **8**, 125–128 (2010). (in Russian)
[13] V.Y. Zaitsev, A.L. Matveyev, L.A. Matveev, G.V. Gelikonov, A.I. Omelchenko, D.V. Shabanov, O.I. Baum., V.M. Svistushkin., E.N. Sobol. Laser Phys. Lett., **13** (11), 115603 (2016). DOI: 10.1088/1612-2011/13/11/115603
[14] V.Y. Zaitsev, A.L. Matveyev, L.A. Matveev, G.V. Gelikonov, A.I. Omelchenko, O.I. Baum, S.E. Avetisov, A.V. Bolshunov, V.I. Siplivy, D.V. Shabanov, A. Vitkin, E.N. Sobol. J. Biophotonics, **10** (11), 1450–1463 (2017). DOI: 10.1002/jbio.201600291
[15] P.Yu. Gulyaev, M.K. Kotvanova, A.I. Omel'chenko. Fizika i himiya obrabotki materialov, **4**, 74–82 (2017). (in Russian)
[16] V.A. Fedulova, A.V. Yuzhakov, O.I. Baum. J. Biomed. Photonics & Engineering, **6** (1), 010302 (2020).
[17] Yu.M. Aleksandrovskaya, O.I. Baum, A.B. Shekhter, O.A. Tiflova, A.K. Dmitriev, E.V. Petersen, E.N. Sobol'. V sb. Lazery v nauke, tekhnike, medicine. Pod red. V.A. Petrova (Moskovskoe NTO radiotekhniki, elektroniki i svyazi im. A.S. Popova, 2019). S. 156–160. (in Russian)