Probe microscopy of biological fluid crystallograms after laser impact

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Abstract. We consider the probe microscopy of crystallograms of biological fluids as a method of revealing structural changes in them under the impact of laser radiation. The experimental data on the influence of laser radiation with different wavelengths on the structure of crystallograms of biological solutions are presented. © 2018 Journal of Biomedical Photonics & Engineering.

Keywords: biological liquid; solution; laser; crystallogram; probe microscopy.

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1 Introduction

At present considerable experimental material is accumulated, that confirms the biostimulating effect of laser radiation [1-3]. However, the mechanisms of stimulating effect and the processes that allow the response of the entire organism to the local action of laser radiation remain poorly studied. The authors of the present paper propose to consider the blood protein complexes as a universal acceptor of laser radiation. It is known [4, 5] that macromolecules in solutions form the so-called clusters, i.e., associates that can comprise tens of molecules. The biological activity of a molecule incorporated in a cluster considerably differs from that of a single molecule, since its active centres can be overlapped by neighbours, and a molecule inside the cluster is not able to interact with the environment at all. The smaller are the clusters, the larger is their relative surface and the higher is the reactivity of the molecules that form the cluster.

If we suppose that the laser radiation interacting with protein clusters causes their disintegration into smaller associates, then we can propose a number of hypotheses on the possible mechanisms of biostimulation. First, the disintegration of clusters and the change of conformation of protein molecules without the disturbance their primary structure allows the explanation of the increased enzymatic activity, the improvement of oxygen transfer, and other effects at the site of laser impact. Second, the transfer of macromolecules “activated” by laser radiation in the composition of blood plasma can be considered as a mechanism of generalisation of the laser effect on the organism as a whole. Third, the presence of clusters having various sizes and configurations provides the absorption in a wide wavelength range, which makes it possible to explain why the biostimulating effect of laser radiation weakly depends on the wavelength [2].

However, the study of laser radiation effect on the structure and activity of macromolecular complexes directly in the organism is a hardy solvable problem, since it requires considering too many factors (the interaction of laser radiation with medical preparations in the blood, the influence of vascular pathologies on the propagation of “exposed” blood, the presence of accompanying diseases). Therefore, to confirm the laser effect on the size of macromolecular clusters we have to use a model medium reflecting the specific features of biological fluids and, at the same time, available for *in vitro* experiments. With these requirements taken into account, we have chosen the solutions of Grippferon and bile as model media.

To estimate the changes occurring under the impact of laser radiation in biological-like fluids we used the method of crystallogram analysis as one of the simplest methods of structure change diagnostics. The crystallograms of biological fluids are formed at the expense of dehydration self-organisation of the solutions of proteins and other substances [6-12] in the process of drop drying on a solid substrate. It is established that the patterns appearing in this case correlate well with the evidences of diseases in patients [6, 9, 11, 12]. The possibility to apply the crystallogram analysis method for assessing the quality of conditioned media, i.e., the nutritive media containing the waste products of cells preliminarily cultivated in them, is also discussed. Such media are considered as potential drugs for regeneration medicine [13].

Therefore, the method of crystallogram analysis can be used to detect the changes that occur in biological fluids under the impact of laser radiation. In Ref. [14], the possibility of detecting the laser-induced changes in the blood serum and plasma was demonstrated. The possible mechanism of the induced changes is related to the size reduction of the clusters of bioorganic molecules (first of all, macromolecules) caused by laser radiation [14].

Usually crystallograms are analysed using an optical microscope. The geometric dimensions (transverse and longitudinal) of the characteristic patterns of the crystallogram are commonly estimated, while the relief (surface profile) of the patterns is usually lost.

The aim of the present paper is to get information on the three-dimensional crystallogram pattern and to estimate the changes that occur in the structure of liquid media (biological-like fluids) subjected to laser irradiation with different wavelengths by analysing the crystallograms using the probe microscopy method.

2 Materials and methods

As mentioned above, the possible mechanism of biological fluid changes induced by laser radiation is related to the reduction of the size of bioorganic molecular clusters, therefore, for study we have chosen biological-like media containing organic molecules and, first of all, macromolecules. If for the chosen objects of study the probe microscopy analysis of crystallograms shows the presence of changes in the their three-dimensional structure and the results agree with earlier optical microscopy ones, then we are able to make more definite statements on the reliability of earlier proposed mechanism and its universal character.

One of the chosen objects of study was Grippferon solution. The main component of this solution is interferon alfa-2b, the protein-nature factor that provides antivirus immunity.

The other object of study was 20% aqueous solution of medical conserved bile, consisting of natural bovine and porcine bile, as well as auxiliary substances (rectified ethanol, formalin, and spirituous solution of Furacilin). The natural bile itself contains cholesterol, bile acids and their salts, phospholipids, conjugated bilirubin, protein, and inorganic ions.

It is worth noting that the results of the study of the laser radiation effect on the processes of crystal formation in volume colloidal and gel-like media by the example of human bile have been already presented in Ref. [15]. Initially the bile crystallisation after the laser impact was studied with the purpose to clarify the possibility of using the laser radiation for preventing crystal formation in hepatobiliary system. The results obtained at that time allowed an idea that the laser radiation affects the processes of crystal formation only in the volume of white bile. No effect of laser radiation was detected in the volume of natural pigmented bile. Probably, this was because the presence of pigment caused sufficiently strong absorption of the radiation in a thin layer of bile near the cuvette walls and the radiation did not penetrate into the volume. It was found that the morphology of bile crystallograms depends on the absence or presence of destabilisation of the bile colloidal structure, its stage, and the remoteness of the appearance of biliary pathology symptoms [11].

To eliminate the influence of the abovementioned factors on the process of crystal formation we used the medical conserved bile (pigmented bile). To enhance the penetration of radiation into the bile volume, we studied its 20% aqueous solution.

The crystallograms of the corresponding solutions were studied before and after the impact of laser radiation.

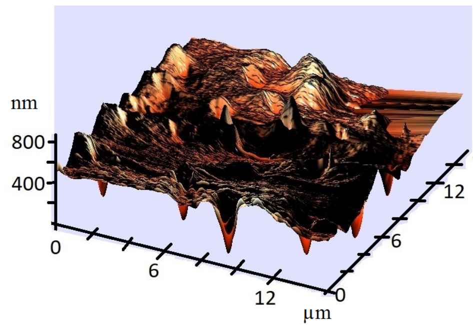
The crystallograms were prepared in the following way. We applied a drop of the studied liquid (the samples to be exposed to laser radiation and the control ones) on the defatted glass substrate and kept dust-protected at room temperature 20-25ºC and relative air humidity 65-70% during 18-24 hours to obtain dry films, i.e., the crystallograms. Then these crystallograms were studied using the scanning probe microscope **NT-MDT Solver**. The contact scanning mode was used.

The Grippferon solution in the cuvette was exposed to laser radiation (having the wavelength 650 nm or 530 nm and the intensity about 80 mW/cm^2) during 1, 2, 5, and 10 minutes. The aqueous bile solution was exposed to the radiation of a semiconductor laser with the wavelength 650 nm during 2 minutes, the power density of radiation being about 250 mW/cm^2.

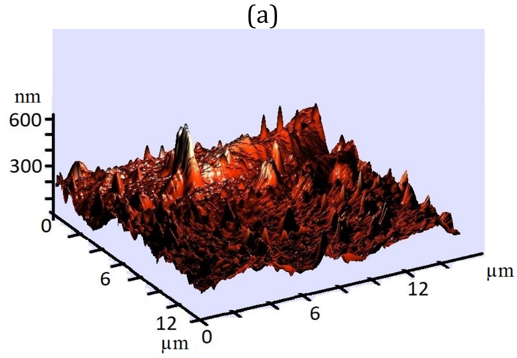
Then using the probe microscope, we constructed 3D models of the crystallogram relief. Separately three parallel sections of the relief were plotted as the segment height dependence on the coordinate. To estimate the changes of a crystallogram segment size in each section numerically, the minimal and maximal height was found, and their difference divided by two was taken as the mean height of the layer. The segment size was determined by averaging the widths of all "peaks" at the height, equal to the mean height of the layer. Note that the described procedure of numerical estimations allows one to assess on the qualitative changes, but is not sufficient to construct correct numerical dynamics, since it considerably reduces the information volume inherent in the 3D model and is expected to give rise to essential variance of the estimate. Therefore, in the present paper the measurement error was not estimated.

3 Results

The 3D models of the crystallogram relief, obtained by means of probe microscopy, are presented in Figs. 1 and 2. Figure 1 presents the results for the Grippferon solution.



(a)



(b)

Fig. 1 3D model of the crystallogram relief for the Grippferon solution. (a) – before the impact (control); (b) – In 10 minutes after the impact (the wavelength 530 nm, the total energy density 48 J/cm^2).

As a result of laser irradiation with the wavelength 530 nm, the decrease of the characteristic size of the pattern was observed already in 5 minutes of the exposure (the total energy density 24 J/cm^2), and after 10 minutes of exposure the size of the inhomogeneity decreased by two times. Besides that, the mean height of the layer (the level above which the peaks rise), as seen from Fig. 1, amounts to about 500 nm for the control solution and nearly 250 nm for the exposed solution. Similar results were obtained for the action of the laser radiation with the wavelength 650 nm, namely, the changes of the crystallogram characteristics size also began from 5 minutes of exposure, but the change relative to the initial size was smaller. To our opinion, higher efficiency of green irradiation is due to the greater energy of quantum that allows greater energy transfer to the clusters and more efficient destruction of the bonds stabilising them.

It is worth attention that the crystallograms were obtained at discrete moments of time, i.e., the laser exposure time changed stepwise. At small values of the exposure time (less than 5 minutes), both the visual and the numerical estimation showed insignificant changes. On the contrary, in the crystallograms obtained from the liquid after 10 and more minutes of laser exposure the changes were significant as compared to the control samples, but slightly differed from each other. Thus, under the conditions of our experiment one can conclude that the changes occur abruptly and correspond to the exposure time of nearly 5 minutes. Therefore, the threshold energy density can be assessed to equal 20-25 J/cm^2, independent of the wavelength. The present result agrees well with the estimates obtained from the crystallogram studies using optical microscopy [16].

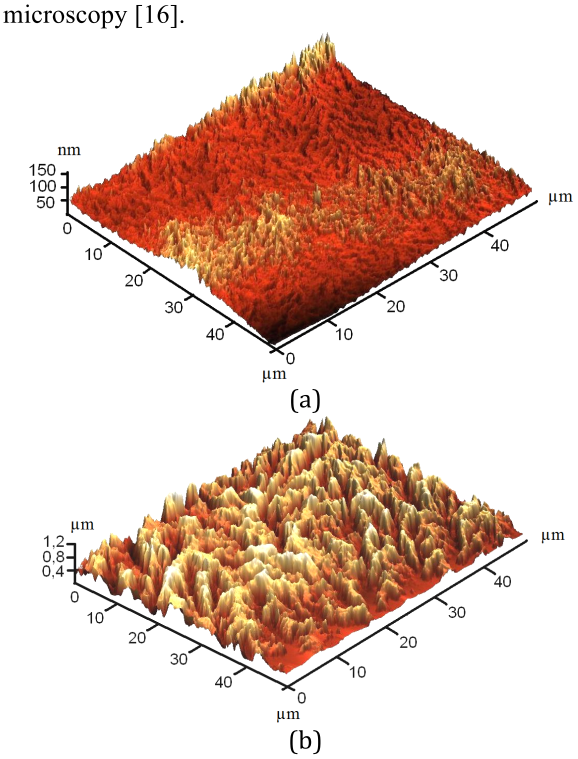


Fig. 2 3D model of crystallogram relief for the bile solution. (a) – before the impact; (b) – After 2 minutes of exposure (the wavelength 650 nm, the total energy density 30 J/cm^2).

The study of crystallograms of irradiated and unirradiated aqueous solutions of bile has shown that their structures also significantly differ both qualitatively and quantitatively (Fig. 2). In particular, it was found that in the crystallogram from the unirradiated solution the structure (peaks) possesses the height smaller by nearly an order of magnitude (0.15 µm for the unirradiated solution and 1.2 µm for the irradiated one). The level difference between the individual crystallogram patterns is also smaller by nearly an order of magnitude and amounts to approximately 35-40 nm upon average. Alongside with these facts, the crystallogram patterns for the unirradiated solution are arranged much more densely, in contrast to the crystallogram of the irradiated solution, although the mean transverse structure size in both cases differs slightly an equals nearly 1-2 µm (Fig. 3).

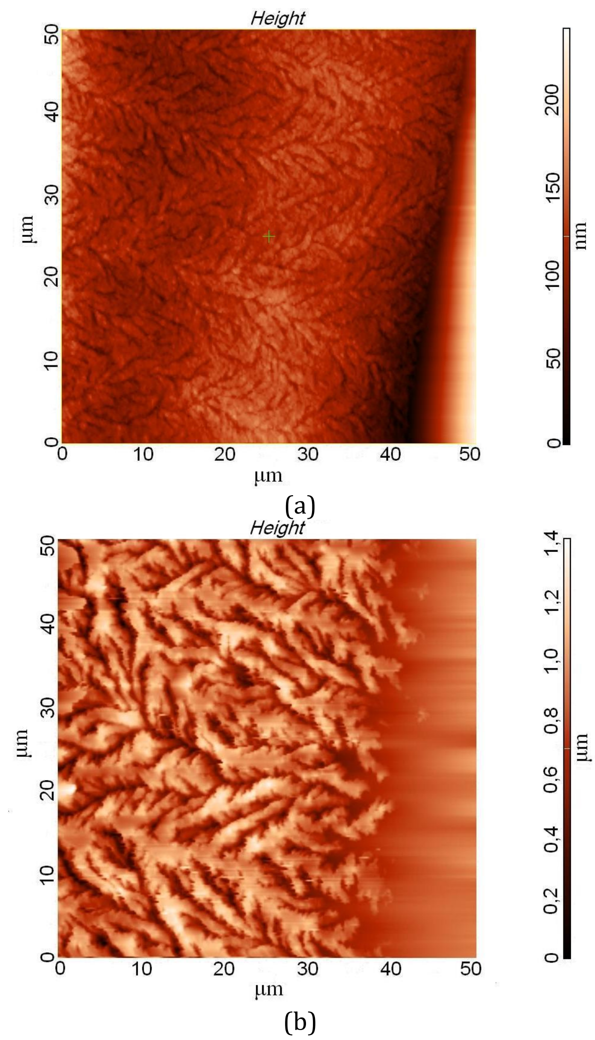


Fig. 3 2D image of the crystallogram surface. (a) – Before the impact; (b) – After 2 minutes of exposure (the wavelength 650 nm, the total energy density 30 J/cm^2).

A possible mechanism of the laser radiation effect on the crystallisation processes in volume and thin

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layers of organic solutions consists in the decomposition of large molecular associates that otherwise could be nucleation centres into smaller associates [14, 15]. Similar to the case of Grippferon solution, under the irradiation with relatively low power the validity of this hypothesis is demonstrated by both the reduction of the characteristic crystallogram size and the decrease of the mean layer thickness.

However, in the case of bile, the considerable radiation power density as compared to that used in Refs. [14, 15] to act on the solution, caused the increase of the solution temperature, the substrate staying "cold". Therefore, the enhancement of the film crystal formation took place, in contrast to the crystallisation of unirradiated solution. Since the crystallisation occurred in a thin layer, together with the temperature difference it additionally accelerated the crystallisation, leading to the difference in the density of arrangement of crystalline structures between the irradiated and unirradiated solutions.

4 Conclusion

Thus, it is experimentally shown that under the effect of laser radiation the structure of Grippferon protein solution changes, which manifests itself in the characteristic size of the crystallogram segments. These changes can be not only detected qualitatively, but also determined quantitatively using the 3D models of the crystallogram surface, obtained by means of a probe microscope. Further improvement of the quantitative estimation procedure is required to consider the entire amount of information contained in the 3D model and to automate the image processing procedure.

The laser radiation of green range produces more significant structure change as compared to that produced by red radiation, which is probably due to the greater quantum energy.

The analysis of crystallograms of bile aqueous solutions also allows a suggestion that the laser radiation affects the crystallisation processes in bile preparations. The mechanisms of the effect are not studied yet, and its hypothetic explanation is related to the changes of the biological fluid structure that occur under the irradiation of the solution [11].

Disclosures

The authors have no relevant financial interests in this article and no potential conflicts of interest to disclose.