On the mechanism of the gallstone nucleus formation and the impact of laser radiation on its growth

Alexander N. Malov[1*], Andrey A. Vaychas[2], Eugenia A. Novikova[3]

[1] Irkutsk National Research Technical University, 83 Lermontova Str., Irkutsk 664074, Russia

[2] Irkutsk Branch of the Moscow State Technical University of Civil Aviation, 3 Kommunarov Str., Irkutsk 664047, Russia

[3] Irkutsk State Medical University, 1 Krasnogo Vosstaniya Str., Irkutsk 664003, Russia

*** e-mail:** cohol2007@yandex.ru

Abstract. Results of the experimental study of laser radiation impact on the process of crystal formation in bile preparations are presented. The possible mechanism of laser effect on the crystal formation process in the volume of biological fluids, in particular, the human bile, is discussed. © 2017 Journal of Biomedical Photonics & Engineering.

Keywords: Biological solution; Laser; Cluster; Pathogenic mineral formation; Biominerals; Bile.

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1 Introduction

Processes of pathogenic mineral formation are known to occur in the human organism leading to the growth of stones, e.g., in the urinary system, dental and salivary stones. To date the growth of gallstone morbidity rate is recorded in many countries, and the number of patients in the world at least redoubles during each decade. Therefore, the issues of diagnostics, treatment and prophylactics of stone formation remain actual [1].

Cholelithiasis is a polyetiologic disease characterised by the formation of stones in bile passages. The major site of stone formation due to developing cholelithiasis is the gallbladder, but they can also appear in all other parts of the bilious system [2].

The following types of gallstones are differentiated [3]:

1. Homogeneous stones (cholesteric, pigmental (bilirubin), calciferous).

2. Mixed stones (80% of all gallstones). The core consists of organic substance, coated by layers of three major elements: cholesterol, bile pigment, and calcium salts.

3. Complex stones, a combination of both forms, are observed in 10% of all cholelithiasis cases. The stone core contains cholesterol, and its shell has mixed composition (calcium, bilirubin, cholesterol).

The bile is a liquid with complex composition. Besides the cholesterol, it includes bile acids and their salts, phospholipids, conjugated bilirubin, protein and inorganic ions [3]. Combined with cholesterol, these substances make it water-solvable, forming the so-called macromolecular (lipid) complex that plays the role of a transport system (in the form of complex micelles and vesicles) and provides the transfer of all components of bile and the conservation of its colloidal stability [4]. Under the normal physiological conditions, all components of bile are completely solvable in it. The disturbance by some factors of the colloid system stability that keeps its components in solvable state, leads to the transition of some components into unsolvable state and sedimentation. If the process progresses, the stones are formed [2].

Although the change in the stability of the bile colloid system is of great importance for the explanation of gallstone formation, the mechanism of this process cannot be considered as completely studied [5]. There is no unique concept explaining the stone formation in the gallbladder. It can be considered as established that the factors that disturb the colloid stability include infections, stagnation of bile, and impairment of cholesterol metabolism. To none of these factors the exclusive significance can be attributed [2, 3]. There are also other biochemical processes that violate the bile colloid equilibrium [2].

On the other hand, the capability of many biological liquids (saliva, blood, urine, bile) to crystallise is used in the diagnostics of diseases. In such diagnostics different patterns formed as a result of the crystallisation of a liquid droplet on a substrate (crystallograms) are analysed [6].

In Ref. [7] the planar crystallisation of human bile was studied experimentally. It was shown that the morphology of crystallograms depends on the destabilisation stage of the bile colloid structure and the remoteness of the appearance of biliary pathology symptoms.

The authors of Ref. [8] studied the influence of laser radiation on the processes of planar crystallisation of blood serum. It was shown that the laser irradiation of the liquid leads to the reduction of the mean size of the planar crystals (lamellar rays) compared to the samples not exposed to laser radiation. The effect of laser radiation on protein solutions was studied in Ref. [9]. It was shown that as a result of the exposure of protein solution to laser radiation the craquelure structure (rupture pattern) of its dried film is changed. The mean size of the elements in such pattern is reduced as compared to the non-irradiated solution. It is worth noting that the possibility to affect polymer gels of biological macromolecules was also shown in the course of hologram recording in gelatine gel-colloid systems, in particular, at the expense of the exposure of gel-colloid layer to the infrared (IR) radiation [10].

The experimental *in vitro* studies of the effect of the helium-neon laser (the wavelength 630 nm, the power 5, 10, and 15 mW) on the physicochemical properties of bile have shown that in the bile samples from the patients with cholelithiasis the laser irradiation reduces the viscosity and pH of the solution and increases the nucleation time [11].

However, according to the present data about the influence of laser radiation on a biological liquid planar crystallisation processes and even on the nucleation in the bile volume, the laser radiation is still not used to prevent mineral formation in the hepatobiliary system. Probably, this is due to the experimental data deficit about the laser radiation impact on the processes of crystal formation in the bile volume, the insufficient development of theory about the mechanism of such impact, as well as the incomplete understanding of the stone nucleation process itself.

At present, the high-energy laser radiation is used for the fragmentation of gallstones (laser lithotripsy) [12], while the low-energy laser radiation has found application in the therapy of inflammation diseases of hepatobiliary system, e.g., cholangitis [13]. Some inflammatory processes are due to the cholelithiasis.

Therefore, the study of physicochemical processes of stone nucleation in colloid and gel-like media is still important both for deeper understanding of gallstone formation and searching for the methods to prevent lithogenesis.

The aim of the present study is to consider the possible mechanism of crystal nucleation in colloid and gel-like media without boundary surfaces and the experimental study of laser radiation impact on the processes of lithogenesis in the volume of these media, in particular, human bile.

2 Biomineral nucleus formation

Basing on the general knowledge of the colloid structure and biochemistry, as well as on the results of the studies of crystallisation dynamics in bile samples [5], we assume that a few mechanisms of the biomineral nucleus formation are possible.

2.1 Associates of macromolecules

The crystal appears and the growth in size process begins due to the appearance of a nucleus. The nucleation mechanism for the formation of crystals on a solid substrate is known [14], but in contrast to the crystal formation on a substrate, in the real biotissue there are no distinct nonuniformities like interfaces between media. Therefore, the nucleation may be initiated by certain mechanical inclusions or local inhomogeneities (clots) in the gel solution. The studies of crystallisation dynamics and crystal morphology in the course of sedimentation in samples of natural bile and in model media, carried out in Ref. [5], have shown that the rate of nucleation is determined by the degree of bile inhomogeneity, which manifested itself in the form of clots having the size to 0.5 mm. It was shown that just on the clots the mass crystallisation of cholesterol and carbonate phases occurs. In the clot-free space, the stones appeared significantly later [5].

It is known that the solidification of gel solution is accompanied by the formation of intermolecular hydrogen bonds, i.e., the gelatination occurs [15, 16]. Even in semidiluted solutions of polymers (with the polymer concentration 1% and less) the macromolecules in the statistical coil molecule conformation, overlap, penetrating into each other with their chain fragments. The chains of adjacent macromolecules can interact and form “bridges” between macromolecules.

The intermolecular bonds are weak. They permanently decay under the impact of thermal fluctuations and arrear at new sites. Therefore, such bonds are typically formed all over the volume of the gel solution and can hardly give rise to stone formation. In the worst case, the entire solution can approach the glassy state under the conditions of growing polymer concentration [15, 17].

Therefore, to our opinion, the formation of stone nucleus is more probable due to the entanglement of adjacent chains of macromolecular coils [15, 17]. Such entanglement of chains can occur also for different other macromolecules, such as proteins and organic molecules. The quasi cross-linkage of macromolecules is topologically more stable than the usual bridge bonds. They join two macromolecules into a united coil molecule conformation. With time in such heterogeneous gel solution, the quasi cross-linkage can serve as a preferable site of sedimentation for other macromolecules, including those having the quasi-crystalline globular conformation. Finally, the microscopic stone is formed that plays the role of a nucleation centre for a big stone.

2.2 Fringed micelles

In is possible that the mechanism of inhomogeneity formation is based on the appearance of a fringed micelles in the bioorganic solution, which can be a gallstone nucleus. The fringed micelle is a macromolecule, in which the crystalline and amorphous segments alternate [18]. As a result of intermolecular interaction that arises between such macromolecules the formation of an intermolecular fringed micelle becomes possible, in some regions of which the macromolecules are packed parallel to each other (the crystals with unwound chains), i.e., the conditions for partial crystallisation are created. These highly ordered regions can be interrupted by the regions with irregular arrangement of molecules. Since the length of macromolecules exceeds that of crystallites, the same polymer chain participates in the construction of a few crystallites, i.e., it spreads through a few crystalline and amorphous regions. Thus, the individual crystallites are linked via the amorphous regions [19].

Most probably, a single macromolecule, even fold into a fringed micelle, can change or invert its conformation. However, if several such molecules join to form a cluster, it is more difficult to transform them into a coil molecule state. That is why the formed cluster can play the role of a stone nucleus.

3 Laser radiation impact on the solutions of bile preparations

3.1 Experimental technique

To clarify the effect of laser radiation on the processes of crystal formation in bulk bioorganic media we experimentally studied the influence of laser radiation on the formation of crystal nucleation in the preparation of bile using the method of bile crystal growth in silica cuvettes. The experimental protocol on biomedical research was compiled as per the Helsinki Declaration, 200.

In the experiment we used the following preparations: i) white bile (free of pigment), gallstones after cholecystectomy in the case of gallbladder hydrops; ii) natural bile, gallstones after cholecystectomy in the case of cholecystitis.

The gallstones were hanged on a nylon thread in the silica plane-parallel cuvette with mat walls, filled with bile, so that the stone had no contact with walls or bottom. The cuvettes were closed with cover glass.

For the experiment, two cuvettes with white bile and two cuvettes with natural bile were prepared. During the experiment, one cuvette with white bile and one cuvette with natural bile were irradiated with laser light. The second cuvette of each pair was not exposed to laser radiation. The cuvettes were kept under the atmospheric pressure p = 705–735 torr and the temperature 35^0C, insignificantly differing from that of gallstones inside the human organism.

The gallstone that was neither placed in bile, nor exposed to any external influences played the role of a control sample.

For the bile irradiate we used the semiconductor laser with the wavelength 532 nm, the power 30 mW, and the beam diameter 5 mm. In the experiment the laser radiation, incident on the depolished glass wall of the cuvette, was scattered and affected the bile with the sample merged in it. The bile was exposed to radiation 5 minutes every day. The experiment was carried out during 65 days and was immediately finished after the complete evaporation of bile from the cuvette.

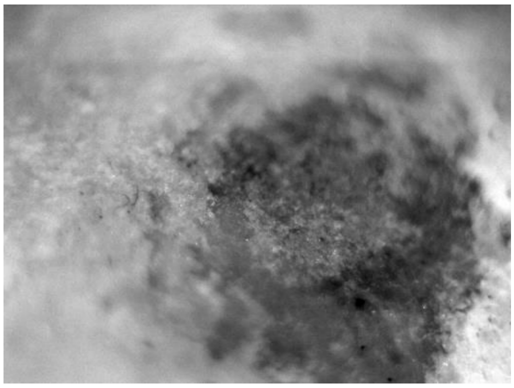
3.2 Results

After the experiment, the following objects were visually studied: i) the gallstone kept in cuvette and not exposed to laser radiation; ii) the gallstone kept in cuvette and exposed to laser radiation; iii) the cuvette with bile remainders not exposed to laser radiation; iv) the cuvette with bile remainders exposed to laser radiation.

In the preparations of natural bile, no qualitative changes were noticed both in the bile remainders in the cuvettes and in the sample gallstones that were kept in theses cuvettes. The studied samples of gallstones from both cuvettes were identical to the control sample.

In the preparations of white bile, strong qualitative changes were observed.

a



b

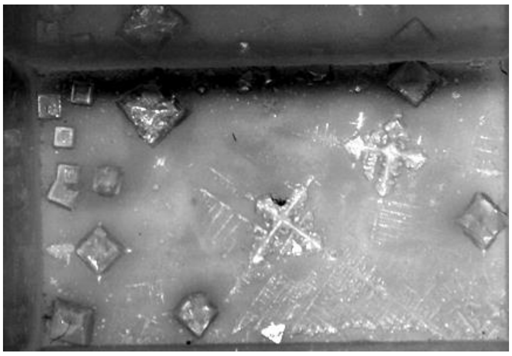
Fig. 1 Deposition on the gallstone kept in the cuvette with bile, not exposed to laser radiation: a) nonuniformity distribution of the deposition; b) surface defects. The magnification was 60×.

Fig. 2 The surface of white coating on the gallstone kept in the cuvette exposed to laser radiation. The magnification was 60×.

In contrast to the control samples and the samples from the cuvettes with natural bile, the gallstones from both cuvettes were coated with white deposition. The sample from white bile, not exposed to laser radiation had nonuniformity deposition with surface defects well seen in the microscope (Fig. 1).

The gallstone kept in the bile exposed to laser radiation had uniform coating without surface defects (Fig. 2). The coating thickness was nearly 0.1-0.2 mm.

During the visual investigation of the cuvette that contained white bile we found parallelepiped-shaped crystals having the thickness of nearly 0.2-0.3 mm at the bottom (Fig. 3).



5 mm

Fig. 3 The crystals grown at the bottom of the cuvette with bile, not exposed to laser radiation.

One of the crystals was extracted for detailed study. The microscopy revealed the presence of concentric rings around one centre, conceivably the nucleus (Fig. 4).

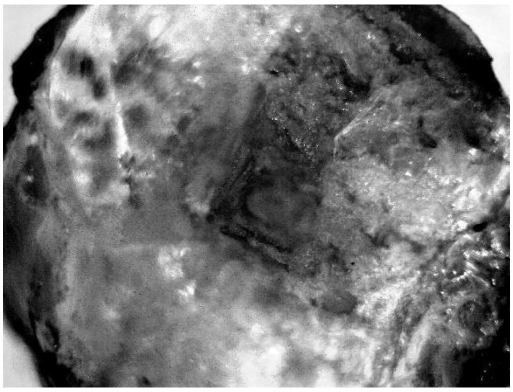


Fig. 4 The crystal with rings around a certain centre observed by means of microscopy (the image is magnified and additionally contrasted).

The study of the crystal in polarised light has shown the presence of anisotropy properties. When the crystal was placed between two crossed polarisers, the observed field of view was cleared in the region of the crystal.

During the visual study of the cuvette, in which during the experiment the white bile exposed to laser radiation was kept, we have found a thin transparent layer of homogeneous viscous colloid liquid with yellowish colour and the thickness of nearly 0.2 mm at the bottom of the cuvette. No crystals like those that appeared in the unexposed cuvette were found. Between two crossed polarisers we found slight triangle-shaped yellowish clearings in the field of view along the bottom edges near the walls. The clearings were caused by small crystalline formations having the thickness of nearly 0.2 mm and the longitudinal dimensions considerably smaller than those of the crystals found in the non-exposed cuvette.

3.3 Discussion

Our results allow the assumption that the laser radiation affects in some way the processes of crystal formation in the volume of white bile.

Our hypothesis is that the mechanism of such influence of the electromagnetic radiation on the solutions of organic substances, including the colloid ones, can be related to the specific behaviour of the interface monomolecular layers of polar solvent near the surface of macromolecules or particles. If the monomolecular hydrate film on the colloid particle surface consists of dipolar molecules, densely covering the particle surface, with similar charged ends directed to the particle, then the film forms a double electrical layer. This double layer is spontaneously polarised (due to the interaction between water molecules themselves and of water molecules with the particle), and the degree of this polarisation grows with the particle or macromolecule size [20]. The unavoidable spontaneous polarisation of the 2D layer of polar molecules on the particles surface occurs, and a gigantic dipole can arise, which agrees with the experimental data [20].

Thus, the possible mechanism of the laser radiation impact on the mineral formation in the bile solution consists in the specific features of the interaction of electromagnetic waves with the organic liquids, namely, in the solution the large clusters that could be the nucleation centres are divided into smaller associations. Due to this reason, in the cuvette exposed by laser radiation the homogeneous viscous mass with minor crystalline inclusions was left, while in the cuvette not exposed to laser radiation the large-size crystals were formed.

The difference between the stones in the uniformity and homogeneity of deposition is most probably also because under the laser exposure, the molecular clusters are broken into smaller parts, and smaller groups of molecules implement the sedimentation. Thus, the deposition layer acquires more uniform thickness than in sedimentation of larger molecular clusters.

Possibly the structure of the liquid, modified by laser radiation will return to the initial conformations after the impact termination via a chain of intermediate structure modifications, rather than instantaneously. In the case of biological molecules, this process can take macroscopic time (minutes or hours). Therefore, the liquid structure changes initiated by the laser radiation could be conserved during long time under the multiply repeated laser impact. This is particularly important, when the laser radiation acts on biological liquids in the human organism *in vivo* and demonstrates clearly expressed positive effect [21].

4 Conclusion

Thus, the possible mechanism of gallstone nucleation can be related to the appearance of local inhomogeneities (clusters) of the gel solution, in which the adjacent chains of different proteins and organic molecules interacting with each other with the participation of water can form stable links. The experimental results allow the assumption that the exposure of white bile by laser radiation affects to the crystal formation processes in it.

The hypothetic mechanisms of the effect are the division of larger molecules associates into smaller ones in the field of laser radiation, which, in turn, reduces the viscosity of the solution and inhibits the stone growth in the gel-colloid medium.

The practical significance of the obtained results consists in hypothetic possibility of using the laser radiation for prophylactics of pathogenic stone formation in the hepatobiliary system. More confident statements will be possible if further experiments will show the stable inhibition of stone formation in the preparations of natural bile, including the dependence on the parameters of the used laser radiation.