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The bactericidal effect of continuous wave laser with strongly absorbing coating at the fiber tip

Vadim Elagin*
Institute of Biomedical Technologies
Privolzhsky Research Medical University
10/1 Minina Square, Nizhny Novgorod 603005, Russia

Laboratory of Laser Nanomodification of Materials
Institute of Applied Physics Russian Academy of Sciences
46 Ulyanova Street, Nizhny Novgorod 603950, Russia
elagin.vadim@gmail.com

Anton Smirnov
Laboratory of Laser Nanomodification of Materials
Institute of Applied Physics Russian Academy of Sciences
46 Ulyanova Street, Nizhny Novgorod 603950, Russia
antonsmirnov@ufp.appl.sci-nnov.ru

Vladimir Yusupov
Institute of Photon Technologies of Federal Scientific Research Centre
"Crystallography and Photonics" of the Russian Academy of Sciences
2 Pionerskaya Street, Moscow, Troitsk 108840, Russia
iouss@yandex.ru

Alexey Kirillov
Department of Innovative Program
Institute of Applied Physics Russian Academy of Sciences
46 Ulyanova Street, Nizhny Novgorod 603950, Russia
kir@ufp.appl.sci-nnov.ru

Nadezhda Ignatova
Institute of Biomedical Technologies
Privolzhsky Research Medical University
10/1 Minina Square, Nizhny Novgorod 603005, Russia
n.i.evteeva@gmail.com

*Corresponding author.

Olga Streltsova
Department of Urology
Privolzhsky Research Medical University
10/1 Minina Square, Nizhny Novgorod 603005, Russia
strelzova_uro@mail.ru

Evgeniy Grebenkin
Department of Urology
Privolzhsky Research Medical University
10/1 Minina Square, Nizhny Novgorod 603005, Russia
evgen-fifa@rambler.ru

Vladislav Kamensky
Laboratory of Laser Nanomodification of Materials
Institute of Applied Physics Russian Academy of Sciences
46 Ulyanova Street, Nizhny Novgorod 603950, Russia
vlad@ufp.appl.sci-nnov.ru

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The bactericidal effect of laser radiation with a quartz fiber-based transmission system with a strong absorption coating converter against bacteria associated with urological stones has been studied. Gram-negative rod *Escherichia coli* and the Gram-positive coccus *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Enterococcus faecium* were used in this study. Each bacterial species was treated by continuous-wave near infrared laser coupled with bare fiber tip or strongly absorption coating fiber tip. After treatment, the temperature of bacterial suspension was measured. In addition, the temperature distribution was analyzed. It has been shown that using laser with a strongly absorption coating fiber tip results in significant bactericidal effect. The decrease of the amount of *E. coli* and *S. epidermidis* was 100% after treatment with an output power of 6 W of radiation at a wavelength of 0.97 μm for 40 s. Number of *S. aureus* and *Ent. faecium* colony-forming unit was reduced to 70% after same exposure. The peak temperature of bacterial suspension was 86°C after treatment by laser with a strongly absorption coating fiber tip. Laser with a strongly absorption coating fiber tip provides large-scale hydrodynamic flows directed away from the fiber tip. The laser with a strongly absorption coating fiber tip has bactericidal effect. The main role is associated with the effect of high temperature, which, in the form of flow in a liquid medium, affects bacteria.

Keywords: Laser; strongly absorbing coating; bacteria; bactericidal effect; laser-induced hydrodynamic.

1. Introduction

The incidence of urolithiasis in the world is steadily growing. Urinary stones may develop as a result of urinary tract infections. Infection stones are formed as a result of persistent infections caused by urease-producing bacteria, and urinary tract obstructions are frequently involved. In industrial countries, approximately 10–15% of urinary stones are infection stones.[1] In addition, a colonized stone culture associated with a sterile urine culture was

found in 25% of the patients.[2] Complete stone removal is the main objective of treatment. Traditional lithotripsy procedure includes fragmentation of a stone and washing out of the urinary tract the remaining pieces. Energy of laser used for traditionally lithotripsy vaporizes the medium creating an expanding cavitation bubble that leads to an acoustic pressure wave. The process of creating vapor plasma (pressure) requires specific conditions necessary to achieve laser-induced shock wave lithotripsy. Eradication of the associated urinary tract infections is only possible after the stone has been completely removed, since stones contain bacteria and the number of bacteria on the stone surface will increase despite antibiotic therapy.[3] It was shown that removal of stone from the renal pelvis provokes pyelonephritis in 87.5% of the cases.[4] Long-term antibiotic treatment must be administered in connection with lithotripsy. The goal of antibiotic therapy is to lower the concentration of bacteria in the urine and on stone fragments. Antimicrobial therapy is not very efficient because of the development of resistant species. In addition, the limited penetration of drugs into bacterial biofilm results in reduced susceptibility to this kind of treatment. Thus, new techniques for aseptic destruction of urinary stone are required.

A new technology based on diode laser with strongly absorbing coating (SAC) on a fiber tip is being developed. The use of laser with SAC fiber tip provided the break of a stone along the marked line and excluded small stone fragmentation.[5] The SAC fiber tip had working temperature at 2000 K. It is suggested that this technique may have bactericidal effect due to high temperature. Laser with SAC fiber tip in liquid is known to create directional jet flows, which, besides being of high temperature, contain vapor bubbles.[6] Previously, it has been shown that the effect of such jets on cancer cells in culture *in vitro* leads to cell death.[7] There are some authors claiming bacteria killing with red and near-infrared (NIR) light.[8] For example, Nussbaum *et al.*[9] reported a bactericidal effect at 630 nm for *Pseudomonas aeruginosa* and *Escherichia coli*. In addition, the bactericidal effects of visible light could be attributed to high amounts of reactive oxygen species generated by endogenous photosensitizers in the bacteria. Visible and NIR light can be absorbed by cellular photosensitizers such as cytochromes, flavins/riboflavins and NADP. Light absorption by photosensitizers causes their excitation and subsequent relaxation by transferring electrons to O_2, thereby generating reactive oxygen species.[10]

Thus, new laser lithotripsy technology is a combined effect of three damaging factors on biological objects in liquid: a hot fluid jet, vapor bubbles and laser radiation scattered by optical inhomogeneities of the fluid jet and bubbles. The goal of this study was to assess bactericidal effect on stone-associated bacteria of a laser with SAC fiber tip.

2. Materials and Methods

2.1. *Bacterial culture*

The study was performed on Gram-negative rod *E. coli* and the Gram-positive coccus *Staphylococcus epidermidis*, *S. aureus*, *Enterococcus faecalis* and *Ent. faecium*. These microorganisms were used in this study because they are closely associated with urinary tract infection and calculus. In this experiment, *E. coli* was cultured in the Luria–Bertani (LB) broth and agar; *Staphylococcus* and *Enterococcus* were cultured in the nutrient broth and agar. Before starting the experiments, the frozen (−80°C) bacterial samples were thawed and incubated for 16 h on a solid culture medium at 37°C under aerobic conditions. One of the grown bacterial colonies of each species was then harvested, put into nutrient broth and incubated for an additional 16 h at 37°C under aerobic conditions to ensure the logarithmic phase of growth for the bacterial suspension. Afterward, bacterial suspensions were diluted with sterile phosphate buffer saline according to the turbidity standard corresponding to 2.5×10^8 CFU/mL. One milliliter aliquots of each bacterial suspension were placed into microcentrifuge tubes and treated by laser. Thereafter, each sample was diluted 10^5-fold and 20 μL of the suspension was transferred into a 6 cm Petri dish with LB agar or nutrient agar. The colony counts were made after 16 h of incubation at 37°C. The number of colonies was routinely counted manually on photographs taken with a digital camera. For each sample, three plates were cultured and counted.

2.2. *Laser irradiation*

One milliliter of bacterial suspension placed in microcentrifuge tube was treated by laser LSP-0.97/10

(IPG IRE-Polus, Russia), emitting continuous-wave (CW) NIR radiation at a wavelength of 0.97 μm. The output powers were 3 W and 6 W, which had been selected previously[11]; the exposure time was 20 s and 40 s. During exposure, the laser radiation was continuously delivered to the sample. The silica fiber was dipped into the bacterial suspension to a distance of 5 mm from liquid surface. Each bacterial species was treated by laser either with bare fiber tip or with SAC fiber tip.

The SAC was a mixture of graphite powder (5–20%) with fractions less than 0.5 μm and silicone varnish (80–95%) based on polymethylphenylsiloxane resin. The procedure of applying the SAC onto the fiber tip was as follows: a drop of the mixture was placed on a glass surface and the fiber tip was dipped into it. Then the laser scalpel was switched on at 0.5–1 W for up to 1 min to dry the applied layer. The SAC on a fiber tip absorbed from 30% to 50% of initial laser irradiation was reported previously.[12] The absorption coefficient of SAC is 700 cm^{-1}.

After laser exposure, the temperature of the bacterial suspension was measured with a universal multimeter equipped with a thermocouple.

2.3. *Studying the laser-induced hydrodynamic processes*

Thirty milliliters of LB broth at room temperature were poured into a 9 cm diameter plastic Petri dish. Absorption coefficient of LB broth measured with Shimadzu UV1800 (Shimadzu, Japan) is 0.53 cm^{-1} at 0.97 μm. Laser radiation at an output power of 6 W was delivered via an optical fiber inserted through a hole made in the side wall of the Petri dish. The fiber tip was in the liquid column. The temperature distribution on the surface was recorded with a thermal imager Optris PI400 (Optris GmbH, Germany) at a rate of 27 frames per second. The Optris PI Connect software was used to display the thermograms and to calculate the temperature distribution characteristics (average surface temperature). The instrument error of the thermal imager is 2°C according to its specification. The value of ambient temperature was set to 24°C. The emissivity of the suspension was adjusted to achieve correspondence with thermocouple in the range of 17–70°C. For $\varepsilon = 0.92$, the difference between these methods was less than 1°C.

The directed two-phase jet flows formed near the fiber tip were recorded with a high-speed camera Fastcam SA-3 (Photron, Japan) operating at 2000 frames per second.

2.4. *Statistical analysis*

The mean number of colonies and standard deviation for each sample were calculated. Also for the results of temperature measurement, the mean ± standard deviation was calculated. Data were then analyzed with analysis of variance followed by multiple comparisons using the Bonferroni correction at a level of significance of 5% ($p < 0.05$). Statistical processing of the results was performed using the Statistica 10 software (GraphPad Software Inc., La Jolla, CA, USA).

3. Results

At first stage of the study, the effect of laser radiation on Gram-negative bacteria was analyzed. Figure 1 shows the result of *E. coli* treated with laser with an output power of 3 W. Decrease of number of CFUs by 40% compared with the control was found after laser exposure with a bare tip for 20 s. At the same time, laser with SAC tip induced 57% reduction of CFU after 40 s exposure. Note that both lasers with a bare tip after 40 s and with SAC tip after 20 s had a negligible stimulation effect. However, the number of CFUs in these cases had no significant difference, compared with the control.

The significant reduction in the mean number of CFUs was revealed after treatment by laser with SAC tip for 40 s. Therefore, in order to achieve a bactericidal effect, the output power of the laser was increased up to 6 W and duration of treatment

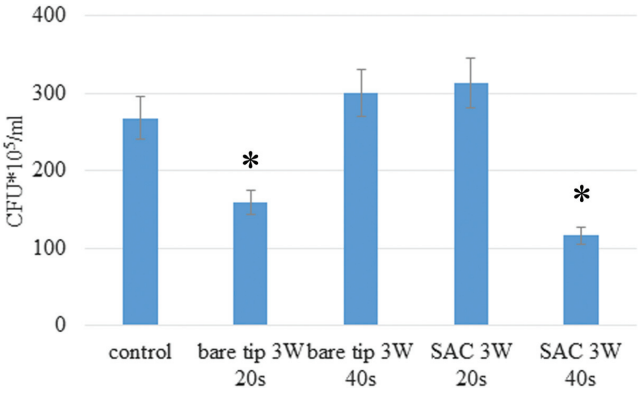

Fig. 1. The number of CFUs in *E. coli* suspension irradiated at 3 W for 20 s and 40 s. *Data show significant difference compared with the control, $p \leq 0.05$.

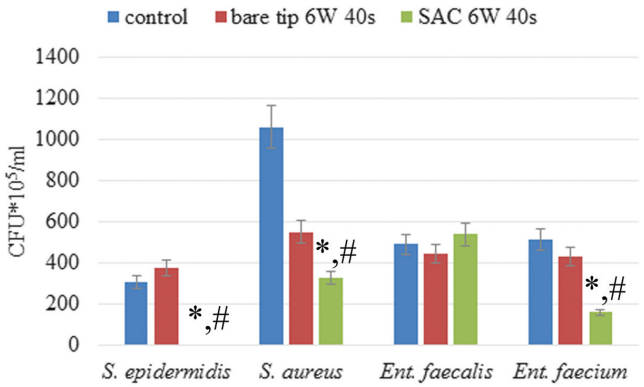

Fig. 2. The number of CFUs in *S. epidermidis*, *S. aureus*, *Ent. faecalis*, *Ent. faecium* suspension irradiated at 6 W for 40 s. *Data show significant difference compared with the control, $p \leq 0.05$; # Data show significant difference compared with laser with a bare tip fiber, $p \leq 0.05$.

was 40 s. It was found that both lasers with bare tip and SAC tip had strongly pronounced bactericidal effect. In both cases, no colonies of bacteria on Petri dish were detected.

Based on these results, Gram-positive cocci were laser-treated with 6 W output power for 40 s (Fig. 2). It was found that the number of *S. aureus* and *Ent. faecium* decreased by 70% after treatment by laser with SAC tip. In the case of *S. epidermidis*, the laser with a SAC fiber tip had 100% bactericidal effect. In contrast, treatment of *Ent. faecalis* did not lead to any changes in number of bacteria.

The main mechanism of NIR laser action on organisms is thermal injury. Therefore, to reveal it, temperature of bacterial suspension was measured before and directly after laser treatment. Initial temperature was 24°C in all cases. The final temperature values are demonstrated in Fig. 3. It was found that peak temperature (86°C) was detected after treatment by laser with a SAC fiber tip 6 W

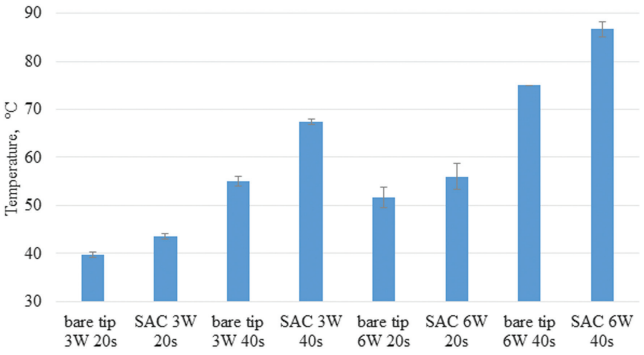

Fig. 3. The temperature values of the bacterial suspension at various irradiation modes.

output power for 40 s. Using bare tip fiber with the same laser parameters led to rise in temperature up to 75°C. Action of laser with 3 W output power resulted in increasing temperature up to 65°C after treatment with SAC fiber for 40 s. The value of temperature in other cases was not sufficient to damage the bacteria.

Figure 4 shows the temperature distribution on a liquid surface in a Petri dish during treatment by laser with a bare tip fiber (Fig. 4(a)) and with a SAC fiber tip (Fig. 4(b)). The fiber was passed through the dish wall at a depth of 3 mm and placed horizontally. In case of using bare tip, the temperature directly above the fiber tip was much higher. For fiber with SAC tip, large-scale hydrodynamic flows propagating from the fiber were detected. The flows had the mean velocity vector directed away from the fiber tip. For using fiber with SAC tip, the rapid heating of the liquid was due to the powerful mixing. Therefore, the integral temperature of region liquid placed far from the fiber tip (Fig. 4(a)) increased faster in case of using SAC fiber tip. The fiber with a SAC tip heated the bulk of liquid in the dish compare with the fiber with a bare tip. The different liquid volumes regularly moved near the

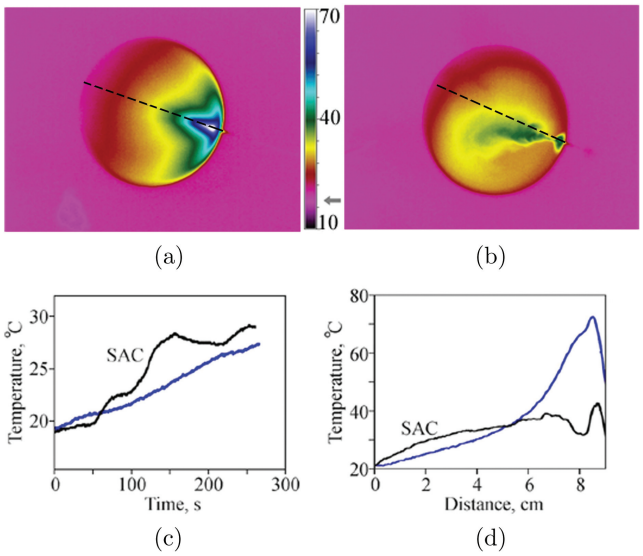

Fig. 4. Processes occurring in a liquid under the action of laser radiation. The temperature distribution on the liquid surface after treatment by laser with a bare fiber tip (a) and a SAC fiber tip (b) for 2 min. The integral temperature in an assign is shown. Dashed line marks axis of the fiber. The change of the integral temperature in the assign region (c) and the distribution of the surface temperature along the fiber axis (d). Blue line corresponds to laser with a bare fiber tip and black line corresponds to a SAC fiber tip.

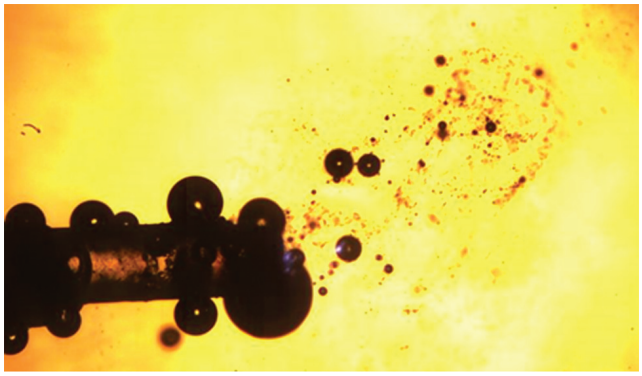

Fig. 5. Directed two-phase jet flows formed near the fiber tip during irradiation by laser with a SAC tip at 6 W. Recording rate is 2000 frames per second.

SAC fiber tip during laser treatment. This movement led to a significantly greater bactericidal effect of the laser with a SAC fiber tip.

It is well known that laser with SAC fiber tip can create directional jet flows, which, besides being of high temperature, contain vapor bubbles. A high-speed camera was used to detect jet flows and vapor bubbles. Jet flows contain a large number of vapor bubbles presented in Fig. 5, which were detected during treatment by laser with SAC fiber tip. Besides, directed two-phase jet flows formed near the fiber tip can have a negative effect on cells. Using laser with bare tip did not result in form jet flows as well as vapor bubbles.

4. Discussion

In this paper, the bactericidal effect of a near-infrared CW laser with the bare tip fiber and the SAC tip fiber was investigated on calculus specific bacteria. Hamasuna *et al.*[13] reported that bacteria were isolated from 72% of patients with obstructive pyelonephritis as a result of urolithiasis. Ho:YAG laser lithotripsy for urolithiasis is the gold standard technique. It is known that stone dusting and stone fragmenting are basic methods of lithotripsy.[14] However, 37.4% of endourologists actively retrieved only large fragments but not small fragments.[15] Small fragments of stone may contain bacteria; therefore, they should be completely removed as well as antimicrobial therapy should be performed. It was shown that use of laser with SAC fiber tip provided the break of a stone without small fragments.[5] In addition, high working temperature of fiber tip may lead to bacteria death.

The significant bactericidal effect for *E. coli*, *S. epidermidis*, *S. aureus* and *Ent. faecium* observed in the study is determined by several components. The first is the thermal damage to bacteria. The temperature value of the bacteria suspension was 75°C for a bare tip and 86°C for a SAC tip. The second component is laser light. The endogenous fluorophores can absorb NIR laser irradiation with subsequent generation of reactive oxygen species or with direct destroying of their molecule.[10] However, low-intensity NIR laser may have growth-stimulated effect on *E. coli*.[16] One possibility of light action is increased electron transfer in the respiratory chain as a result of a change in the redox properties of the carrier due to the photoexcitation of their electronic states. Probably, stimulating effect demonstrated in Fig. 1 may provide this mechanism. The third component is mechanical action of jet flows and vapor bubbles formed near SAC fiber tip. The absorbing coating rapidly heated due to strong absorption of laser light and powerful hydrodynamic flows arose near the tip.[6,17] Recently, it has been shown that the effect of such jets on cancer cells in culture *in vitro* leads to cell death.[7] The oscillations of bubble are shown to induce fluid flow and mechanical elimination material.[18,19] The fourth component is acoustic noise. It was shown that such sound could provide a protective effect on stem cells.[20] The stimulating effect of the laser with SAC fiber tip at 3 W for 20 s is probably due to broadband sound generation. Acoustic noise from the proposed system was studied in papers.[21,22] It was shown that CW laser with SAC fiber tip did not allow the formation of a "shock" wave. In contrast, Er:YAG laser used for lithotripsy is known to induce cavitation bubbles with different sizes depending on fiber tip geometry. The cavitation bubble size determines the acoustic transient amplitudes.[23] Thus, using laser with SAC fiber tip for lithotripsy allows to decrease number of bacteria on urinary stone surface.

A large number of bacteria are known to be found in renal stones, especially coral-like stones.[24,25] Laser lithotripsy in "dusting" mode, which had the effect of breaking off exceedingly small fragments from the stone, promotes to release stone-associated bacteria.[26] Currently, a technique of renal calculus lithotripsy using a diode laser with SAC at the fiber tip is being developed.[5] This technique allows us to break stones up into parts with controlling size. The current paper shows that a diode laser with a SAC

at the fiber tip has the bactericidal effect on renal calculus specific bacteria. We guess that microorganisms located on the surface of a stone as well as in the interior will be affected by laser during lithotripsy. Application of the technique can reduce the number of viable microorganisms dispersed in the kidney during lithotripsy and therefore reduce the likelihood of infection.

5. Conclusion

The present study clearly demonstrated that the laser with a SAC fiber tip at 6 W output power has antibacterial effects against *E. coli*, *S. epidermidis*, *S. aureus* and *Ent. faecium*. The bactericidal effect is determined by all the factors discussed earlier (temperature, jet flows containing vapor bubbles, direct action of laser light and acoustic noise). In the case of using CW NIR laser with SAC fiber tip, high-temperature flows played the main role in disinfection. In the future, the bactericidal effect of this laser on infected calculus extracted from patients will be studied. Moreover, the bactericidal effect of intermittent radiation will be investigated.

Conflict of Interest

The authors have no conflicts of interest related to the present study.

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