Determining Optimal Exposure Parameters of Femtosecond Laser to Enhance the Effectiveness of Pharmaceutical Nano-Emulsions on Staphylococcus Aureus Bacteria

Nastaran Kahrarian[a], Atoosa Sadat Arabanian[a,*], Zinab Moradi Alvand[b], Hasan Rafati[b], and Reza Massudi[a]

[a]Laser and Plasma Research Institute, Shahid Beheshti University, Tehran, Iran

[b]Research Institute of Medicinal Plants and Raw Materials, Shahid Beheshti University, Tehran, Iran

Corresponding author email: a_arabanian@sbu.ac.ir

Regular Paper-Received: June 17, 2024, Revised: Oct. 08, 2024, Accepted: Oct. 13, 2024, Available Online: Oct. 15, 2024, DOI: to be added soon

ABSTRACT— The modification of cell surface structures has become a focal point in cell biology, with methods like drugs, chemicals, and non-destructive techniques such as laser light exposure being utilized. In particular, exposure to femtosecond laser pulses has been found to increase cell permeability to formulations without causing thermal damage. This study aimed to observe and document the changes in the structure of *Staphylococcus aureus* bacteria when they were optically trapped and subjected to femtosecond laser pulses, along with the application of a medicinal substance, over 20 minutes. The research successfully determined the optimal power and exposure time of the laser light on the bacterial surface and demonstrated the ability of femtosecond laser pulses to enhance the efficacy of the medicinal substance.

KEYWORDS: Bacteria, cell membrane, femtosecond laser pulses, optical tweezers.

I. INTRODUCTION

Every cell in our body relies on the exchange of essential substances with its external environment to survive [1]. The cell membrane plays a crucial role in this process, facilitating the transfer of drugs, chemicals, and genetic material into the cell. Researchers are currently exploring ways to manipulate the membrane to improve the transfer of substances from outside the cell. One promising method being studied involves the use of femtosecond laser pulses on the cell membrane's surface [2]. Femtosecond lasers operating in the near-infrared spectral region offer several advantages for this application. They have low absorption by water and low photon energy, making them less likely to cause damage to the cells when used in ultra-short pulses with a single pulse energy of approximately nanojoules or less [3]. These pulses create tiny pores on the cell surface, temporarily allowing foreign substances to enter the cytoplasm. This technique has numerous applications in bioengineering, tissue engineering, and stem cell therapy [4]. Researchers can use optical tweezers to accurately monitor the exposure of the sample surface to femtosecond pulses. This non-destructive and precise method of trapping cells is widely used in various fields, including analyzing cell responses and studying changes in the cell surface structure [5].

In our study, we established an experimental setup to investigate the enhanced permeability of pharmaceutical nanoemulsions in destroying *Staphylococcus aureus* (ATCC 6538) bacteria under femtosecond pulse irradiation. We determined the optimal power and time irritation for the laser pulse radiation and used optical tweezers to trap the target bacteria and observe the immediate effects of the medication.

II. THEORY

The cell membrane is composed of proteins and lipids. Lipids give the membrane flexibility, while proteins control chemical processes within the cell and help transport molecules across the membrane [1] (Fig. 1).

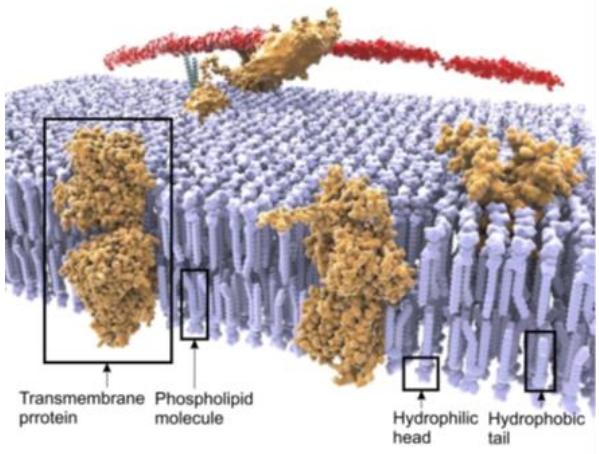

Fig. 1. A schematic view of components of the cell membrane [1].

Focusing femtosecond laser pulses on the cell membrane can facilitate the transfer of external components into the cell. These pulses increase the photon density at their peak to over 1012 W/cm^3, which is extremely intense. This intense energy can disrupt the membrane layer, creating tiny pores on its surface. These pores establish a pathway for exchange between the cell's internal and external environments. It is important to note that the membrane is made up of phospholipids, as shown in Fig. 1. These phospholipids have two components: the hydrophilic heads and the hydrophobic tails. When the membrane is disrupted, these components quickly reconnect, allowing the cell to return to its original state. To closely study this phenomenon, optical trapping is used. During the trapping process, the net force acting on the cell must be zero. Since the biological sample is suspended in a liquid, it is affected by two forces: gravity (Fg) and buoyancy (Fb). To counteract these forces and achieve a net force of zero, the optical force exerted by the laser beam is used. The optical force on the particle is determined using geometrical optics. When the laser beam strikes the surface of the sphere, it is refracted due to the difference in refractive index between the sphere and its surroundings. This refraction splits the beam into reflected (rr) and transmitted (rt) parts [6]. This change in momentum leads to a force being exerted on the sphere, as shown in Fig. 2.

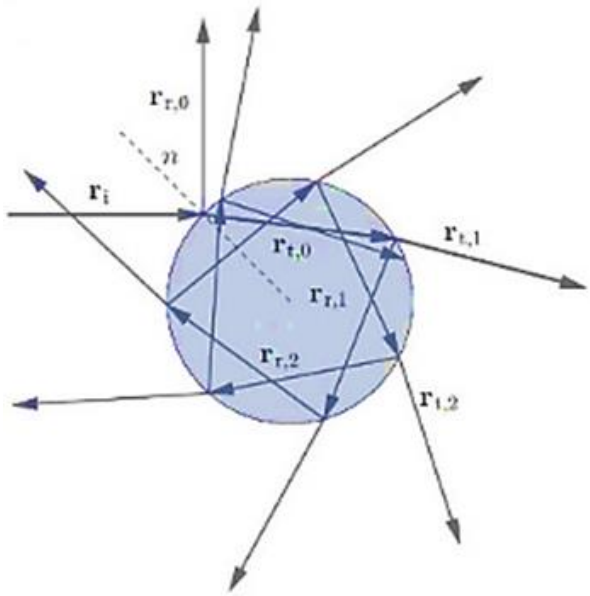

Fig. 2. Sequential reflections of a single beam inside the dielectric sphere [6].

In order to explain energy transfer, we use a set of light rays. These rays are vectors that are perpendicular to the wavefront and aligned with the direction of electromagnetic energy flow. By doing this, we can calculate the force exerted on a sphere with a specific refractive index using Eq. 1 [3]:

$$\mathbf{F}_{GO} = \sum_m \mathbf{F}_{ray}^{(m)} = \sum_m \left[\frac{n_i P_i^{(m)}}{c} \hat{\mathbf{r}}_i^{(m)} - \frac{n_i P_r^{(m)}}{c} \hat{\mathbf{r}}_{r,0}^{(m)} - \sum_{n=1}^{\infty} \frac{n_i P_{t,n}^{(m)}}{c} \hat{\mathbf{r}}_{t,n}^{(m)} \right] \quad (1)$$

where $\hat{\mathbf{r}}_i^{(m)}, \hat{\mathbf{r}}_{r,0}^{(m)}$, and $\hat{\mathbf{r}}_{t,n}^{(m)}$ represent vectors in the direction of the incident, nth reflected, and nth transmitted rays, respectively. $P_{t,n}^{(m)}$, $P_r^{(m)}$, and $P_i^{(m)}$ also, correspond to the powers of the incident beam, *m*, the first reflected beam, and the nth transmitted beam, and *c* is the speed of light in the vacuum. In terms of the particle, the incoming rays are focused by a lens on its center of mass, and the FGO force is exerted on

the particle under the interaction of converging rays $\hat{\mathbf{r}}_f^{(m)}$ at point o, as shown in Fig. 3.

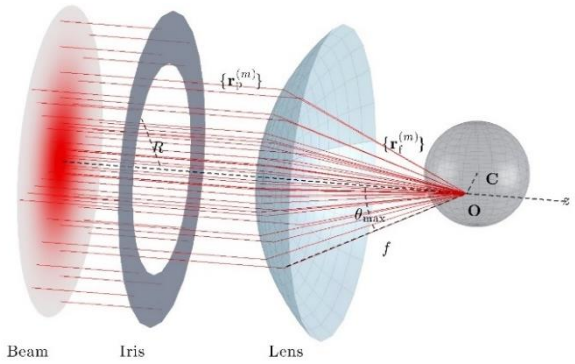

Fig. 3. Focusing of paraxial rays [6].

The optical force (FGO) acts upon the particle to oppose the other forces acting on it at the laser focal point. This results in a net force of zero (FGO=Fb+Fg). When these conditions are met, the particle remains still and becomes effectively trapped at the laser focal point.

III. Optical Setup

A schematic of the experimental setup has been demonstrated in our previous work [7].

A continuous wave laser with a wavelength of 980 nm was used to perform the trapping. Additionally, we utilized a femtosecond laser from Noor Abi Laser Company, specifically the Vira-fs-1040 model, which had a pulse width of 200 fs, a wavelength of 1040 nm, and operated at a repetition rate of 23 MHz. We employed this laser to study the impact of laser pulses on compound permeability.

To ensure the beam diameter of both lasers matches the aperture diameter of the objective lens, we employ two Galilean telescopic systems in the beam path. These systems allow both beams to enter the polarization beam splitter (PBS) orthogonally and align with each other at its output. From there, the combined beam is directed to the objective lens using a flat mirror (M2) with 90% reflection for 980nm and 1040nm.

We use an oil-immersed objective lens with a numerical aperture of 1.25 and 100X magnification to focus the two laser beams on the sample. To capture images, we illuminate the sample with an LED, and the reflected light is then directed to the eyepiece and camera through another flat mirror (M2).

To control the average power of the laser and fine-tune the pulse energy delivered to the sample, we incorporate a half-wave plate and a polarizing beam splitter (PBS) into the beam path. Additionally, a shutter regulates the exposure time of the laser on the sample during examination.

IV. Preparation of Bacterium

Gram-positive bacteria strains (*Staphylococcus aureus*) were used to evaluate the effect of femtosecond laser pulses on increasing the antibacterial effect of the nanoemulsion approach in the laboratory. The bacteria were cultured in Mueller Hinton agar for a full day at 37°C [8]. Then, a colony was selected and cultured in sterilized Nutrient Broth (NB) medium after two hours of incubation at 37°C and 250 rpm. Absorbance was measured at a wavelength of 600 nm (OD600 nm=0.400), indicating bacterial growth. Bacterial suspension was used to investigate the effect of femtosecond laser pulses on accelerating the delivery of the formulations to them.

V. Experimental Results

In the first part of the experiment, we investigated the duration of effectiveness of a medicinal nanoemulsion on bacteria. To do this, we introduced the medicinal nanoemulsion into the bacterial solution. Subsequently, we employed an optical tweezer with a diode laser power of 36.8mW to capture a bacterium at the focal point of the laser beam. We then proceeded to observe and document the degradation process of the surface membrane of the trapped bacteria at specific intervals: t = 0, 4, 8, and 20 minutes. Based on these findings, we deduced that the nanoemulsion substance typically requires an average time of 2 to 4 minutes to penetrate the bacterial cytoplasm.

We used femtosecond pulse radiation to disrupt the surface layer of the bacterial membrane to enhance the bactericidal effectiveness of the Nano-emulsion. First, we determined the

optimal power and exposure time for laser light irradiation on the bacterial surface. The goal was to create small holes in the membrane's surface without killing the bacteria outright, to facilitate delivery of the Nano-emulsion. We then exposed the bacteria to different powers and durations of laser irradiation and assessed their viability rates. This information is illustrated in Fig. 4.

Data on bacteria survival rates when exposed to varying power levels show that femtosecond pulses within the 14-15 mW range for 20-40 seconds do not kill the bacteria. However, these laser pulses have the potential to enhance the delivery of these compounds by creating openings on the bacterial surface. Therefore, we explored the impact of femtosecond laser pulses on the effectiveness of nanoemulsions for bacterial killing.

For more clarity, we observed and recorded the process of surface changes of the trapped bacterium after irradiating by femtosecond laser pulses at average powers of 10 mW, 15mW, and 20mW with an exposure time of 40 seconds (Fig. 5). The results show that the 15mW power can be optimal for delivering medicine to bacteria.

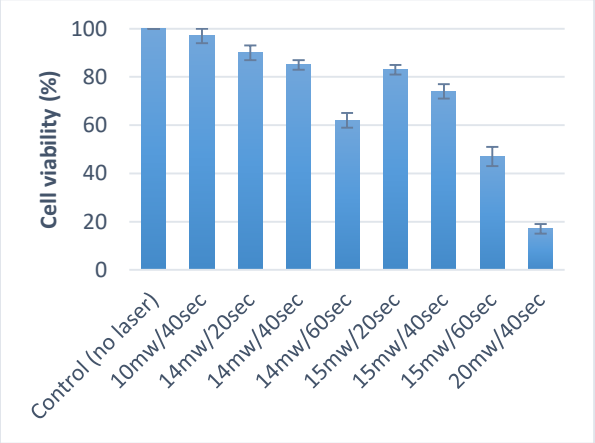

Fig. 4. Diagram of bactria viability rate under the irradiation of femtosecond laser pulses with different powers and durations in the absence of Nano-emulsion.

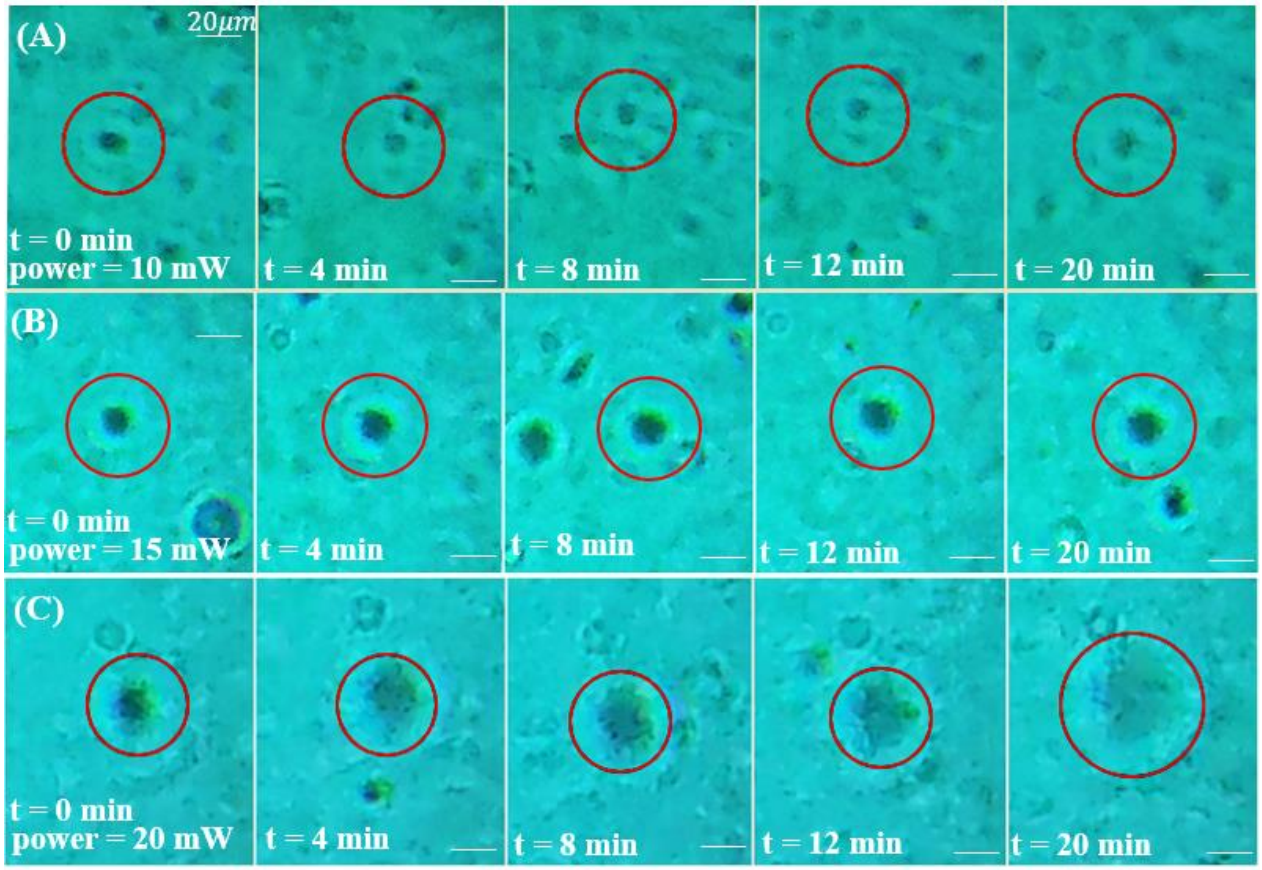

Fig. 5. Degradation process of the surface membrane of the trapped bacteria for different average powers of the femtosecond laser of (A) 10mW, (B)15 mW, and (C) 20 mW, in an exposure time of 40 s.

In Fig. 6, we compared the inhibition of bacterial cells using nanoemulsion delivery with and without femtosecond laser irradiation. Microscope images of the bacteria trapped within the Nano-emulsion were recorded after being irradiated for 40 seconds by a femtosecond laser with an average power of 13.4 mW, which is equivalent to 0.62 nJ per pulse. As illustrated in Fig. 6, femtosecond pulse exposure could reduce the time needed to disrupt the bacterial surface structure to just 8 minutes, in contrast to the slower inhibition of bacteria without irradiation. The destruction of bacteria commenced before the fourth minute, suggesting that the Nano-emulsion penetrated the sample more rapidly when combined with femtosecond laser radiation than in previous observations without it.

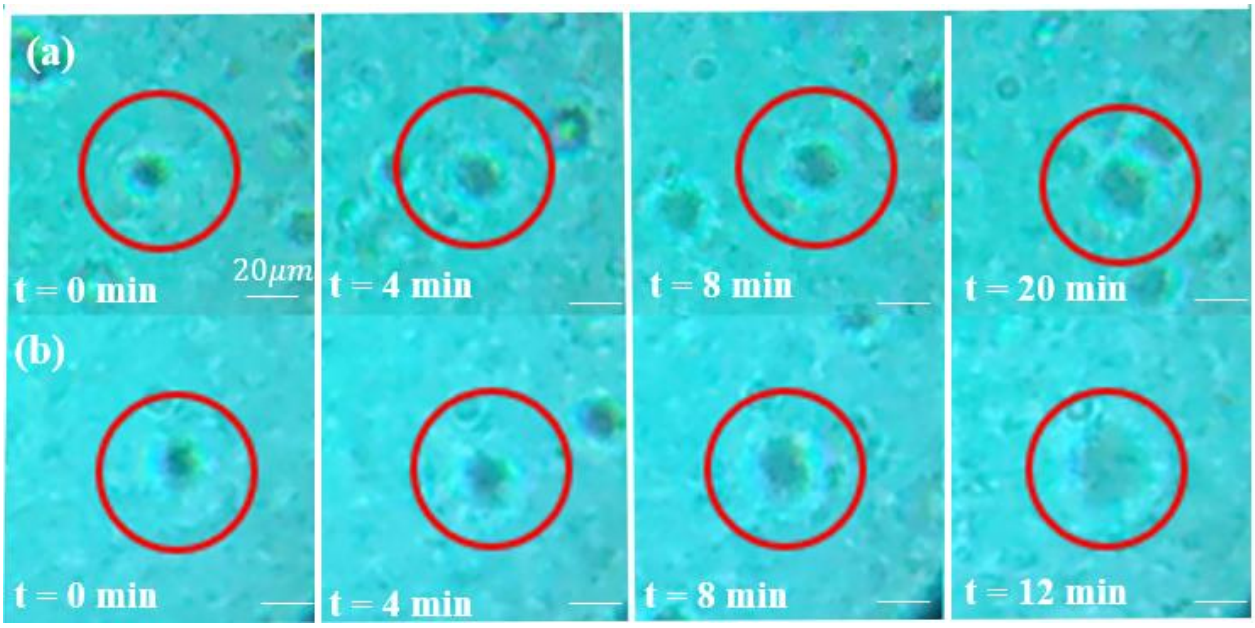

Fig. 6. Investigating the effect of femtosecond laser pulses on accelerating the effectiveness of nanoemulsion on bacteria (a) Treatment of bacteria with nanoemulsion (b) Treatment of bacteria with nanoemulsion and femtosecond laser pulse.

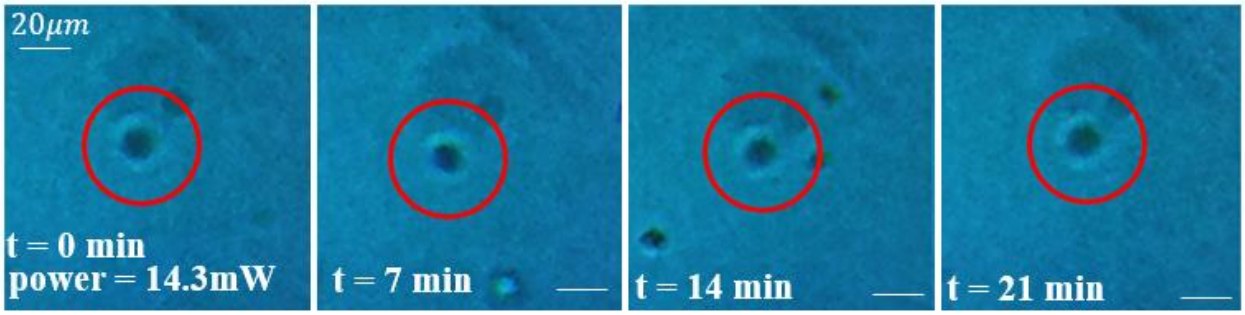

Fig. 7. Investigating the effect of ultra-short pulses without the presence of nano-emulsion on bacteria.

Furthermore, to confirm that ultra-short laser pulses with pulse energy of 0.62 nJ alone have no destructive effect on bacterial structure, we conducted an experiment where we trapped a bacterium without adding any Nano-emulsion substances to the solution. We then exposed it to femtosecond pulses at the same laser intensity for 40 seconds. As shown in Fig. 7, it was observed that without Nano-emulsion substances, femtosecond laser pulses cannot destroy or kill the bacteria, and there is no noticeable change in the surface morphology of the bacteria.

VI. Conclusion

In this study, we used optical tweezers to immobilize *Staphylococcus aureus* (ATCC 6538) bacteria. We then investigated the effects

of the formulation delivery with and without femtosecond laser pulse irradiation. We found that femtosecond laser pulses, when used within a specific range (14-15 mW power and 20s-40s exposure time), improved the effectiveness of the formulation on the bacterial membrane surface. Importantly, these pulses did not harm the bacteria's viability within the specified parameters. Therefore, combining femtosecond laser and pharmaceuticals can enhance the effectiveness of Nano-emulsion and lead to better therapeutic outcomes.

VII. REFERENCES

[1] G.M. Cooper, *The Cell: A Molecular Approach* 4th Ed., Vol. 1, Sunderland, MA: Sinauer Associates, Inc., pp. 44–71, 2006.

[2] M. Waleed, S.-U. Hwang, J.-D. Kim, I. Shabbir, S.-M. Shin, and Y.-G. Lee, "Single-cell optoporation and transfection using femtosecond laser and optical tweezers," Biomed. Opt. Express, Vol. 4, pp. 1533-1547, 2013.

[3] M. F. Yanik, H. Cinar, H. N. Cinar, A. D. Chisholm, Y. Jin, and A. Ben-Yakar, "Functional regeneration after laser axotomy," Nature, Vol. 432, p. 822, 2004.

[4] H. He, S.K. Kong, and K.T. Chan, "Transfection and cell fusion by femtosecond lasers," J. Innovative Opt. Health Sci., Vol. 4, no. 2, pp. 113-125, 2011.

[5] Z. Zhang, T.E.P. Kimkes, and M. Heinemann, "Manipulating rod-shaped bacteria with optical tweezers," Sci. Rep., Vol. 9, pp. 19086(1-9), 2019.

[6] A. Ashkin, "Acceleration and trapping of particles by radiation pressure," Phys. Rev. Lett., Vol. 24, pp. 156-159, 1970.

[7] L. Parseghian, N. Kahrarian, A.S. Arabanian, Z.M. Alvand, R. Massudi, M. Rahimi, and H. Rafati, "Berberine-loaded nanoemulsions as a natural food preservative; the impact of femtosecond laser irradiation on the antibacterial activity," Heliyon, Vol. 10, pp. e37283(1-15), 2024.

[8] A. Jafari-Sales, B. Jafari, H. Khaneshpour, and M. Pashazadeh, "Antibacterial effect of methanolic extract of rosa damascena on standard bacteria Staphylococcus aureus, Bacillus cereus, Escherichia coli and Pseudomonas aeruginosa in vitro," IJNLS, Vol.4, pp. 40-46, 2020.

Nastaran Kahrarian received her BSc in engineering physics focusing on optics and lasers from Kermanshah University of Technology (KUT) in 2021. She worked as a Master's student at the Femtosecond Lasers Research Laboratory from 2021 to 2024 at Shahid Beheshti University (SBU). Her research interests include lasers, biophotonics, drug delivery, cell fusion, optical tweezers, and nonlinear optics**.**

Atoosa Sadat Arabanian was born in Tehran, Iran, in 1980. She received her BSc degree in applied physics from the Azad University of Karaj, in 2003, her MSc degree in photonics from the Laser and Plasma Research Institute, Shahid Beheshti University, Tehran, Iran, in 2006, and her PhD degree of Photonics in September 2013 from the Laser and Plasma Research Institute, Shahid Beheshti University, Tehran, Iran. She is currently a faculty member of the Laser and Plasma Research Institute of Shahid Beheshti University. Her research fields include technology of femtosecond fiber lasers and continuous fiber lasers, femtosecond laser micromachining, quantum technology, and biophotonics

Zinab Moradi Alvand received her PhD from Shahid Beheshti University, majoring in Phytochemistry. The research field of PhD studies was investigation of biological activities for natural essential oils nanoemulsions through microfluidic systems, which include especially cytotoxic and antibacterial properties of the mentioned formulations.

Hasan Rafati received his PhD from Tabriz University of Medical Sciences, majoring in Pharmacy, pharmaceuticals England. The research field of the studies was in the microencapsulation of peptide-protein drugs and natural compounds. The present investigations include the use of nanotechnology findings in the design of modern drug delivery systems with an emphasis on anticancer drugs, antibiotics, antiviral drugs and on CNS diseases.

Reza Massudi received his BSc from Sharif University of Technology in 1988, his MSc from Isfahan University of Technology in 1992, and his PhD in laser physics from Laval University, Quebec, Canada in 1999. He has been a faculty member of the Laser and Plasma Research Institute, Shahid Beheshti University, Tehran, Iran since 2000. His interests are ultrashort and fiber lasers and their applications, such as biophotonics. He has published different papers in related journals and presented his research works at national and international conferences.

THIS PAGE IS INTENTIONALLY LEFT BLANK.