EVALUATION OF LED EMISSION OF BLUE AND VIOLET SPECTRA ON MULTIDRUG-RESISTANT STRAINS OF *PSEUDOMONAS AERUGINOSA IN VITRO*

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Abstract

Nowadays, the influence on the microbial ability to form biofilms and formed daily biofilms is regarded as a promising new strategy for antimicrobial therapy. The impact of LED on the destruction of microbial biofilms and prevention of biofilms formation *in vitro* is of particular importance. The study of the effect LED emission of blue and violet spectrum *in vitro* on *Pseudomonas aeruginosa* formed biofilms found that after 10 minutes exposure a decrease of biofilm optical density took place compared with the optical density of *Pseudomonas aeruginosa* biofilms before exposure, indicating a violation of the integrity in formed by isolates biofilms. It was established that in daily biofilms formed by *Pseudomonas aeruginosa* after the action of LED emission of blue and violet spectra planktonic cells production decreased in comparison with control. And, *Pseudomonas aeruginosa* planktonic cells after exposure to LED emission of blue and violet spectra for 10 minutes were unable to form dense biofilms. This fact is very important in prevention of *Pseudomonas aeruginosa* colonization and admitting of adequate combine antimicrobial therapy.

Background

For a long time, studies on finding methods for blocking the formation of nosocomial pathogens biofilms and preventing the secondary biofilms formation as colonization factor of *Pseudomonas aeruginosa* multi-resistant strains are carried out. Nowadays, the influence on the microbial ability to form biofilms [1] and formed daily biofilms is regarded as a promising new strategy for antimicrobial therapy. A large number of laboratories conduct research of methods and study the influence of various physical factors that inhibit the formation of biofilms [2]. The impact of LED on the destruction of microbial biofilms and prevention of its formation *in vitro* is of particular importance. It is established that the effect of LED emission of blue and violet spectra on biological macromolecules is their degradation with inhibition of biocatalytic activity [3]. But at present there is no data about effect of LED emission of blue and violet spectra on biofilms of nosocomial multi-resistant strains of *Pseudomonas aeruginosa* with determining the ability of planktonic cells to form new biofilms as a factor of pathogen colonization and persistence.

The aim was to study the effect of LED emission of blue and violet spectra on *Pseudomonas aeruginosa* biofilms.

Methods

The ability to form biofilms was observed on polystyrene plates with primary synchronization of periodic culture of studied strains. Synchronization of bacterial culture was conducted after growth kinetics definition of asynchronous culture by Mitchison and Vincent selection method. Optical density of biofilms was measured using spectrophotometer “Multiskan EX 355” (figure 1) and was shown in absorbance units of optical density.

Figure 1. Spectrohotometer «Multiskan EX 355».

Exposure *in vitro* was made with LED sources of blue (450 – 480 nm) and violet (380 – 430 nm) emission of photon matrix of the Korobov “Barva-Flex” apparatus which has a LED matrix with superluminescent diodes (figure 2) and a power unit. Statistical programs “Statistica” and “Biostat” were used to process the results.

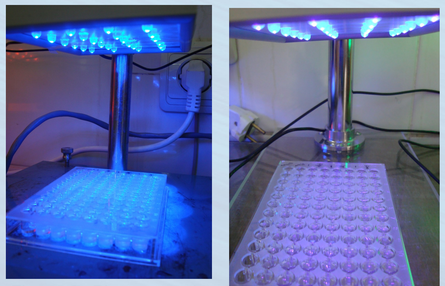

Figure 2. Photon matrix of the Korobov “Barva-Flex” apparatus.

Results

The study of the effect LED emission of violet spectrum *in vitro* on *Pseudomonas aeruginosa* formed biofilms found that after 10 minutes exposure a decrease of biofilm optical density in 4.8 times took place compared with the optical density of *Pseudomonas aeruginosa* biofilms before exposure (0,59 ± 0,03 and 2,81 ± 0,46 units of optical density respectively).

Similar data were obtained during studying of the action of LED emission of blue spectrum for 10 minutes on daily biofilms formed by *Pseudomonas aeruginosa*. Optical density raised down in 3.3 times compared to the same before exposure (0,85 ± 0,07 and 2,81 ± 0,46 units of optical density respectively), indicating a violation of the integrity in formed by isolates biofilms (figure 3).

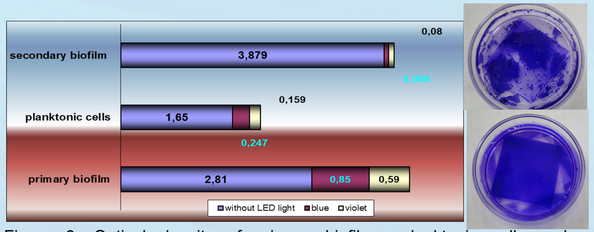

Figure 3. Optical density of primary biofilms, planktonic cells and new (secondary) biofilms under influence of LED emission of blue and violet spectra.

It was established that in daily biofilms formed by *Pseudomonas aeruginosa* after the action of LED emission of blue spectrum planktonic cells production decreased in 6.7 times compared with the control (0,247 ± 0,08 and 1,65 ± 0,06 units of optical density respectively). A similar data were obtained when exposed with LED emission of violet spectrum, such property was less in 10,4 times in comparison with control (0,159 ± 0,06 and 1,65 ± 0,06 units of optical density respectively).

In determining the ability to form biofilms by *Pseudomonas aeruginosa* planktonic cells after exposure to LED emission of blue and violet spectra for 10 minutes it was revealed that removed planktonic cells were unable to form dense biofilms (0,068 ± 0,01 and 0,08 ± 0,02 units of optical density respectively). This fact is very important in prevention of *Pseudomonas aeruginosa* colonization and admitting of adequate combine antimicrobial therapy (figure 4).

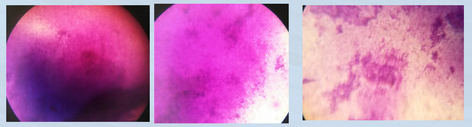

Figure 4. Disorganization of daily biofilms under influence of LED emission of blue and violet spectra.

Conclusion

Biodestructive impact of LED emission on dense biofilms of *Pseudomonas aeruginosa* multidrug-resistant strains has been detected. Based on the conducted research it is suggested to use the LED emission of blue and violet spectra as a part of a complex antimicrobial therapy of pyoinflammatory processes that to prevent *Pseudomonas aeruginosa* colonization and extension of hospital-related infections.

Thus, *Pseudomonas aeruginosa* biofilms exposure to LED emission of blue and violet spectra promotes inhibition of pathogenicity factors, that according to reference data undergoes by changing the structure and function of DNA, proteins photoinactivation and damage of biomembranes. These processes underlie all photoprocesses occurring at the level of cell. Damage of phospholipids in biomembranes membrane proteins enhances the inactivation of membrane enzymes and as a result enzymes of pathogenicity lose their activity.

Photodestruction of proteins and biological membranes that occurs when exposed to LED emission of blue and violet spectra, causes the development of numerous biological effects that lead to lyses of bacterial cell. Crucial event in the biological action of LED emission is a nucleic acids absorption in the blue and violet regions of the spectrum. The main mechanism is implemented by photolysis of DNA double bond, causing inhibition of the DNAase. Under influence of LED emission of blue and violet spectra on proteins destruction of amino acid residues in the active site of the protein takes place together with affecting on protein conformation [4].

In bacteria influence LED emission of blue and violet spectra leads to the change in the kinetics of cell growth and death. Immediately after irradiation and incubation, the optical density of planktonic cells decreases. The cells that survive are not able to continue to form dense secondary biofilm.

References

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