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Determination of the effect of Ag nanoparticles impregnated in medical polyethylene products together with the LED radiation of the red and violet spectra on the ability of microorganisms to form biofilms and on daily biofilms

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Abstract

The paper is devoted to the study of antimicrobial activity of nanocomposites on the example of low density polyethylene and nonwoven polymeric material based on wool and synthetic polyester fiber, which were impregnated with Ag nanoparticles by the original method, in a complex with LED red (610-760nm) and violet (380-430nm) spectra. Formation of microorganisms' biofilms was studied on the surface of microtiter plates for enzyme-linked immunosorbent assay. It was determined that the studied combinations inhibit the process of formation of biofilms by planktonic cells and disorganize the formed daily biofilms, which can promote the penetration of antibacterial agents.

Key words: biofilm formation; Ag nanoparticles; low-density polyethylene; non-woven polymeric material; LED.

Introduction. To increase the effectiveness of antimicrobial therapy LED radiation of various spectra has been used for topical application on the inflammatory focus for quite a long time. Photobiological effects are known to be dependent on the parameters of LED radiation and are quite diverse and specific.

Studies have been conducted for a long time to determine the antimicrobial effect of LED radiation on microorganisms under the action of various drugs. Thus, on the models of biofilms of antibiotic-resistant clinical strains of purulent-inflammatory causative agents processes *in vitro* the effect of amplification by LED radiation of antimicrobial action was shown, both antiseptics containing edetate disodium and antimicrobial drugs; LED radiation in the orange spectrum has been shown to activate phagocytosis and the ability to form extracellular traps by neutrophils [1]. The synergistic effect of LED radiation and antimicrobial drugs on microorganisms biofilms was revealed and the increase of bactericidal effect of antimicrobial drugs under the action of blue and red spectra of LED radiation was established. In the model of localized purulent-inflammatory process *in vivo* it is proved that the combination of antimicrobial drug and blue and red LED radiation is the most optimal in terms of the rate of inflammatory focus healing, restoration of immune and cytokine status and normalization of morphofunctional state of parenchymal organs and immune system organs [2].

Despite intensive study of the antimicrobial activity of silver and copper nanoparticles, the antimicrobial action of silver and copper nanoparticles impregnated in polymeric materials able to prevent the occurrence of purulent-inflammatory complications has not been reliably established by now.

Scientists study the effect of light, namely blue light in the spectrum of 400-470 nm, as the latest non-antibiotic antimicrobial elaboration which is less harmful compared to UV light [3].

Scientists from New Jersey, USA, studied the effect of visible light on Ag NP. They determined that the antimicrobial activity of Ag NP increases due to the release of ions absorbed by microorganisms [4].

A group of researchers from the Chinese Academy of Sciences demonstrated negative correlation between the size of Ag NP and toxicity in case of *Tetrahymena pyriformis*. The scientists also found that under the action of light radiation for 24 hours there is a decrease in the toxicity of small NP and it comprises $32 \pm 0.7\%$, and in large NP it is $10.6 \pm 5.2\%$. This effect was explained by the fact that light irradiation causes the decrease in the amount of Ag ions release, increase in size and aggregation of NP [5].

The effect of LED irradiation (405 ± 5 nm) against *Salmonella spp.* and *E. coli* O157 was studied: H7. It was determined that LED irradiation reduced the amount of Salmonella by 1.2 log and inhibited the growth of *E. coli*. Scientists have described the mechanism of antibacterial action based on DNA oxidation and loss of membrane functions. [6, 7]

Mechanisms of the action of silver and copper nanoparticles impregnated in polymeric materials are of fundamental interest for understanding the antimicrobial action and is of practical importance for the objective justification of the feasibility of these materials using in medical practice. Therefore, the determination of optimal and effective combinations of LED radiation spectra with antimicrobial agents able to inhibit the formation process of microorganisms planktonic cells and inflammatory processes causative agents is quite relevant.

The aim of the study is to determine the complex effect of Ag nanoparticles impregnated in a catheter made of high pressure polyethylene and in a non-woven material based on wool and synthetic polyester fiber with LED radiation of red and violet spectra on the ability of microorganisms to form biofilms and on daily biofilms.

Materials and methods. The objects of the study were:

1. Microorganisms strains: a). reference strains: *Candida albicans* CCM 885, *Escherichia coli* ATCC 25922 (F50) = NCDC F 50, *Klebsiella pneumoniae* NCTC 5055 = SS B 5055, *Pseudomonas aeruginosa* ATCC 27853 = NCDCF-51, *Staphylococcus aureus* ATCC 25923 = NCDC 25923 = F-49, *Streptococcus pyogenes* IIC (№ 1) = PSK 130001 and clinical strains: *Staphylococcus aureus* (n=16), *Klebsiella pneumoniae* (n=2), *Escherichia coli* (n=12), *Pseudomonas aeruginosa* (n=3), *Streptococcus spp.* (n=3), *Candida albicans* (n=12). b). clinical strains were isolated in children with purulent-inflammatory diseases who were treated in the surgical department of Kharkiv Regional Children's Clinical Hospital № 1 and in persons with mycoses who were examined and treated at Kharkiv City Clinical Dermatological and Venereological Dispensary №5 on the day of hospitalization.

2. Samples: a). non-woven polyester material (a mixture of woven fibers/polyethylene terephthalate (PTP) fibers, STEL-TICKS, Ukraine), impregnated with Ag nanoparticles (conc. 0.060%); b). Commercial low density poly (ethylene) (LDPE, 18103-035, Ukraine) with melting index 2,0 (g / 10 min) (2.16 kg / 190°C) in the form of strands with a diameter 1.8 mm, impregnated by Ag nanoparticles.

Researches of biofilm formation (O'Toole G.A. method) were studied by determining the ability of bacterial strains to adhesion on polystyrene surface in polystyrene tablets [8].

Irradiation *in vitro* was performed by LED sources of red (610 - 760 nm) and violet (380 - 430 nm) radiation of the photonic matrix of the Korobov "Barva-Flex" apparatus containing LED matrix with super-luminescent LEDs (24 units) and power supply according to instructions. Main technical characteristics of the photon matrix are: radiation power of each LED - 5 mW; radiation intensity - 100%; continuous mode, exposure time - 10 minutes [9,10], the temperature was 24°C. Inoculated microorganisms in a polystyrene tablet were placed directly under the visible radiation generator at a distance of 10 sm. for irradiation. The statistical program "Statistica 9.0" (StatSoft Ink., USA) was used to process the results.

The study results. When studying the ability of planktonic cells to form microorganisms biofilms under the action of Ag nanoparticles impregnated into a catheter made of high-pressure polyethylene and LED radiation of red (RLEDR) and violet (VLEDR) spectra, it was found that the optical density of the daily biofilm of *Escherichia coli* experimental stains decreases by 10.5 times under the action of Ag and RLEDR and by 43.3 times under the influence of Ag and VLEDR; in strains of *Klebsiella pneumoniae*: by 19.2 times under the action of Ag and RLEDR and by 51.6 times under the influence of Ag and VLEDR; in strains of *Pseudomonas aeruginosa*: by 17.6 times under the action of Ag and RLEDR and by 46.9 times under the influence of Ag and VLEDR; *Staphylococcus aureus*: by 20.2 times under the action of Ag and RLEDR and by 48.9 times under the influence of Ag and VLEDR; *Streptococcus pyogenes*: by 20.4 times under the action of Ag and RLEDR and by 56.8 times under the influence of Ag and VLEDR; *Candida albicans*: by 17.7 times under the action of Ag and RLEDR and by 33.3 times under the influence of Ag and VLEDR compared to control meanings without the influence of Ag nanoparticles and LED radiation and decrease of optical density of formed by isolates biofilms takes place:

Klebsiella pneumoniae: by 3.5 under the action of Ag and RLEDR and by 9.5 times under the influence of Ag and VLEDR; in *Pseudomonas aeruginosa* strain: by 4.6 under the action of Ag and RLEDR and by 12.4 times under the influence of Ag and VLEDR; *Staphylococcus aureus*: by 6.2 times under the action of Ag and RLEDR and by 48.9 times under the influence of Ag and VLEDR; *Streptococcus pyogenes*: by 15 times under the action of Ag and RLEDR and by 14.9 times under the influence of Ag and VLEDR; *Candida albicans*: by 5.3 times under the action of Ag and RLEDR and by 9.9 times under the influence of Ag and VLEDR compared to Ag nanoparticles influence indices without LED radiation (fig.1).

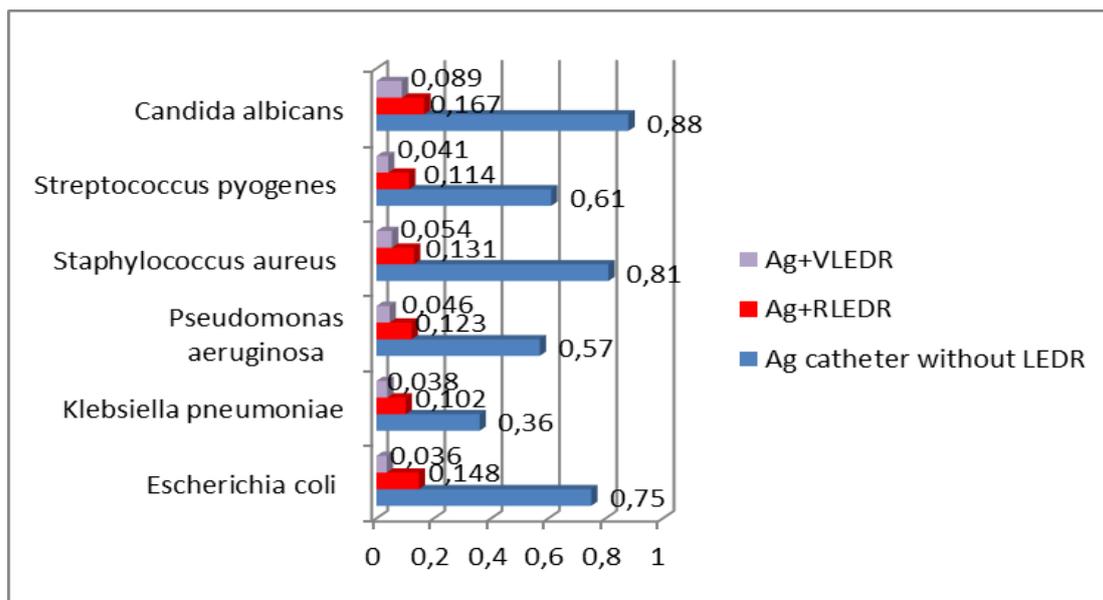


Figure 1. Influence of LED radiation of violet and red spectra with simultaneous action of Ag nanoparticles impregnated in high pressure polyethylene catheter on the ability of experimental strains to form daily biofilms

Microscopic examination revealed that the clinical strains of the experimental microorganisms are located chaotically and in isolated cases sedimentation groups were recorded, with subsequent congregation and formation of small biofilms that do not form dense conglomerates. (fig.2).

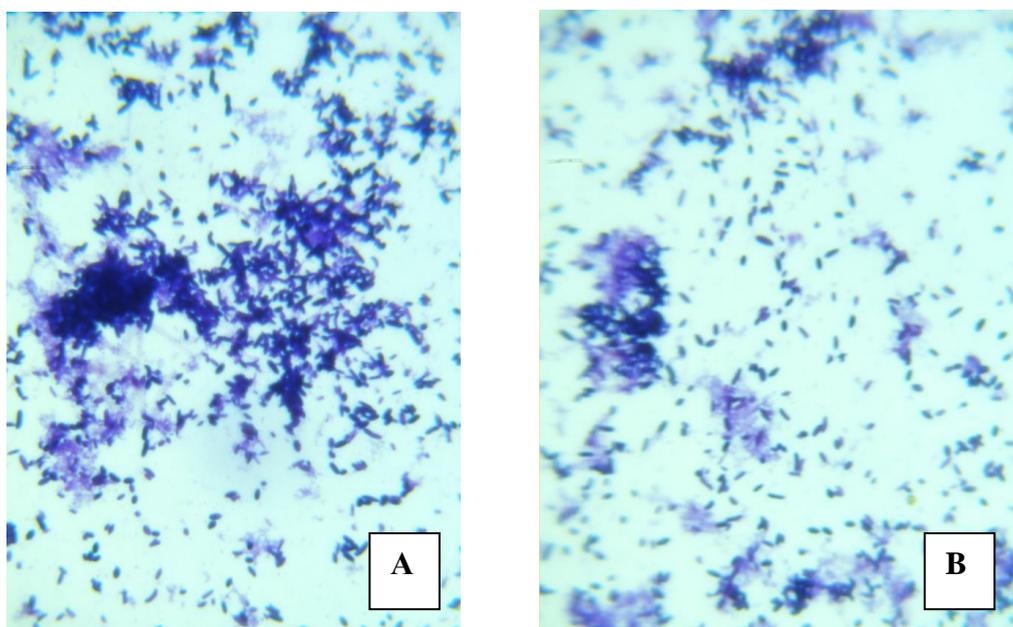


Figure 2. Microscopic study of violet (A) and red (B) spectra LED radiation with simultaneous action of Ag nanoparticles impregnated in high pressure polyethylene catheter on the ability of *Escherichia coli* to form daily biofilms.

When studying the ability of microorganisms planktonic cells to form biofilms under the action of Ag nanoparticles impregnated in non-woven polymer material and RLED and VLED spectra, it was found that the optical density of daily *Escherichia coli* biofilm decreases by 31.8 times under the action of Ag and RLED and by 43.3 times under the influence of Ag and VLED; in strains of *Klebsiella pneumoniae*: by 36.9 times under the action of Ag and RLED and by 57.7 times under the influence of Ag and VLED; in strains of *Pseudomonas aeruginosa*: by 35.4 times under the action of Ag and RLED and by 56.8 times under the influence of Ag and VLED; *Staphylococcus aureus*: by 23.6 times under the action of Ag and RLED and by 62.9 times under the influence of Ag and VLED; *Streptococcus pyogenes*: by 23.8 times under the action of Ag and RLED and by 64.7 times under the influence of Ag and VLED; *Candida albicans*: by 21.3 times under the action of Ag and RLED and by 64.3 times under the influence of Ag and VLED compared to control meanings without the influence of Ag nanoparticles and LED radiation and decrease of optical density of formed by isolates biofilms takes place: *Escherichia coli* - by 4.9 times under the action of Ag and RLED and by 6.8 times under the influence of Ag and VLED; in strains of *Klebsiella pneumoniae*: by 3.4 times under the action of Ag and RLED and by 5.3 times under the influence of Ag and VLED; in strains of *Pseudomonas aeruginosa*: by 6.2 times under the action of Ag and RLED and by 10 times under the influence of Ag and VLED; *Staphylococcus aureus*: by 5.1 times under the action of Ag and RLED and by 13.6 times under the influence of Ag and VLED; *Streptococcus pyogenes*: by 4.7 times under the action of Ag and RLED and by 12.8 times under the influence of Ag and VLED; *Candida albicans*: by 4.8 times under the action of Ag and RLED and by 14.7 times under the influence of Ag and VLED compared to Ag nanoparticles influence indices without LED radiation (fig. 3-4).

Determination of the action of Ag nanoparticles impregnated in a catheter made of high pressure polyethylene simultaneously with the LED radiation of red and violet spectra on the formed daily biofilms of isolates and inflammatory processes causative agents allowed to establish that optical density of *Escherichia coli* experimental strains daily biofilms decreased by 9.7 times under the action of Ag and RLED and by 37.1 times under the influence of Ag and VLED; in strains of *Klebsiella pneumoniae*: by 11.4 times under the action of Ag and RLED and by 42.6 times under the influence of Ag and VLED; in strains of *Pseudomonas aeruginosa*: by 11.7 times under the action of Ag and RLED and by 41.5 times under the influence of Ag and VLED; *Staphylococcus aureus*: by 13.5 times under the action of Ag and RLED and by 36.7 times under the influence of Ag and VLED;

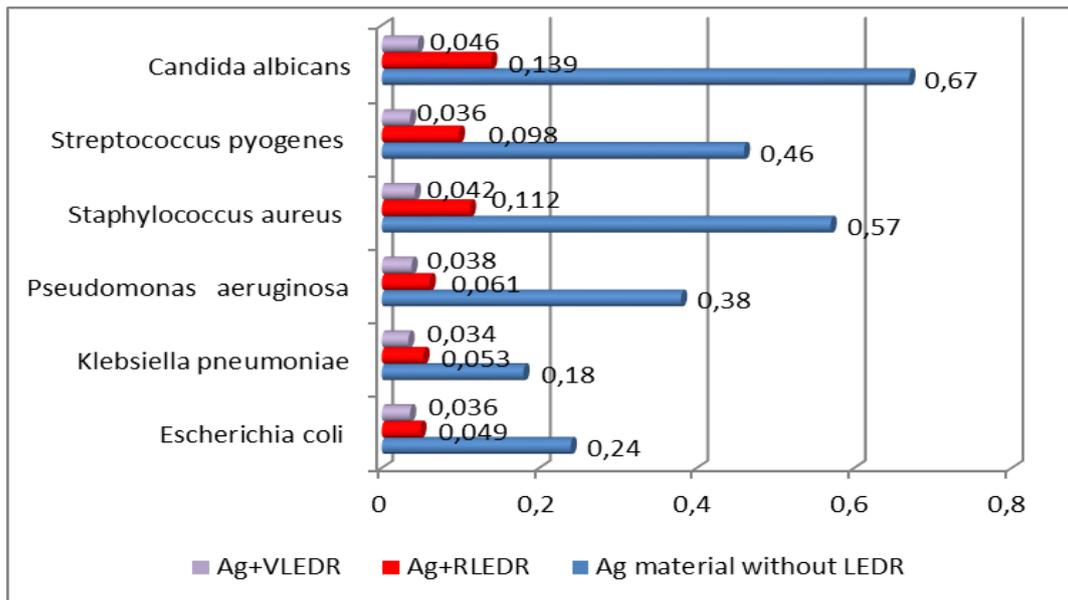


Figure 3. Influence of violet and red spectra LED radiation with simultaneous action of Ag nanoparticles impregnated in non-woven polymeric material on the ability of experimental strains to form daily biofilms

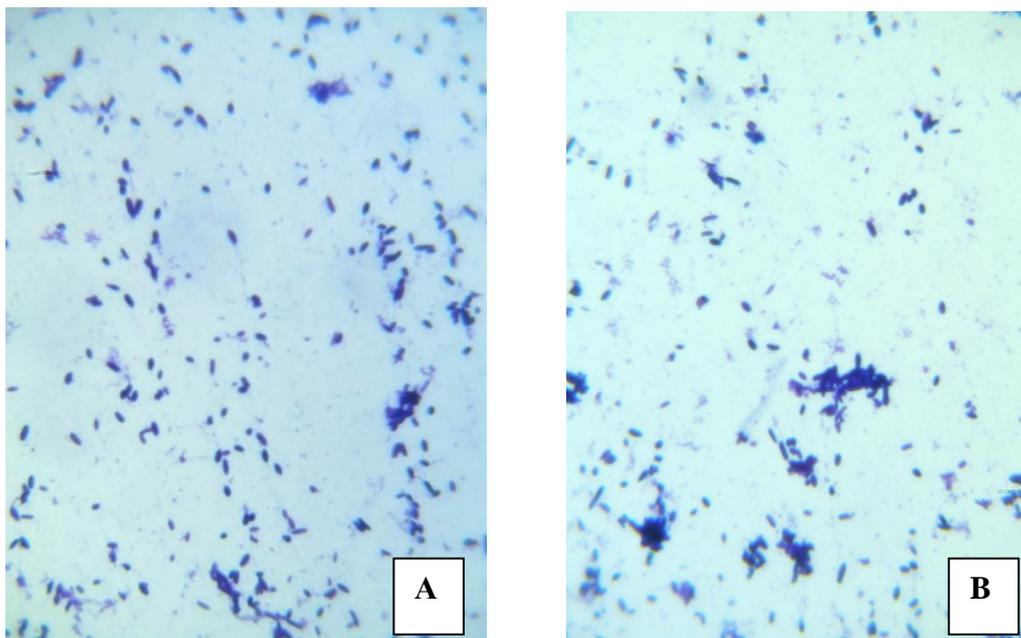


Figure 4. Microscopic study of violet (A) and red (B) LED radiation influence with simultaneous action of Ag nanoparticles impregnated in non-woven polymeric material on the ability of *Escherichia coli* to form daily biofilms

Streptococcus pyogenes: by 14.5 times under the action of Ag and RLEDR and by 36.9 times under the influence of Ag and VLEDR; *Candida albicans*: by 13,3 times under the action of Ag and RLEDR and by 30.2 times under the influence of Ag and VLEDR compared to control

meanings without the influence of Ag nanoparticles and LED radiation and decrease of optical density of formed by isolates biofilms takes place: *Escherichia coli* - by 6.8 times under the action of Ag and RLEDR and by 26.2 times under the influence of Ag and VLEDR; in strains of *Klebsiella pneumoniae*: by 7.2 times under the action of Ag and RLEDR and by 26.7 times under the influence of Ag and VLEDR; in strains of *Pseudomonas aeruginosa*: by 7.9 times under the action of Ag and RLEDR and by 27.9 under the influence of Ag and VLEDR; *Staphylococcus aureus*: by 8.9 times under the action of Ag and RLEDR and by 24.3 times under the influence of Ag and VLEDR; *Streptococcus pyogenes*: by 10.1 times under the action of Ag and RLEDR and by 25.9 times under the influence of Ag and VLEDR; *Candida albicans*: by 8.2 times under the action of Ag and RLEDR and by 18,5 times under the influence of Ag and VLEDR compared to Ag nanoparticles influence indices without LED radiation (fig.5).

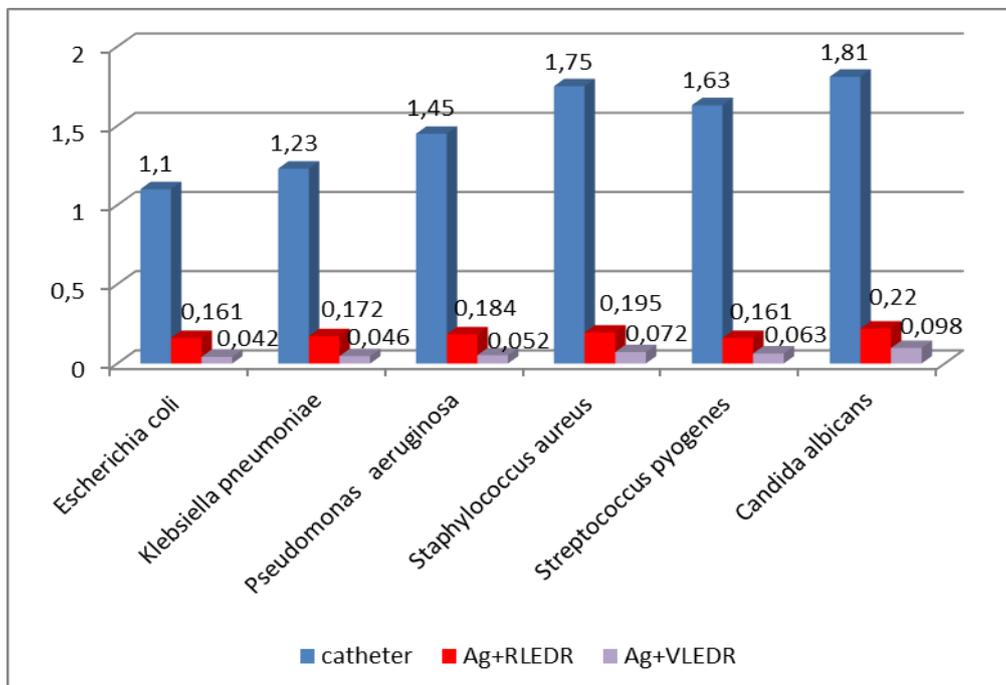


Figure 5. Influence of violet and red spectra LED radiation with simultaneous action of Ag nanoparticles impregnated in high pressure polyethylene catheter and non-woven polymeric material on the ability to destroy daily biofilms

Analysis of the obtained results showed that under the action of LED radiation in combination with Ag nanoparticles impregnated in non-woven polymeric material on the formed daily biofilms of isolates decreases 29.4 times under the action of Ag and RLEDR and 41.1 times under the influence of Ag and VLEDR; in strains of *Klebsiella pneumoniae*: by 21.5 times under the action of Ag and RLEDR and by 47.8 times under the influence of Ag and VLEDR; in strains of *Pseudomonas aeruginosa*: by 25.4 times under the action of Ag and

RLEDR and by 44.1 times under the influence of Ag and VLEDR; *Staphylococcus aureus*: by 15.2 times under the action of Ag and RLEDR and by 40 times under the influence of Ag and VLEDR; *Streptococcus pyogenes*: by 13.8 times under the action of Ag and RLEDR and by 40.2 times under the influence of Ag and VLEDR; *Candida albicans*: by 15.3 times under the action of Ag and RLEDR and by 36.5 times under the influence of Ag and VLEDR compared to control meanings without Ag nanoparticles and LED radiation influence. Microscopic examination revealed that under the action of LED radiation in combination with Ag nanoparticles impregnated in non-woven polymer material, the formed daily biofilms of isolates disorganize daily biofilms with the formation of holes through which the penetration of antimicrobial drugs is possible. (fig.6).

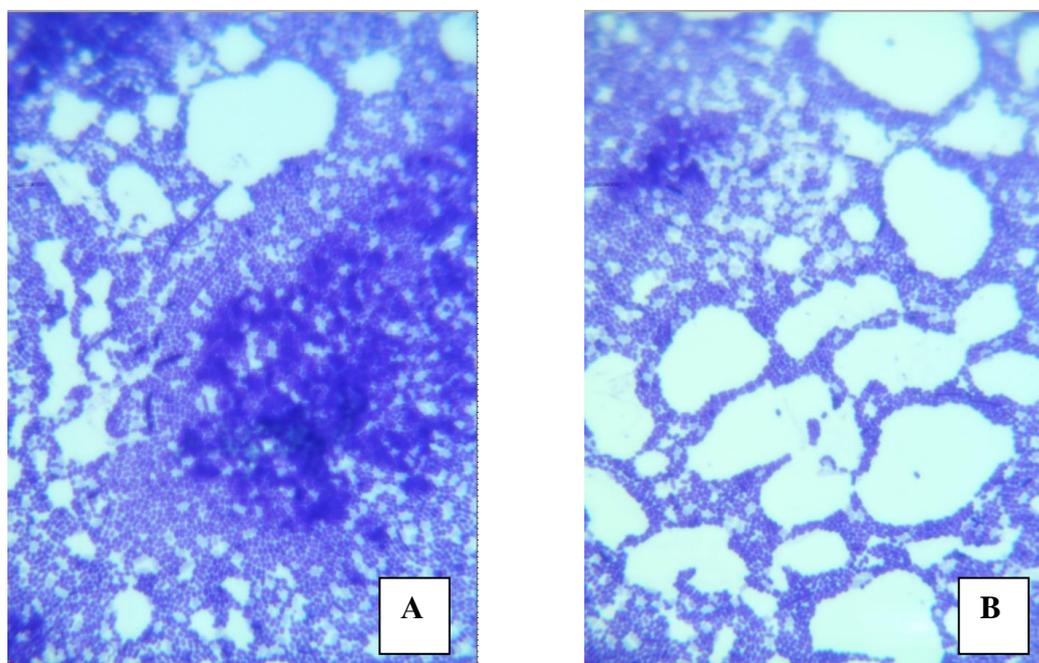


Figure 6. Microscopic study of violet (A) and red (B) LED radiation influence with simultaneous action of Ag nanoparticles impregnated in non-woven polymeric material on daily biofilms of *Staphylococcus aureus* clinical strains

Decrease of formed daily biofilms optical density: *Escherichia coli* - by 18.1 times under the action of Ag and RLEDR and by 25.3 times under the influence of Ag and VLEDR; in strains of *Klebsiella pneumoniae*: by 12.2 times under the action of Ag and RLEDR and by 27.1 times under the influence of Ag and VLEDR; in strains of *Pseudomonas aeruginosa*: by 14.8 times under the action of Ag and RLEDR and by 25.7 times under the influence of Ag and VLEDR; *Staphylococcus aureus*: by 8.7 times under the action of Ag and RLEDR and by 22.9

times under the influence of Ag and VLEDR; *Streptococcus pyogenes*: by 8.3 times under the action of Ag and RLEDR and by 24.3 times under the influence of Ag and VLEDR; *Candida albicans*: by 8.5 times under the action of Ag and RLEDR and by 20.4 times under the influence of Ag and VLEDR compared to Ag nanoparticles without LED radiation influence (fig.7).

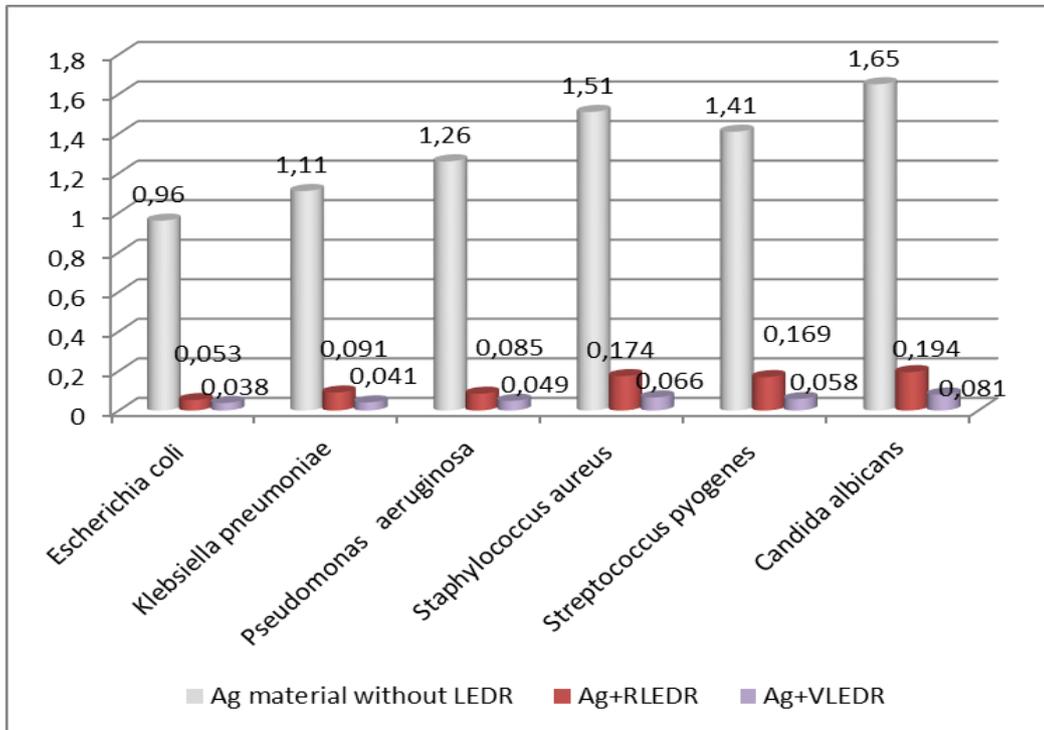


Figure 7. Influence of violet and red spectra LED radiation with simultaneous action of Ag nanoparticles impregnated in nonwoven polymeric material on experimental strains daily biofilms

Data on Ag nanoparticles and LED radiation (LEDR) mechanism of action on bacteria and fungi were systematized and the graphic model was proved on the basis of the conducted researches, analytical review of the newest researches results received by the leading experts of the world concerning influence of Ag on microorganisms (fig. 8).

Thus, based on the literature sources explaining the mechanism of Ag influence on microorganisms cell, special importance is paid on intracellular physico-chemical processes [11], namely: bacteria and fungi protoplasm oxidation and its destruction by oxygen [12]. There are studies proving the formation of nucleic acids complexes with Ag nanoparticles, which causes the disruption of the bacteria stability [13], the number of free radicals increases [14], which leads to inhibition of enzymes and proteins required for ATP production expression and inhibition of respiratory enzymes chain with further separation of the

processes of respiration and oxidative phosphorylation in microbial cells [15], resulting in microbial cell death.

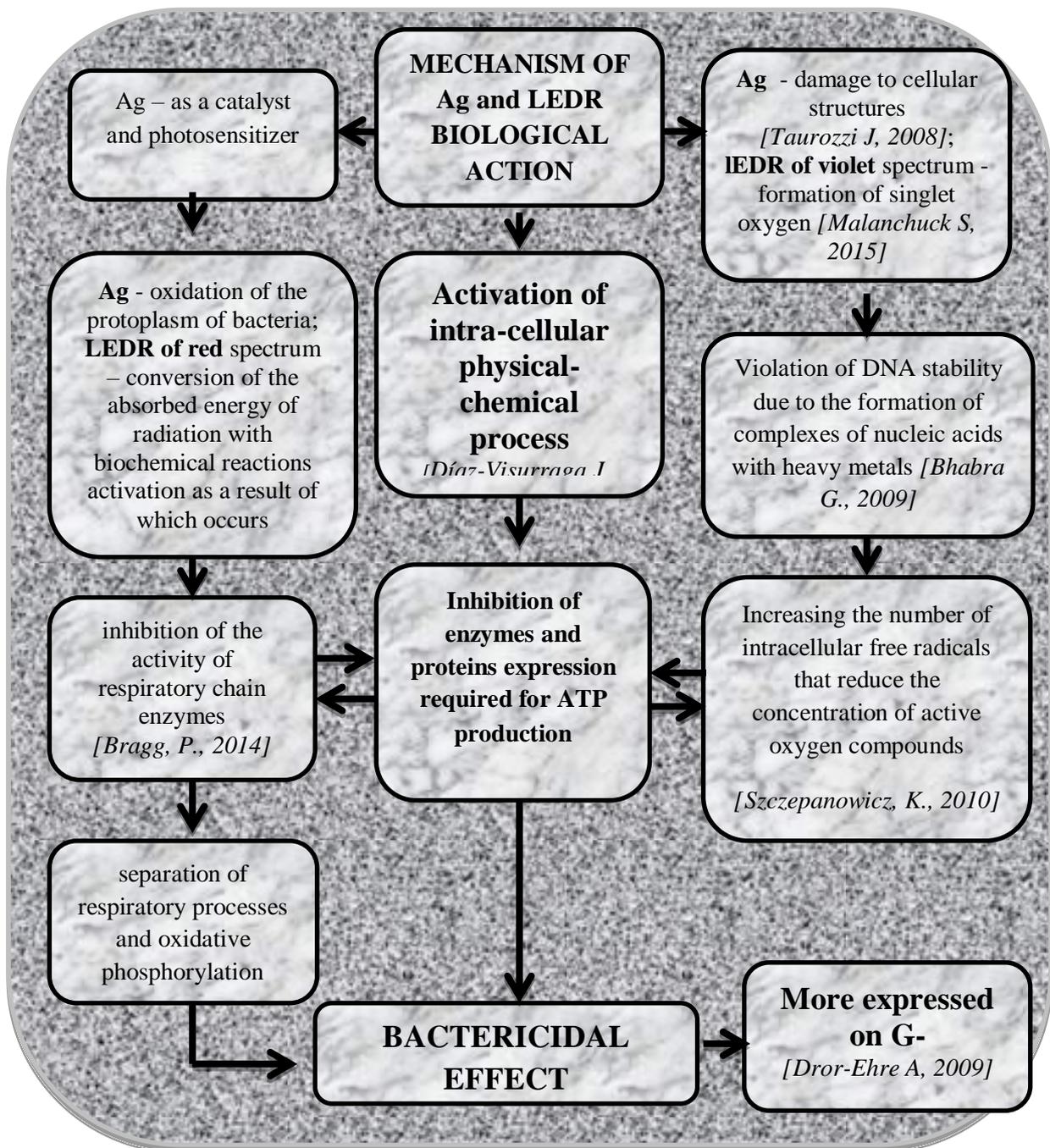


Figure 8. Graphic model of Ag and LEDR influence mechanisms on microorganisms, causative agents of inflammatory diseases

To enhance the antimicrobial effects of Ag nanoparticles LED radiation of the violet and red spectra can be used for effective application of photodynamic therapy, which has recently become one of the new effective antimicrobial techniques. Ag nanoparticles absorbed

by the causative agent of inflammatory processes serve as a photosensitizer and catalyst of biochemical reactions. The photodynamic effect is local in nature and allows avoiding side effects [16].

Thus, the study obtained specific data indicating the feasibility of integrated application of LED radiation of red and violet spectra with Ag nanoparticles impregnated in products for medical use for prophylaxis and photo-dynamic therapy of purulent-inflammatory diseases.

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