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Assessment of the biological effect of Cu nanoparticles impregnated in polyethylene medical devices combined with the effect of 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

For many years scientists around the world have been looking for an alternative to antibiotics due to rapidly growing level of resistance to them. LED light of various spectra and metal nanoparticles are considered as a promising alternative to antibiotics. In this work, we determined the complex effect of copper nanoparticles impregnated in high-pressure polyethylene and non-woven material with the effect of red and violet LED radiation on the ability of microorganisms to form biofilms and on already formed daily biofilms.

Key words: low density poly (ethylene); LED; biofilm formation; Cu nanoparticles.

Introduction. Today the question of finding new compounds with pronounced antimicrobial activity is relevant, which is associated with the ever-increasing resistance of

causative agents of purulent-inflammatory diseases to existing antimicrobial drugs. A promising area is the use of nano-sized copper (Cu) particles with antibacterial activity against bacteria [1] which is of interest for the use of copper nanoparticles in medical devices.

Analysis of the literature shows the prospects for the use of bactericidal activity of Cu nanoparticles, but unlike Ag nanoparticles copper ones have a very low stability due to easy oxidation and therefore they are less studied.

On the other hand, photodynamic inactivation of bacterial and fungal cells by excitation of intracellular porphyrins is of great interest, which leads to the production of cytotoxic active oxygen species [2] and this is a generally accepted hypothesis because mechanism of LED radiation of different spectra is still being studied. [3, 4].

In a joint work of British and German scientists the effect of blue light against *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Enterococcus faecium* та *Klebsiella pneumoniae* isolates was studied. It has been shown experimentally that in 71% of the planktonic form of bacterial existence a decrease in viability at exposure from 15 to 30 minutes was detected. A significant correlation was found between the sensitivity of the planktonic form to light and its ability to biofilm formation, i.e. the most resistant isolates demonstrate the formation of denser biofilms [5]. A group of scientists conducted an experiment *in vivo* on mice simulating burns and infection with a multidrug-resistant strain of *Acinetobacter baumannii*, the lesion focus was irradiated with blue light for 30 minutes and a decrease in bacterial load was determined as well as it was found that after 10 cycles of inactivation with blue spectrum of LED radiation bacteria progressed no resistance [6]. Similar results were obtained by the same scientists in the study of *S.aureus*-MRSA localized infection *in vivo*: blue light contributed to the rapid rate of bacterial load reduction, growth recovery was observed a day after irradiation [7] which requires further research to determine the feasibility of using LED radiation of different spectra in localized purulent-inflammatory lesions. Thus, one of the promising areas in solving this problem is to determine the compatible antimicrobial effects of Cu nanoparticles and LED radiation of the red and violet spectra.

The aim of the study: to determine the complex action of Cu nanoparticles impregnated in a high-pressure polyethylene catheter and in a non-woven fabric based on wool and synthetic polyester fiber, with the action of red and violet LED radiation on microorganisms ability to form biofilms and on daily biofilms.

Materials and methods. The subject of the study was:

1. Strains of microorganisms: 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* ШС (№ 1) = ПСК 130001 and clinical strains: *Staphylococcus aureus* (n=16), *Klebsiella pneumoniae* (n=2), *Escherichiacoli* (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 the Kharkiv Regional Children's Clinical Hospital № 1 and in persons with mycoses who were examined and treated at the Kharkiv City Clinical Dermatological and Venereological Dispensary №5 on the day of hospitalization.

2. Samples: a). non-woven polymeric material (a mixture of woven fibers / polyethylene teraphthalate (PET) fibers, STEL-TICKS, Ukraine), impregnated with Cu nanoparticles (conc. 0,068%). b). Commercial low density poly (ethylene) (LDPE, 18103-035, Ukraine) with melting index 2,0 (r / 10 min) (2,16 kg / 190°C) in the form of strands with a diameter 1,8 mm impregnated by Cu nanoparticles.

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

Irradiation in vitro was performed with LED sources of red (610 - 760 nm) and violet (380 - 430 nm) radiation of the photonic matrix of the Korobov apparatus "Barva-Flex" [9] apparatus containing LED matrix with super-luminescent LEDs (24 units) and power supply according to instruction. The main technical characteristics of a photonic matrix: radiation power of each light-emitting diode - 5 mW; supply voltage - 14 V, radiation intensity - 100%; continuous mode, exposure time - 10 minutes, the temperature was 24^0C. For irradiation, the inoculated microorganisms in a polystyrene tablet were placed directly under the visible radiation generator at a distance of 10 cm. When processing the results the statistical program "Statistica 9.0" (StatSoft Ink., USA) was used.

Results of the study. When studying the ability to form biofilms (Fig. 1) by microorganisms planktonic cells under the action of Cu nanoparticles impregnated in a catheter made of high pressure polyethylene and LED radiation it was found that optical density of the daily biofilm of *Escherichia coli* experimental strains decreases by 9.2 times under the action of Cu and RLEDR and by 28.9 times under the influence of Cu and VLEDR; in strains of *Klebsiella pneumoniae:* by 16.9 times under the action of Cu and RLEDR and by 28/8 times under the influence of Cu and VLEDR; in strains of *Pseudomonas aeruginosa:* by 16.1 times under the action of Cu and RLEDR and by 36.6 times under the influence of Cu and

VLEDR; *Staphylococcus aureus*: by 23.2 times under the action of Cu and RLEDR and by 64.4 times under the influence of Cu and VLEDR; *Streptococcus pyogenes*: by 22.8 times under the action of Cu and RLEDR and by 61.3 times under the influence of Cu and VLEDR; *Candida albicans:* by 22,1 times under the action of Cu and RLEDR and by 44.8 times under the influence of Cu and VLEDR compared to control meanings without Cu nanoparticles and LED radiation influence.

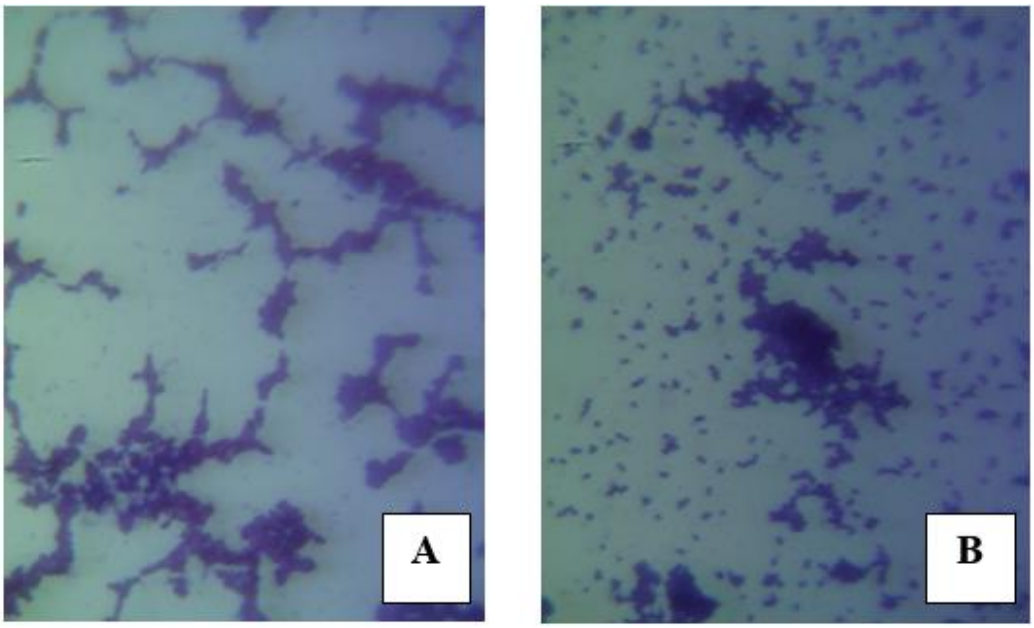

Figure 1. Microscopic study of the influence of LED radiation of violet(A) and red(B) spectra with simultaneous action of Cu nanoparticles impregnated in a high pressure polyethylene catheter on the ability of *Streptococcus pyogenes* to form daily biofilms

There is a decrease in optical density of the formed biofilms by isolates: *Escherichia coli* - by 5.7 times under the action of Cu and RLEDR and by 17.2 times under the influence of Cu and VLEDR; in trains of *Klebsiella pneumoniae:* by 1.4 times under the action of Cu and RLEDR and by 2.4 times under the influence of Cu and VLEDR; in strains of *Pseudomonas aeruginosa:* by 2.6 times under the action of Cu and RLEDR and by 5.9 times under the influence of Cu and VLEDR; *Staphylococcus aureus*: by 3.7 times under the action of Cu and RLEDR and by 10.2 times under the influence of Cu and VLEDR; *Streptococcus pyogenes*: by 3.3 times under the action of Cu and RLEDR and by 8.9 times under the influence of Cu and VLEDR; *Candida albicans:* by 4.3 times under the action of Cu and RLEDR and by 8.6 times under the influence of Cu and VLEDR compared to Cu nanoparticles without LED radiation influence (fig. 2).

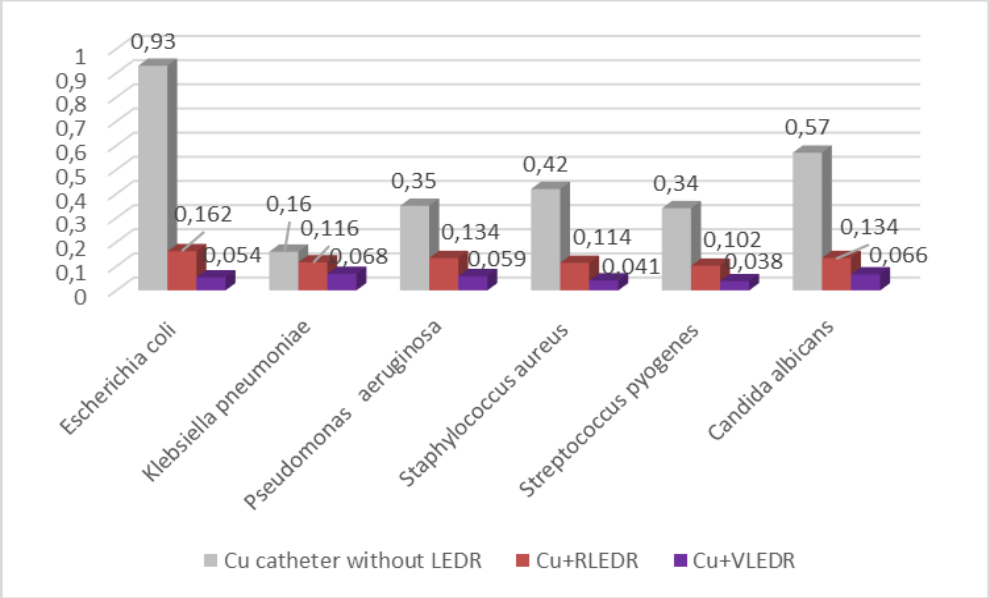

Figure 2. Influence of LED radiation of violet and red spectra with simultaneous action of Cu nanoparticles impregnated in a high pressure polyethylene catheter on the ability of the experimental strains to form daily biofilms

When studying the effect of Cu nanoparticles impregnated in a catheter made of high pressure polyethylene and red LED radiation on the formed biofilms with isolates, it was found that optical density of the daily biofilm of the *Escherichia coli* experimental strains decreases by 7.2 times under the action of Cu and RLEDR and by 9.7 times under the influence of Cu and VLEDR; in strains of *Klebsiella pneumoniae:* by 6 times under the action of Cu and RLEDR and by 9.5 times under the influence of Cu and VLEDR; in strains of *Pseudomonas aeruginosa:* by 8 times under the action of Cu and RLEDR and by 11.3 times under the influence of Cu and VLEDR; *Staphylococcus aureus*: by 21.8 times under the action of Cu and RLEDR and by 41.3 times under the influence of Cu and VLEDR; *Streptococcus pyogenes*: by 19.7 times under the action of Cu and RLEDR and by 40.2 times under the influence of Cu and VLEDR; *Candida albicans:* by 15.3 times under the action of Cu and RLEDR and by 35.7 times under the influence of Cu and VLEDR compared to control meanings without Cu nanoparticles and LED radiation influence and there was the decrease of optical density of formed daily biofilms with isolates: *Escherichia coli* - by 4.2 times under the action of Cu and RLEDR and by 5.7 times under the influence of Cu and VLEDR; in strains of *Klebsiella pneumoniae:* by 3.2 times under the action of Cu and RLEDR and by 5 times under the influence of Cu and VLEDR; in strains of *Pseudomonas aeruginosa:* by 4.9 times under the action of Cu and RLEDR and by 6.9 times under the influence of Cu and VLEDR; *Staphylococcus aureus*: by 12.3 times under the action of

Cu and RLEDR and by 23.3 times under the influence of Cu and VLEDR; *Streptococcus pyogenes*: by 11.7 times under the action of Cu and RLEDR and by 23.8 times under the influence of Cu and VLEDR; *Candida albicans:* by 7.9 times under the action of Cu and RLEDR and by 18.3 times under the influence of Cu and VLEDR compared to Cu nanoparticles without LED radiation influence indices (fig. 3).

When studying the ability to form biofilms by microorganisms planktonic cells under the action of Cu nanoparticles impregnated in non-woven polymeric material and LED radiation it was found that the optical density of the daily biofilm of *Escherichia coli* experimental strains decreases by 13.4 times under the action of Cu and RLEDR and by 17.7 times under the influence of Cu and VLEDR; in strains of *Klebsiella pneumoniae:* by 29.9 times under the action of Cu and RLEDR and by 30.6 times under the influence of Cu and VLEDR; in strains of *Pseudomonas aeruginosa:* by 17.6 times under the action of Cu and RLEDR and by 22.5 times under the influence of Cu and VLEDR; *Staphylococcus aureus*: by 28.1 times under the action of Cu and RLEDR and by 45.5 times under the influence of Cu and VLEDR; *Streptococcus pyogenes*: by 28.8 times under the action of Cu and RLEDR and by 43.1 times under the influence of Cu and VLEDR; *Candida albicans:* by 27.4 times under the action of Cu and RLEDR and by 47.7 times under the influence of Cu and VLEDR compared to control meanings without Cu nanoparticles and LED radiation influence.

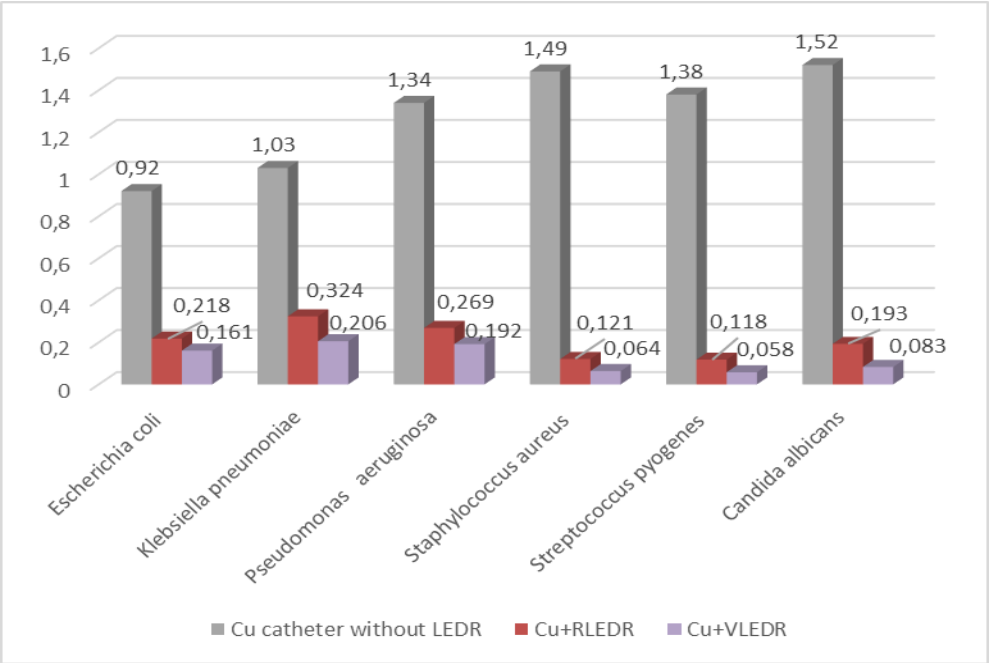

Figure 3. Influence of LED radiation of violet and red spectra with simultaneous action of Cu nanoparticles impregnated in a high pressure polyethylene catheter on the ability of the experimental strains to form daily biofilms

The decrease in the optical density of the formed biofilms was established by isolates: *Escherichia coli* - by 7.4 times under the action of Cu and RLEDR and by 9.8 times under the influence of Cu and VLEDR; in strains of *Klebsiella pneumoniae:* by 1.1 times under the action of Cu and RLEDR and by 1.4 times under the influence of Cu and VLEDR; in strains of *Pseudomonas aeruginosa:* by 2.1 times under the action of Cu and RLEDR and by 2.7 times under the influence of Cu and VLEDR; *Staphylococcus aureus*: by 3.4 times under the action of Cu and RLEDR and by 5.1 times under the influence of Cu and VLEDR; *Streptococcus pyogenes*: by 3.2 times under the action of Cu and RLEDR and by 4.8 times under the influence of Cu and VLEDR; *Candida albicans:* by 4.2 times under the action of Cu and RLEDR and by 7.3 times under the influence of Cu and VLEDR compared to Cu nanoparticles without LEDR radiation influence indices (fig. 4).

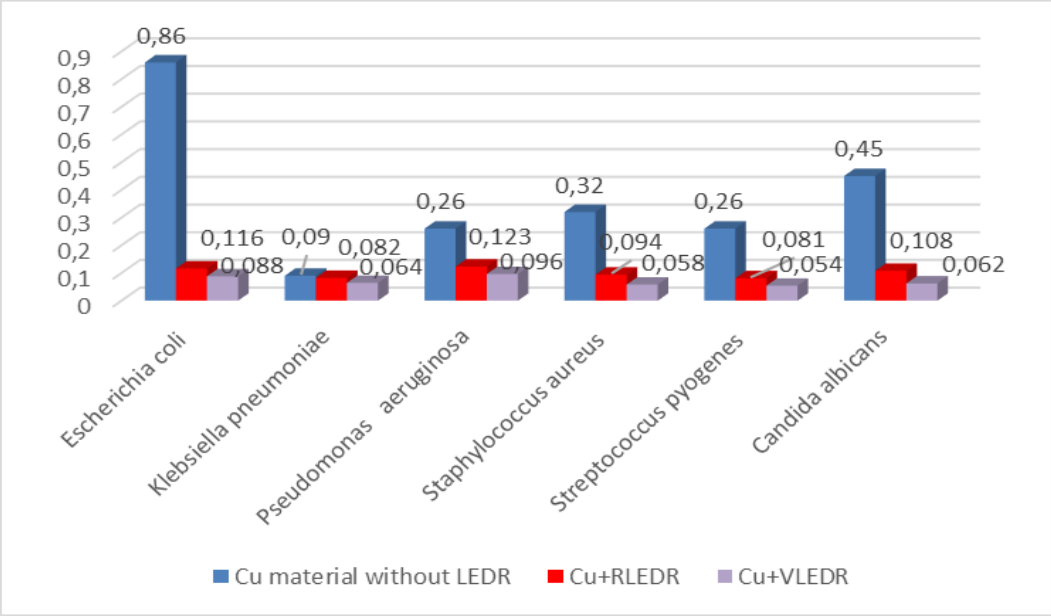

Figure 4. Influence of LED radiation of violet and red spectra with simultaneous action of Cu nanoparticles impregnated in non-woven polymeric material on the ability of the experimental strains to form daily biofilms

Similar results were recorded in determining the effect of Cu nanoparticles impregnated in non-woven polymeric material and the combined effect of LED radiation on isolates daily biofilms. It was stated that optical density of the daily biofilms of the experimental *Escherichia coli* strains decreases by 9.7 times under the action of Cu and RLED and by 11.5 times under the influence of Cu and VLEDR; in strains of *Klebsiella pneumoniae:* by 6.9 times under the action of Cu and RLEDR and by 10.5 times under the influence of Cu and VLEDR; in strains of *Pseudomonas aeruginosa:* by 10.1 times under the action of Cu and

RLEDR and by 13.3 times under the influence of Cu and VLEDR; *Staphylococcus aureus*: by 24.4 times under the action of Cu and RLEDR and by 50.8 times under the influence of Cu and VLEDR; *Streptococcus pyogenes*: by 22.8 times under the action of Cu and RLEDR and by y 52.9 times under the influence of Cu and VLEDR; *Candida albicans:* by 24.1 times under the action of Cu and RLEDR and by 47.7 times under the influence of Cu and VLEDR compared to control meanings without Cu nanoparticles and LED radiation influence. Microscopic examination by scanning electron microscopy revealed the destruction of dense conglomerates that were part of *Candida albicans* isolates biofilms under the action of LED radiation combined with Cu nanoparticles impregnated in non-woven polymeric material (fig.5).

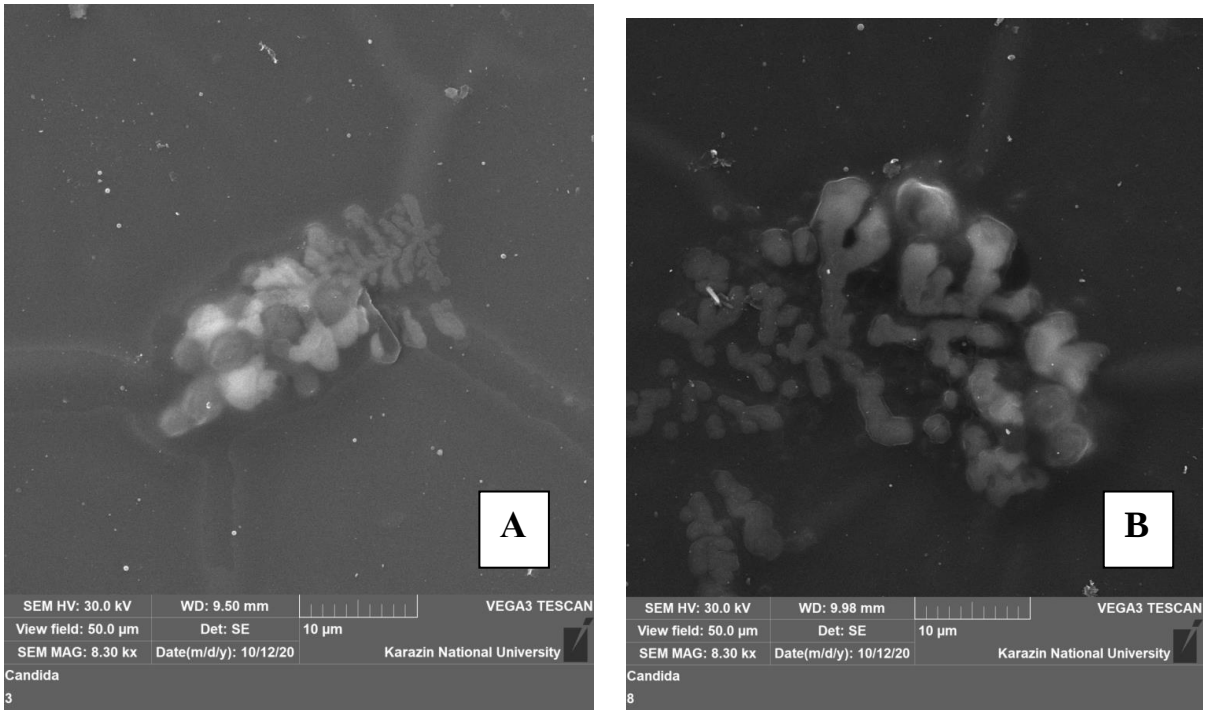

Figure 5. Scanning electronic microscopy of the daily biofilms of *Candida albicans* isolates under LED radiation of violet (A) and red (B) spectra with simultaneous action of Cu nanoparticles impregnated in non-woven polymeric material

There is a decrease in the optical density of the formed biofilms by isolates: *Escherichia coli* - by 4.3 times under the action of Cu and RLEDR and by 6.3 times under the influence of Cu and VLEDR; in strains of *Klebsiella pneumoniae:* by 3.5 times under the action of Cu and RLEDR and by 5.2 times under the influence of Cu and VLEDR; in strains of *Pseudomonas aeruginosa:* by 4.6 times under the action of Cu and RLEDR and by 6.1 times under the influence of Cu and VLEDR; *Staphylococcusaureus*: by 10.4 times under the action of Cu and RLEDR and by 21.5 times under the influence of Cu and VLEDR; *Streptococcus pyogenes*: by 10 times under the action of Cu and RLEDR and by 23.2 times under the influence of Cu and

VLEDR; *Candida albicans:* by 9.3 times under the action of Cu and RLEDR and by 18.4 under the influence of Cu and VLEDR compared to Cu nanoparticles influence without LED radiation indices (fig. 6).

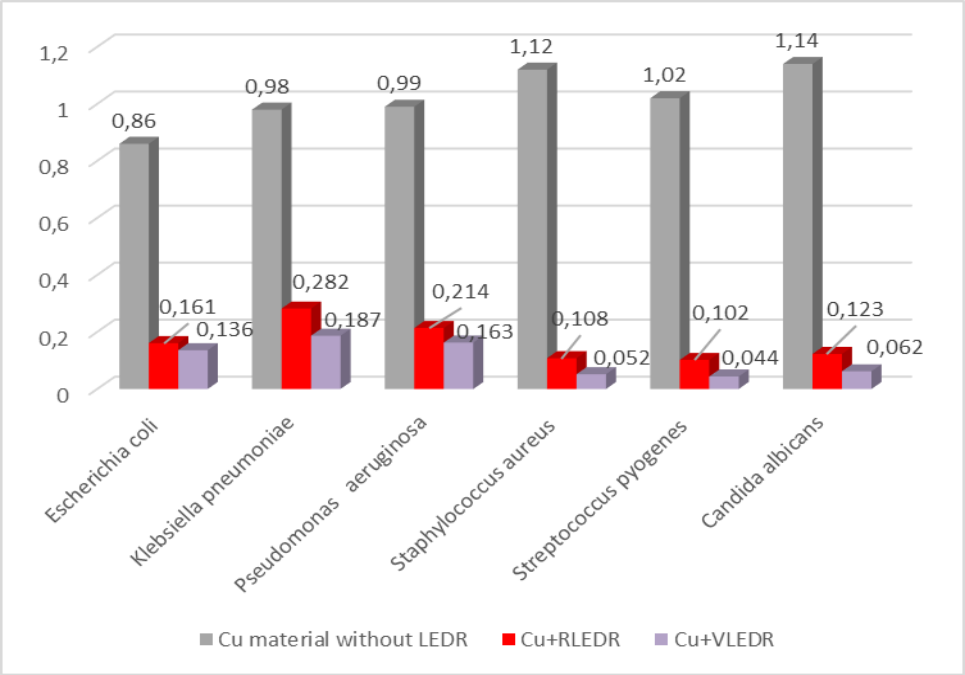

Figure 6. Influence of LED radiation of violet and red spectra with simultaneous action of Cu nanoparticles impregnated in non-woven polymeric material on formed biofilms

It should be noted among the molecular mechanisms mentioned by various researchers [10, 11] that the structure of proteins changes under the influence of Cu nanoparticles and as a result they can not perform their functions, resulting in microorganisms inactivation. Cu nanoparticles increase the activity of free radicals causing the appearance of holes in the cell membranes of bacteria and fungi, thereby disrupting the integrity of cells and cause leakage of basic solutes from the microbial cell [12].

Copper is proved to damage the respiratory chain in bacterial cells, which is associated with impaired cellular metabolism. It has been found that excess copper can affect microbial proteins and microbial enzymes, thereby inhibiting their activity [13]. These potential mechanisms are the subject of constant study by professional researchers around the world.

Thus, the efficiency of complex application of red and violet spectra LED radiation with Ag and Cu nanoparticles impregnated in a catheter made of high pressure polyethylene and in a non-woven polymeric material is proved which reduces the ability to form daily biofilms by microorganisms clinical strains, causative agents of inflammatory processes and destruction of formed biofilms.

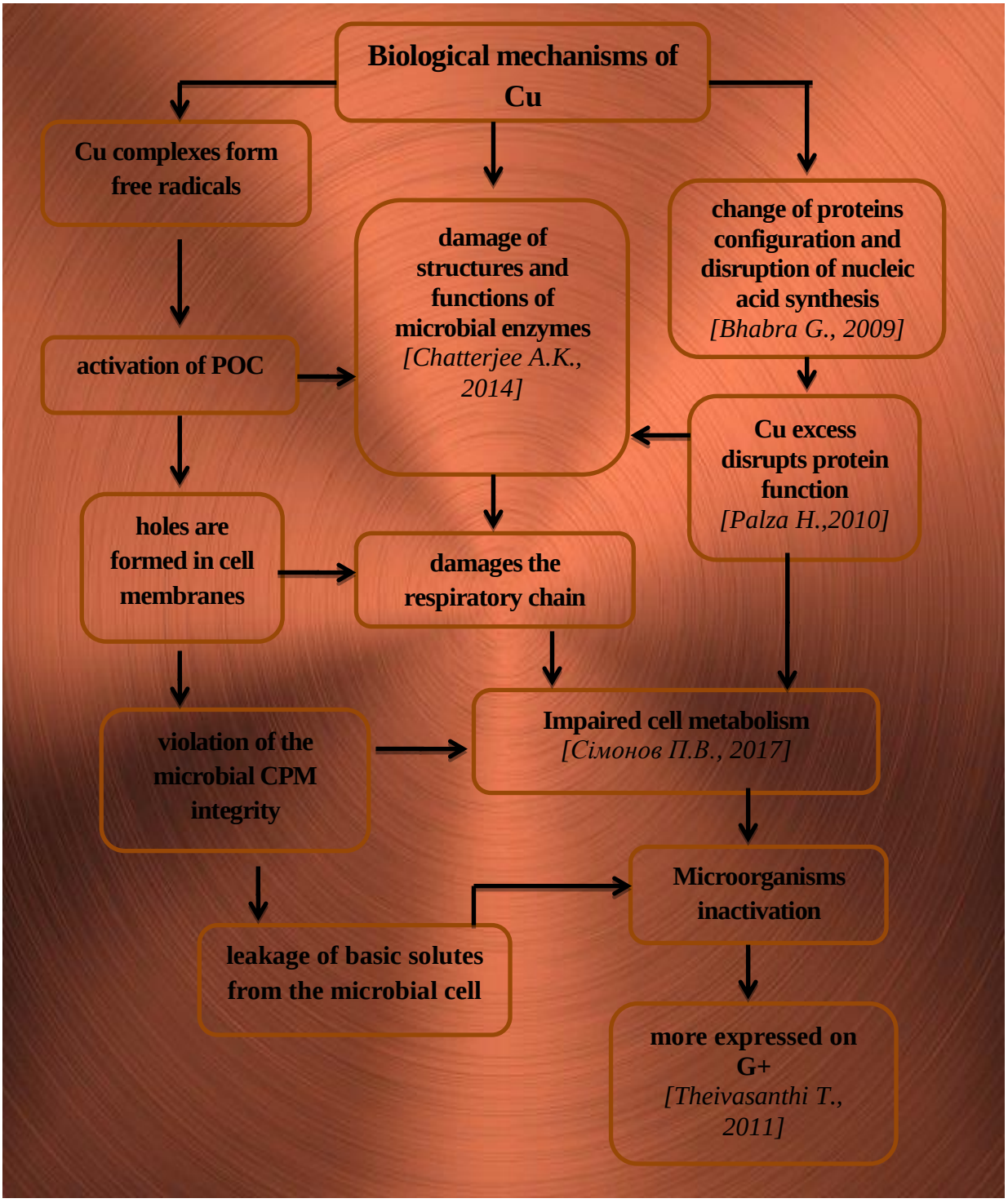

Figure 6. Graphic model of Cu and LEDR mechanisms of influence on microorganisms, causative agents of inflammatory diseases

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