Original Article

Sanitary and hygienic assessment of the dressing material modified with silver nanoparticles

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ABSTRACT

Increasing the antibacterial resistance of microorganisms stimulates the search for new antibacterial drugs. One of the significant advantages of using silver-containing drugs in the treatment of patients is a limited number of side effects when applied topically. In this scientific work, the sanitary and hygienic properties of the dressing material modified with silver nanoparticles were studied. The work used strains of gram-negative microbes (Moraxella spp., Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia, Acinetobacter baumannii, Salmonella enteritidis), gram-positive microbes (Staphylococcus haemoliticus, Staphylococcus aureus) and fungi of the genus Candida (Candida spp.). The reaction of the strains was analyzed by these microorganisms on the main antibiotics widely used in clinical practice in Russia. During the work, it was found that the dressing material modified with silver nanoparticles has high antimicrobial and antifungal activity. In addition, it was found that the effect of silver nanoparticles does not directly depend on the level of sensitivity of the microorganism to antibiotics.

Keywords: Antibacterial effect, Antifungal effect, Dressing material, Silver nanoparticles

Introduction

An increase in the antibacterial resistance of microorganisms is an alarming sign in modern clinical practice. This stimulates the search for new antibacterial drugs [1, 2]. In particular, it is proposed to place substances with high sanitary, hygienic, and antimicrobial effects (antiseptics, antibiotics, sulfonamides, nitrofurans, iodine, etc.) on dressings, and medical devices [3, 4]. So, the primary task is to discover new substances and components that effectively suppress the viability of both Gram-positive and Gram-negative microorganisms, as well as fungi.

The search for new antibacterial and antifungal materials is currently one of the intensively developed areas, which is being created with the cooperation of physicians, chemists, and materials scientists. Preparations containing silver ions are used due to their antimicrobial action, but they have not been widely used in purulent surgery. First of all, this is because such compounds are not effective enough for the local treatment of purulent wound infections.

The use of metallic silver as an antimicrobial agent has been known since ancient times [5-7]. Silver nitrate solutions have been used since the 19th century to treat burns and infections until the first production of silver sulfadiazine ointment [8].

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One of the significant advantages of using silver-containing drugs in the treatment of patients is a limited number of side effects when applied topically [9]. These include silver intoxication or argyrosis, characterized by irreversible pigmentation of the skin, which takes on a silvery or bluish-gray hue [10].

Argyrosis is a dermatological disease that occurs due to the increased intake of silver ions into the body and its subsequent deposition in the skin and its appendages. The main symptom of the disease is the darkening of the skin and mucous membranes up to a bluish-gray shade, in severe cases visual impairment is also observed. There is no treatment for this disease, you can only slightly lighten the skin using the method of medical cosmetology [10].

Silver ions and their colloidal solutions have bactericidal, antiviral, antifungal, and antiseptic effects against more than 500 pathogenic microorganisms, yeast fungi, and viruses. Their antimicrobial effect is slightly stronger than that of penicillin, biomycin, and other antibiotics due to their inhibitory effect. However, silver is a heavy metal. Drinking water with silver ions is not worth it. With prolonged oral administration of silver preparations, neurological disorders and the deposition of diffuse silver in the internal organs are noted. In addition, when exposed to large amounts of silver, kidney damage and foundry fever develop [11]. At the same time, the safety of silver for human skin has been proven [12].

The development of nanotechnology opens up new prospects for the use of silver and its compounds in medicine. It has been proved that metals converted to a nanostatic state exhibit their inherent properties more vividly or acquire new ones. Thus, the effect of silver nanoparticles on microorganisms and fungi differs significantly from the nature of the impact of a compact metal. We can talk about a significant increase in bactericidal or fungicidal efficacy [13, 14].

It has been established that silver nanoparticles exhibit unusual physical, chemical, and biological properties [15]. They are used as photosensitive components, catalysts, and for physicochemical analyses [16].

It can be expected that the use of silver nanoparticles for the treatment of purulent wounds will be promising vectors for the development of nanomedicine in the coming years. The application of metal nanoparticles to dressings or other carriers prevents their aggregation and makes it possible to obtain a homogeneous material with a set of specified properties [17, 18].

One of the environmentally friendly and effective methods for obtaining mono- and bimetallic nanoparticles and materials based on them is the method of metal-vapor synthesis [19]. Previously, it was proved that silver-containing composite materials for medical purposes can be obtained by metal-steam synthesis [20]. It was assumed that metal-steam synthesis would also be effective for the preparation of wound coatings of natural or synthetic origin modified with silver nanoparticles.

In this regard, the widespread introduction of dressings containing silver nanoparticles can play a significant role in improving the results of treatment of purulent wounds in an era of increasing antibiotic resistance of microorganisms.

The purpose of this study was to study the sanitary and hygienic properties of silver nanoparticles, namely, to determine the antibacterial and antifungal effect of a new dressing material based on a medical gauze bandage containing silver nanoparticles obtained by metal-vapor synthesis.

Materials and Methods

A medical gauze bandage (GOST 1172-93) produced by Navtex LLC, Russia was used in the work. The use of silver nanoparticles in medical practice requires the use of methods for the synthesis of "green" chemistry, excluding reagents harmful to the environment. In addition, particles used in medicine must be hydrophilic, as they are used in an aqueous environment. Silver nanoparticles up to 10 nm in size are capable not only of adsorbing on the cell membrane but also of penetrating bacteria. To obtain silver nanoparticles, the Keri-Lee method was used in the work, where ferrous sulfate plays the role of an Ag^{+} reducing agent, and sodium citrate stabilizes the formed particles. The reaction is carried out at room temperature. The growth of particles occurs, presumably, by an aggregate mechanism, and the higher the mixing rate of the solution, the lower the aggregation of nanoparticles and the greater the monodispersity of the solution. Sols obtained by the Keri-Lee method are superior in their characteristics to conventional silver citrate sols. The disadvantage of the method is the use of high concentrations of reagents in the classical synthesis scheme. This leads to the need for a series of sequential cycles of precipitation by centrifugation and redispersion of metal particles.

A nanocomposite material based on a medical gauze bandage and silver nanoparticles was studied by X-ray diagnostics methods - X-ray photoelectron spectroscopy (XPS), small-angle X-ray scattering (SAXS) and X-ray absorption spectroscopy (EXAFS/XANES) using synchrotron radiation.

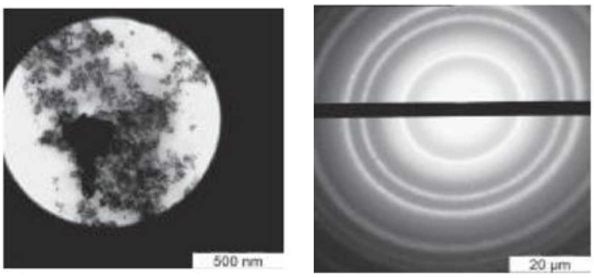
The results of EXAFS/XANES on the K-edge of silver showed that metal nanoparticles are present in the silver/bandage sample, in which the charge state and interatomic metal-metal distances differ insignificantly from massive silver. Surface analysis by the XPS method showed that the binding energy of the Ag $3d_{5/2}$ peak is 368.8 eV, which is 0.5 eV more than that of massive silver. This is due to the dimensional effect and the presence of metal nanoparticles in the material.

The size and size distribution of nanoparticles in the samples were estimated based on SAXS data. For a sample of 0.24% (wt.) Ag/bandage, an asymmetric distribution was obtained with a maximum of 3.5 nm (particle radius) and a wide tail towards large sizes - up to 40 nm. This maximum indicates the existence of a distance between metal particles, which is 7.6 nm, which is close to the calculated doubled particle radius. This indicates that silver nanoparticles appear to form pairs or even small fragments of tightly packed ensembles.

The analysis of silver organosol in isopropanol used in the modification of the bandage, performed by transmission electron microscopy, showed that metal nanoparticles after removal of the organic reagent are aggregates in the form of a "bunch of grapes" consisting of individual elements with an average size of 8-12 nm **(Figure 1a)**.

The photoelectronic spectra were captured on an XSAM-800 spectrometer manufactured by Kratos (Great Britain). A magnesium anode with a characteristic radiation energy of 1253.6 eV was used as an excitation source.

Microscopic studies were performed on an LEO 912AB OMEGA transmission microscope, Zeiss (Germany) **(Figure 1b)**.



a) b)

Figure 1. a) TEM micrograph of silver organzole in isopropanol; b) Diffractogram of silver nanoparticles in isopropanol

The work used strains of gram-negative microbes (*Salmonella enteritidis, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Acinetobacter baumannii, Moraxella spp.*), gram-positive microbes (*Staphylococcus aureus, Staphylococcus haemoliticus*) and fungi of the genus Candida (*Candida spp.*). All of them were obtained from purulent wounds in surgical patients branches of Rostov-on-Don, Russia.

Wound samples were taken from patients of the Rostov-on-Don City Hospital (Russia) with purulent wounds for microbiological examination. Standard disposable sterile swabs were used to take samples. A pure culture of the microbe was isolated in the microbiological laboratory, and the sensitivity of each microorganism to antibiotics and antifungal drugs was determined. The sensitivity of bacteria to the six most commonly used antibiotics (amoxicillin, cephalexin, gentamicin, ciprofloxacin, cefazolin, erythromycin) is presented in **Figure 2**.

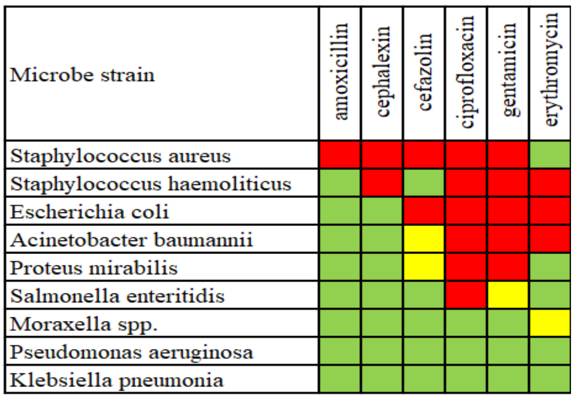


Figure 2. Antibacterial sensitivity of microbial strains used in research, [green] - the microorganism is resistant to this antibiotic, [yellow] - there is a moderate resistance of the microorganism to this antibiotic, [red] - the microorganism is sensitive to this antibiotic

The determination of antibacterial and antifungal sensitivity was carried out by diffusion into agar using discs.

The *Candida spp.* fungus used in the study was sensitive to amphotericin B and fluconazole, resistant to itraconazole, nystatin and ketoconazole.

Then the isolated culture of the microbe was sown on a mown meat-peptone agar, after daily cultivation, flushing with a sterile 0.85% NaCl solution (5 ml) was carried out, and dilution to the desired concentration with the same solution by sequentially sowing on Petri dishes with agar of different concentrations of the microorganism. The required concentration corresponded to the formation of a 0.1 ml microbe suspension after seeding with a measuring pipette and placing a Petri dish in a thermostat for 24 hours of about 100 colony-forming units (CFU). The following concentrations were used in the study: 0.5×10^{-5} for *S. haemoliticus* and *A. baumannii*, 1×10^{-5} for *Candida spp.*, 0.5×10^{-6} for *S. aureus*, *S. enteritidis, P. mirabilis, K. pneumonia* and *E. coli*, for *Moraxella spp.* 1×10^{-6} and for *P. aeruginosa* 1×10^{-7}.

0.1 ml of the resulting suspension of microorganisms was seeded onto a Petri dish with meat-peptone agar. Then 2 strips of gauze medical bandage, measuring 1.5 × 4 cm, were placed on each cup. A standard medical gauze bandage was used as a control, and a medical gauze bandage containing silver nanoparticles was used in the experimental groups. After that, all Petri dishes were placed in a thermostat at a temperature of 37.0 ℃ for a day for cultivation. After 24 hours, colony-forming units were counted in both directions from the edge of the bandage at a distance from the edge equal to the diameter of one colony of ad oculus using a binocular magnifier.

Statistical processing of the results was carried out using the Statistica 6.0 program.

Results and Discussion

The number of pathogenic microorganisms studied was calculated for a standard sterile dressing and a sterile dressing modified with silver nanoparticles. The measurement results are shown in **Table 1**. The number of repetitions is n=12.

Table 1. The number of colony-forming units of the studied microorganisms along the edge of the bandage at a distance on both sides from the edge is equal to the diameter of one colony.

Microbe strain	Control (regular bandage)	A bandage containing silver nanoparticles
Staphylococcus aureus	7.4 ± 0.32	0.8 ± 0.02
Staphylococcus haemoliticus	11.2 ± 0.54	6.8 ± 0.24
Salmonella enteritidis	15.8 ± 0.75	1.2 ± 0.05
Proteus mirabilis	5.2 ± 0.24	1.4 ± 0.06
Pseudomonas aeruginosa	4.9 ± 0.19	2.1 ± 0.09
Klebsiella pneumonia	6.4 ± 0.28	2.6 ± 0.12
Escherichia coli	15.8 ± 0.69	6.8 ± 0.28
Acinetobacter baumannii	9.6 ± 0.42	4.7 ± 0.21
Moraxella spp.	7.4 ± 0.39	3.2 ± 0.14

Candida spp.	15.7 ± 0.62	2.3 ± 0.11

It was noticed that the data of the control groups of different strains differ. Therefore, to compare the antibacterial effect of a bandage containing silver nanoparticles against various microorganisms, a percentage reduction indicator was calculated. To identify a possible relationship between antibiotic resistance and resistance to silver nanoparticles, in **Figure 3** we also displayed the number of antibiotics (out of six studied) to which each strain is resistant and calculated the correlation coefficient between these values and the percentage reduction values. A graphical representation of the results of a percentage decrease in the number of colony-forming units relative to the control and the number of antibiotics to which the strain is resistant are shown in **Figure 3**.

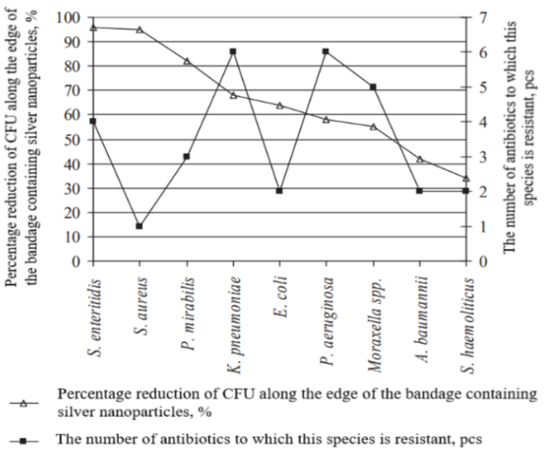


Figure 3. Percentage decrease in the number of colony-forming units relative to the control and the number of antibiotics to which this species is resistant

Figure 3 shows that the maximum percentage reduction and, consequently, the maximum antibacterial effect was observed in *S. enteritidis, S. aureus,* and *P. mirabilis* strains (>81%), the weakest effect was in relation to *A. baumannii* and *S. haemoliticus*. The correlation analysis of the curves of the percentage decrease in the number of colony-forming units and the number of antibiotics showed that there is no statistically significant correlation between them (the correlation coefficient is -0.031). Therefore, there is no connection between antibiotic resistance and resistance to silver nanoparticles, and silver nanoparticles represent a worthy alternative to modern antibacterial drugs.

It should be emphasized that in seven of the nine bacterial strains, the percentage decrease in CFU was more than 50%, and in the remaining two - more than 33%. For *Candida spp.*, the percentage decrease was 86%, while all indicators were statistically significant. This indicates a pronounced antibacterial (antifungal) effect of a medical gauze bandage containing silver nanoparticles.

The mechanism of antibacterial action of silver nanoparticles has not been definitively established.

The studied bacterial strains can be divided into two large groups: gram-positive and gram-negative. The difference between them lies in the structure of the cell wall. If we assume that silver nanoparticles affect only the cell wall, then the studied dressing material should have shown a more pronounced antibacterial effect in relation to any one group, which is not observed. Thus, representatives of the genus *Staphylococcus* in **Figure 3** (percentage decrease in CFU) took one of the first (*S. aureus* - 95%) and the last place (*S. haemoliticus* - 34%). Therefore, it is impossible to explain the effect of silver nanoparticles only by acting on the cell wall.

A medical gauze bandage containing silver nanoparticles suppressed not only the growth of bacterial colonies but also fungal ones, which was demonstrated by the example of *Candida spp.* This shows significant advantages of using silver nanoparticles compared to the traditional combined use of antibacterial and antifungal drugs.

Silver nanoparticles have the following bactericidal properties: high antibacterial activity against a wide range of pathogens, including those with multidrug resistance; moderate fungicidal activity; and antiviral activity.

In moderate doses, silver nanoparticles do not exhibit pronounced acute and chronic toxicity in various routes of entry, and do not have mutagenic, carcinogenic, neurotoxic, and embryotoxic effects.

The antibacterial properties of silver nanoparticles make it possible to use them in the treatment of bacterial-infected wounds, burns, ulcers, and inflammations, as well as other diseases of the mucous membranes and skin of bacterial etiology [21]. Silver (Ag^+) cations bind strongly to groups of electron donors in biological molecules containing silver, oxygen, or nitrogen. The cell surface of a pathogenic microorganism contains a large number of sulfur-containing proteins. Interacting with them, silver ions have an overwhelming effect on the bacterial cell [22, 23]. At the same time, it is impossible to explain the mechanism of action of silver nanoparticles only by the release of silver ions from them. The reason is that the silver nanoparticles themselves have different physical, chemical, and other properties than the solid silver from which they are obtained [24, 25].

Several authors suggest that the antibacterial effect of nanoscale silver particles is due to the electrostatic interaction between negatively charged bacterial cells and positively charged nanoparticles [26].

Conclusion

The nanocomposite material silver+bandage has pronounced antimicrobial activity against both pathogenic microorganisms and fungi. It was found that the viability of the studied bacteria is largely suppressed by the action of silver nanoparticles. Thus, it can be argued that the level of sensitivity of microorganisms to silver nanoparticles does not directly depend on their sensitivity to the most common antibiotics.

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Conflict of interest: None

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Ethics statement: All studies of purulent wound samples were conducted with the written consent of patients and their attending physicians.

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