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Bactericidal, fungicidal and virucidal properties of nanosilver. Mode of action and potential application. A review

Właściwości bakterio-, grzybo-, wirusobójcze nanosrebra.
Mechanizmy działania i potencjalne zastosowanie. Praca przeglądowa

Summary. Silver has long been known to show antibacterial activity but it was only the development of the nanotechnology which allowed to create substances and materials of the new generation. Preparations with embedded silver nanoparticles exhibit even stronger biocidal effects against a wide spectrum of harmful microorganisms, i.e. bacteria, fungi and viruses (even HIV). Multidirectional activity of nanosilver compromises the induction of microbial defensive mechanisms and stops the development of bacterial resistance. Nanostructured silver damages the cell structure, affects energy metabolism and the genetic material of microorganisms. The nanosilver parameters are dependent on its shape, size and an engineering mode. Nanoscale silver bound to solid surface has been incorporated in wound dressings as it was demonstrated to reduce wound bioburden, prevent or treat local infection. The studies are also conducted on silver nanoparticles application in implantology and tissue engineering as well as in food industry for disinfection of air and food production areas. The evidence-based scientific assessment of nanosilver highlights its relatively low toxicity to humans.

Key words: nanosilver, disinfection, toxicity

INTRODUCTION

Nanotechnology proves to be a field of growing scientific interest as the properties of engineered nanomaterials can be utilized in a broad spectrum of applications. Today, disinfectants used for human hygiene purposes make an essential component of human health care approach as they aim to remove and destroy harmful and undesirable microorganisms. These activities are absolutely imperative not only in medicine and public health care but in food industry and the related branches as well. However, there is a growing concern about increasing resistance of some microorganisms to disinfecting agents, especially those used in healthcare settings. Therefore, the current research efforts focus on developing novel disinfectants of chemically stable properties and high biocidal effectiveness. Silver has been well recognized for its antimicrobial capacity but only the development of nanotechnology facilitated creating substances and materials of the new generation. Preparations incorporating nanosilver demonstrate strong inherent anti-bacterial activity towards a wide range of harmful microorganisms, i.e. bacteria, fungi and viruses.

Methods of nanosilver synthesis and stability

Nanosilver is metallic silver whose particles range between 1 and 100 nm in size. Its molecules, subject to a chosen synthesis method, can show different structural architecture, from oval, triangular, hexagonal shape to nanowire forms [Panigrahi *et al*. 2004]. The most popular chemical method to obtain nanosilver is the reduction of silver salts with a strong reducing agent; overall the procedures are easily performed and cost-effective. The most common source of silver atoms are inorganic salts like, silver nitrate (AgNO3), silver chlorate (AgClO4) or silver tetrafluoroborate (AgBF4) [Malina *et a*l. 2010]. Example reducing agents include sodium citrate, ascorbic acid, ethanol or glucose. Changing the reagent concentration and the process conditions result in obtaining molecules of varying size [Panigrahi *et al.* 2004]. Stability is a prominent characteristics of the nanocompound manufacture process. Nanoparticles possess natural ability for aggregation which seriously declines their biocidal properties and therefore, various polymers are added to the solution of the obtained nanomolecules to get high yield of non-aggregated particles. The most frequently stabilizers used are polyvinylpyrrolidone (PVP), sodium dodecylsulfate (SDS) or polyvinyl alcohol (PVA) [Malina *et al*. 2010].

There are also physical and biological methods applied for nanosilver synthesis. The physical methods are based on the properties of electromagnetic radiation, laser ablation or irradiation-reduction of silver ions [Panigrahi *et al*. 2004]. As for biological methods for silver nanocrystals formation, there are applied substances produced by living organisms, such as proteins, enzymes, vitamins, amino acids [Klaus *et al.* 1999]. Silver nanoparticles can be rapidly synthesized by treating silver ions by plant extracts, in that a *Capsicum annuum* extract [Li *et al.* 2007]. Some bacteria are also reported to possess ability for nanosized material production. Klaus *et al*. [1999] studied the phenomenon of biosynthesis in a bacterial strain *Pseudomonas stutzeri* AG259. The cells incubated for 48h in the liquid medium with silver nitrate exhibited the presence of silver-based crystals with sizes from 20 up to 50 nm. The authors associate the deposits with microbial resistance mechanism against toxic silver concentration.

Bactericidal activity of nanosilver

Colloidal solution may be composed of three forms of silver, that is metallic silver Ag 0, free silver ions Ag + and silver ions Ag + adsorbed on the nanoparticle surface. The continuous release of silver cations from the nanostructured surface proves to be a notable determinant responsible for efficient antibacterial activity [Malina *et al.* 2010].

The mechanisms of nanosilver toxicity against bacteria still remain unknown. More recently, the studies have thoroughly examined the character of biocidal activity [Lok *et* *a*l. 2006, Wzorek and Konopka 2007] that is largely associated with silver nanoparticle physico-chemical parameters, i.e. specific surface area (expressed as surface-to-volume ratio), high adsorption ability, chemical reactivity and catalytic properties [Elechiguerra *et al*. 2005, Choi and Hu 2008].

Cell wall

Nanostructured silver targets the bacterial cell wall which serves several functions and is a protective barrier against some substances. Currently, it is well known that nanoparticles sized less than 10 nm in diameter can bind to bacterial cell wall to cause its perforation which finally leads to cell death. These changes are observed in both, Gram-positive and Gram-negative bacteria and confirmed by the electron microscopy images [Feng *et al.* 2000]. Nanosilver with average particle size ca.12 nm cause specific damage to the *E. coli* cells, i.e. formation of irregular-shaped pits in the bacterial cell membrane. According to the studies of Feng *et al*. [2000], silver ions can also make the cell membrane detach from the cell wall but the mechanism of this operation has not been defined yet.

Disturbance in normal functioning of the cell wall may be also associated with catalytic behavior of nanosilver. Oxygen adsorbed on nanoparticle surface can remove a hydrogen atom from the thiol groups (-SH) of cysteine composing the bacterial cell wall peptides. The thiol groups deprived of hydrogen form disulfide bonds -S-S- in the bacterial cell wall and thus, may block the pathways of electron transfer through the respiratory chain [Wzorek and Konopka 2007]. The catalytic properties of nanosilver and the presence of generated reactive oxygen species contribute to the damage of not only cell wall peptides but genetic material in cells as well [Lok *et al*. 2006, Choi and Hu 2008].

Evidence of the interaction between nanostructured silver and cell wall was based on some regularity observed in Gram-positive and Gram-negative bacteria. It was found that Gram-positive bacteria, with some exceptions, are more resistant to nanosilver activity compared to Gram-negative ones [Egger *et al.* 2009]. Bacterial cell wall of Gram-positive is made up of much more murein, peptidoglycan material which is negatively charged. Due to the negative charge of Gram-positive cell wall, many more silver cations are kept within the wall and that prevents their penetration into cells [Wzorek and Konopka 2007].

Cell membrane

Cell membrane, alike the cell wall, is the boundary separating bacterial cell from the external environment, yet its role is far more complex than this. Numerous cell membrane-anchored proteins have essential functions in fundamental life processes of bacteria, like nutrient transport, cell wall synthesis, energy production and removal of redundant and harmful substances from the cell inside [Feng *et al.* 2000].

Nanosilver accumulation within the cell membrane leads to rapidly increased cell permeability and ultimately, cell death [Sondi and Salopek-Sondi 2004]. Likewise the case of cell wall, the underlying detailed mechanism is unclear. There are some theories attempting to explain the destructive effect of nanosilver on cell membrane that stress the interaction of silver ions with cell membrane proteins [Holt and Bard 2005].One of such theories indicates that silver nanoparticles may bind to bacterial cell membrane through electrostatic attraction, while another one suggests that they may potentially generate free radicals that damage its structure [Choi and Hu 2008].

Biochemical activity

Bacterial cell wall can make up an effective barrier against nanosilver, but according to Morones *et al.* [2005], the particles under 10 nm in size are able to penetrate into cytoplasm where they disturb cell metabolism and biochemical processes. The critical points in bacterial cell metabolic activity proves to be respiration and the mechanism of obtaining energy to perform all the energy-demanding life processes. In aerobic respiration, energy generation relies on the respiratory enzyme complexes associated with the respiratory chain. It was found that silver ions are likely to disturb its functions. Holt and Bard [2005] investigated the interaction between silver ions and respiratory chain enzymes in *E. coli* and concluded that silver ions bind to functional groups of amino acids making up enzymes and that activity inhibits the efficient electron transport via the respiratory chain. At the same time, the authors stated that during the initial stages, small doses of silver (1–5 µM Ag^+) can increase cellular respiration rate which they attributed to the reduction of oxygen atoms that occurs in the early stage of the respiratory chain. The process is inefficient and leads to the formation of toxic reactive oxygen species. Silver affects the further electron transport through the respiratory chain and blocks it by inactivation of this protein complex. The final effect is the complete stoppage of electron transport on oxygen and thus, the blockage of phosphorylation of ADP to ATP. The authors also bring attention to the NADH dehydrogenase complex as a potential target for silver ions activity.

Besides, nanosilver is known for its capacity to work as a catalyst within all the protein structures. The catalytic behavior is mainly manifested by binding silver ions with functional groups of amino acids. and the aforementioned ability for reaction catalysis between the –SH groups of neighboring protein amino acids and formation of -S-S- bonds between them. In the normal structure of some proteins, disulfide bonding (disulfide bridges) contributes to increased physical spatial stability of peptide structure. Formation of additional –S-S- bonds may induce molecular changes that lead to protein inactivation and in the case of enzymes, to their deactivation [Wzorek and Konopka 2007].

The studies of Lok *et al*. [2006] recognize nanosilver as a strong stressogenic agent towards bacteria. The authors reported that *E. coli* under silver influence showed enhanced synthesis of heat shock proteins and outer membrane proteins, such as OmpA, OmpC, OmpF, OppA, MetQ. Induction of heat shock proteins evidenced high intensity of stressogenic factors. Besides, the authors determined that even small amount of nanosilver disturbs cytoplasmic membrane potential to generate ATP. Due to defects in energy production, bacterial outer membrane proteins cannot be efficiently transported to their destination – the outer membrane and consequently, they accumulate in the cell cytoplasm.

Another characteristics of nanosilver is generation of reactive oxygen species (ROS), which reacting with some substances in bacterial cell seriously damage other molecules and the cell structures [Choi and Hu 2008]. Under the aerobic respiration conditions, small quantity of ROS may occur in cell where molecular oxygen is reduced to carbon dioxide. Generally, a properly functioning cell produces enzymes like, superoxide dismutase (SOD) and catalase, that work as catalysts to scavenge toxic reactive oxygen species. Superoxide dismutase catalyses the conversion of superoxide anion into hydrogen peroxide which is broken down to oxygen and water. The studies on nitrifying bacteria carried out by Choi and Hu [2008] revealed that silver nanoparticles sized only 15 nm produce the increase in intracellular ROS level and the concentration correlated to bacterial growth inhibition rate ($r^2 = 0,86$).

Formation of reactive oxygen species is dependent to some extent on the aforementioned catalytic activity of nanoscale silver, yet it is noteworthy that it is a typical light-in catalytic behavior tuned with nanoparticle size (i.e. more intensive only with small-scale particles). ROS generation is initiated mainly as an outcome of the respiratory enzymes and respiratory chain dysfunction [Choi and Hu 2008].

Genetic code

Numerous researches support the fact that a considerable target site for nanostructured silver is the genetic material of the bacterium [Feng *et al.* 2000, Kim *et al.* 2010]. It was found that DNA loses its replication ability once the bacteria are treated by nanoscale silver, which is associated with the silver ion capacity for binding to phosphorane residues of DNA molecules [Morones *et al.* 2005]. Moreover, silver ions also affect gene expression. In *E. coli*, silver was observed to stop S2 protein expression, a component of 30S ribosomal subunit and its denaturation. At the same time, the expression of genes encoding other proteins and enzymes involved in energy reactions, in that ATP synthesis was stopped [Gogoi *et al*. 2006].

The studies on mammalian cells showed that nanoparticles may directly damage DNA and chromosome molecules [Kim *et al*. 2010]. As far as bacteria are concerned, detrimental activity consists in deactivation of enzymes responsible for DNA replication and repair processes. One of the defense mechanisms of the bacterium genetic material is overproduction of proteins that accumulate in the cell centre, around nucleoids. Such changes were reported by among others, Feng *et al*. [2000] in *Escherichia coli* and *Staphylococcus aureus*.

Factors affecting bactericidal activity

Bactericidal capabilities of nanoscale silver may vary and are dependent on many factors ,such as particle size, shape, engineering mode, contact time and bacteria species. The studies on *Escherichia coli* indicated that nanoparticles of a triangular shape displayed stronger biocidal action than oval ones or in nanowire form [Pal *et al*. 2007]. Besides, their bactericidal effect is notably size-dependent. Their toxicity was found to decrease with increasing particle diameter which is associated with impaired particle penetration into the cell cytoplasm, smaller specific surface area and reduced release of Ag^+ ions from the particle surface [Choi and Hu 2008].

The researches on *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli* and *Pseudomonas aruginosa* concluded that bactericidal properties of nanostructured silver are also influenced by their engineering mode. Nanosilver synthesized by the chemical reduction with glucose as a reducing agent had a shorter killing time (until 15 min) as compared to particles produced with polyethylene glycol. Interestingly, the opposite trend was noted comparing the MIC values (minimal inhibitory concentration) [Kheybari 2010].

Other determinants of nanosilver antimicrobial activity in liquid suspensions are the use of a stabilizing agent and choice of appropriate surfactant. Kvitek *et al.* [2008] reported that sodium dodecyl sulphate (SDS) additive as a surfactant significantly reduced the MIC value, whereas enriching the suspension with substances lowering surface tension, in that Tween 80, did not impact the preparation biocidal properties. In both cases, an important factor influencing nanosilver activity was a species of the bacterium under investigation.

Fungicidal activity of nanosilver

Silver applied in varied chemical forms has been proven highly effective as a fungicide. Likewise in the case of bacteria, the major target sites of fungicidal action are fungal cell wall and membranes [Gajbhiye *et al.* 2009]. The fungicidal activity is dependent on both, a type of chemical compound and fungus developmental stage. The studies of Wright *et al.* [1999] showed that wound dressing incorporating nanocrystalline silver turned out to have far more enhanced properties than silver sulphadiazine or silver nitrate. Taking into account high toxicity of silver nitrate, nanosilver may be effective and relatively safe for prevention and control of fungal wound infection.

Fungicidal activity also relies on nanosilver concentration. Falkiewicz-Dulik and Macura [2008] studying the footwear materials with nanosilver have reported complete growth inhibition of different fungal species, in that dermatophytes at as low as 20 ppm nanosilver concentration. Lowering concentration to 2 ppm inhibited fungal growth of 61% examined strains. The authors highlighted that nanosilver addition to shoe glue markedly reduced mould development, while dermatophytes – to some degree. However, Egger *et al.* [2009] state that as for fungi, higher resistance is exhibited by filamentous fungi.

Kim *et al.* [2008] compared the IC80 values (amount of substance responsible for 80% growth inhibition in the examined microorganism) for nanosilver and commonly applied fungicides. The authors showed that nanoscale silver exhibited evidently better antifungal activity against most tested fungi than amphotericin B and fluconazole. In the test with *Candida albicans*, IC80 values were 2–4 μg/ml, 5 μg/ml and 10–16 μg/ml, respectively. Besides, the authors observed that nanoparticles had influence on the fungus developmental cycle through blocking one-celled forms to group as micelles and thus, deprived them of the capacity to induce infection. Gajbhiye *et al.* [2009] stressed that combination of nanosilver and antifungal agents, in that fluconazole, can enhance antifungal efficacy of disinfectants and widen their operation spectrum.

Virucidal activity of nanosilver

Antimicrobial activity of nanosilver is not limited to only bacteria and fungi as nanostructured silver can fight some viruses as well. The researches made by Mehrbod *et al.* [2009] on cell-cultured influenza virus treated by nanosilver indicated a decrease of infected cell numbers as compared to control. A direct contact of virus with nanoscale silver in in vitro conditions caused the decline of isolated viral DNA load. The same studies showed that nanoparticles can also prevent binding of viral coat protein to antibodies as well as counteract virus penetration into cells.

Importantly, nanosilver has been shown to be effective against HIV virus as well. Particles sized ca.16 nm can bind through gp120 glycoproteins knobs on HIV-1 to the

CD4 receptor sites on the host cell. Here, similarly to the case of bacteria, the most probable sites for interaction are the sulfur-bearing residues of the gp120 glycoprotein knobs [Elechiguerra *et al*. 2005].

Nanosilver application

There have already been developed several applications of silver nanoparticles, especially for disinfection of air and production areas. Nanosilver as liquid disinfectant has good disinfecting properties but its drawback is limited use in the contaminated environment. Their antimicrobial efficacy, according to Wzorek and Konopka [2007] is lowered at the presence of high load of organic matter as it is reported at some production lines at food industry. The ongoing scientific efforts aim to improve disinfectant formulation to obtain stable and permanent properties of preparations.

Nanopreparations are most commonly applied in medicine and health protection as in these fields their potential to cope with hazardous microorganisms is known to be powerful. In hospital environment, properly performed disinfection makes up a major component of biosecurity. It should be stressed however, that conventional disinfecting agents used most frequently have become inefficient against bacteria like, *Pseudomonas aeurginosa* or *Staphylococcus aureus* that have been shown to develop resistance to disinfectants very rapidly. Nanosilver is one of the nanomaterials used most commonly in medicine because it can be applied as colloidal solution or particles directly bound to solid surfaces or materials. Today, novel wound dressings combine hydrogel and silver nanoparticles to enhance wound healing process and prevent infections. The wound dressings show improved bactericidal activity attributed to nanosilver and are relatively safe for human health [Egger *et al*. 2009].

Combination of nanoparticles with various materials, including polymers or mineral compounds, heightens their toxicity. Immobilization of nanoparticles on polymer or other porous materials overcomes their natural tendency to agglomerate, especially unwanted in the case of colloidal silver nanoparticle solution. Composite materials are highly desirable in implant production [Egger *et al.* 2009]. Silver incorporation prevents bacterial colonization and biofilm formation on surfaces of medical implants essential for long-term performance in patient`s body. Currently, the studies have been conducted on dental materials with nanostructed silver [Liao *et al.* 2010]. The research results indicate benefits of combination of titanium plates with nanosilver that exhibits high activity against bacteria inducing periodontal diseases, such as *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*; besides it reduces bacterial adhesion to dental implant surfaces.

Tissue engineering is a rapidly developing research field in medicine that facilitates laboratory culture of tissue or even entire inner organs. Engineering a tissue or organ requires specially constructed scaffolding for the anatomical shape reconstruction and making growth medium for cultured cells.

During the cell multiplication process, it is vital to keep the culture sterile and prevent microbial contamination. According to the studies by Li *et al.* [2009] nanosilver may prove very useful in this field as well. The authors tested a biocomposite composed of neutral for the recipient immune system PLLA (poly-L-lactic acid) polymer and nanosilver. Additive of silver nanoparticles sized 30-100 nm showed antimicrobial activity against Gram-negative and Gram-positive bacteria as well as prevented the development of harmful microorganisms on the tested material surface.

Nanosilver has another beneficial application in the treatment of air from harmful microorganisms. Yoon *et al.* [2008] reported that small fragments of air filters with carbon fiber and nanostructured silver of 12–15 nm size exhibited complete growth inhibition of *Escherichia coli* and *Bacillus subtilis* bacteria fairly quickly, i.e. the 10-minute contact.

Nanosilver containing materials are also used in the food industry where appropriate air quality status is of considerable prominence. The promising research results were presented by Kowalski *et al.* [2010] when nanosilver particles were impregnated into fiber filtration bags used in the slaughterhouse air-conditioning system. The most important advantages are simplicity, low-cost exploitation and high efficiency. At the early study period, the authors obtained the 85% removal rate of bacteria and complete reduction of fungi.

The livestock nanosilver has some use for disinfection, food, plant-transport of the animals, and some of the technological equipment in livestock buildings [Ahmadi 2009, Dobrzański *et al.* 2010]. Since recently is testing the possibility of using nanosilver, or composites containing nanosilver to reduce ammonia emissions from livestock manure [Myczko and Kołodziejczak 2008, Dobrzański *et al.* 2010].

Nanosilver toxicity to human

Regarding the widespread use of silver and its compounds in developing industry branches, it has become imperative to study its potential negative impact on human health. Therefore, alike drugs and other potentially harmful substances, the silver toxicity testing has been conducted using cell cultures and animal models.

Generally, an increasingly common application of nanosilver in industry and protection of public health increases the probability of human exposure to this element. The major routes of nanoscale silver and silver compound penetration into human body are the respiratory tract, gastrointestinal system or the skin. It is known that ingestion of large doses of colloidal silver or long-term inhalation of silver dust result in permanent and irreversible discoloration of the skin (argyria) [Kim *et al.* 2010]

These changes are recognized in patients who were exposed to prolonged chronic contact with silver, e.g. occupational exposure of workers involved in silver mining or patients using colloidal silver as regular dietary supplement. Silver doses under the values inducing argyria are considered relatively low toxic [Kim *et al.* 2010].

Korani *et al*. [2011] assessed toxicity of silver penetrating human organism through the skin, besides nanosilver toxicity was compared to that of silver nitrate. The experiments involved guinea pigs. The authors showed that even large doses of nanosilver up to 10000 µg/ml did not result in death of experimental animals or macroscopic lesions in the animal internal organs. Both, nanoscale silver and silver nitrate caused reduced thickness of epidermis and, noteworthy, a raised dose increased the toxic responses of the skin. The long-term dermal contact with nanostructured silver triggered inflammatory response manifested by the elevated count of Langerhans cells. Besides, the authors indicated declined thickness of the reticular layers and raised collagen levels of the dermis. Nanosilver and silver nitrate were found to produce microscopic changes in the liver and spleen. The histopathologic changes of the liver include inflammatory infiltrations, hepatocyte degeneration, overproduction of Borowicz-Kupffer cells responsible for immune responses.

The investigations by Kim *et al.* [2010] showed that nanosilver taken up through oral route caused only slight changes within the intestines and liver. Whereas the kidneys of the experimental animals exhibited nanoscale silver accumulation, yet even a dose exceeding 125 mg/kg bw did not affect internal organs functioning. It was found that silver nanoparticles having penetrated the organism via the inhalation route accumulate in the lungs. Similarly, intensity of pulmonary lesions increased with a raising dose and exposure time [Stebounova *et al.* 2011].

Toxic effects of silver nanoparticles at the biochemical level, i.e. genotoxicity, protein inactivation or generation of reactive oxygen species, may be similar in both, microorganisms and higher organisms. The studies on gene expression revealed that human cell cultures under silver nanoparticles influence tend to activate encoding genes that are of importance in cell defense against oxidative stress. Nanosilver can produce chromosome damage, disturbed mitotic progression and changes in cell morphology [Xu *et al.* 2012].

RESUME

Antimicrobial activity of silver combined with the unique properties of nanomaterials have given rise to the new generations of materials and disinfectants. Nowadays, due to increasing bacterial resistance to conventional disinfecting agents, there is a progressive demand for novel solutions in this field. Effective disinfection practices and development of prevention procedures to control harmful microorganisms growth are crucial not only for health care functioning but for food industry or agriculture as well. Numerous scientists recognize nanosilver as a disinfectant relatively safe for health, yet we should look to a variety of its potential applications with moderate optimism.

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Streszczenie. Srebro jest znane ze swoich antybakteryjnych właściwości od dawna, jednak rozwój nanotechnologii umożliwił opracowanie substancji i materiałów nowej generacji. Preparaty z dodatkiem nanosrebra wykazują silne właściwości bójcze w stosunku do wielu szkodliwych mikroorganizmów, zarówno bakterii grzybów, jak i wirusów (nawet wirusa HIV). Wielokierunkowe działanie nanocząstek srebra utrudnia mikroorganizmom uruchamianie mechanizmów obronnych i nabywanie przez nie odporności. Nanosrebro uszkadza struktury komórkowe, wpływa na metabolizm energetyczny i materiał genetyczny mikroorganizmów. O jego właściwościach decydują takie czynniki, jak kształt, wielkość czy sposób wytwarzania. Nanocząstki srebra związane ze stałą powierzchnią znalazły już zastosowanie w produkcji opatrunków, gdzie zapobiegają rozwojowi szkodliwej mikroflory. Prowadzone są również badania nad wykorzystaniem ich w implantologii i inżynierii tkankowej i w przemyśle spożywczym do dezynfekcji powietrza i powierzchni produkcyjnych. Zaletą nanosrebra, jak dowodzą badania, jest jego stosunkowo niska toksyczność dla ludzi.

Słowa kluczowe: nanosrebro, dezynfekcja, toksyczność