INTERACTION BETWEEN BACTERIA AND METAL NANOPARTICLES

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ABSTRACT

Metal nanoparticles have found larger application in the last years with unique qualities, they demonstrate. Basic problem in medicine is the fight with microbial infections in hospitals and resistance of pathogenic microorganisms to the most of current antibiotics. The bactericidal effect of Ag is well known from the ancient time and nowadays nanotechnology creates the huge diversity of metal nanoparticles with a different structures and strong effects that can substitute antibiotics and other toxic disinfectants in the battle with bacteria. Plenty of physical, chemical and microbiological approaches are used to investigate the qualities of metal nanoparticles. The obtained results depend on the size, structure and synthesis of nanoparticles, as well as on the type of microorganisms. Different cell wall structure is the main reason for the sensitivity of microorganisms to the nanoparticles.

Key words: *nanoparticles, microbiological methods, antibacterial activity*

INTRODUCTION

Metal nanoparticles have found larger application in the last years with unique qualities, they demonstrate. Basic problem in medicine is the fight with microbial infections in hospitals and resistance of pathogenic microorganisms to the most of current antibiotics.

Nanoparticles have different qualities depending on the shape and size from 1-100nm. Their use in medicine and industrial products has grown in the last years. They have application in cosmetics, drug carriers, diagnostics, fabrics with antibacterial effects, filters etc.

The bactericidal effect of Ag is well known from the ancient time and nowadays nanotechnology creates the huge diversity of metal nanoparticles with a different structures and strong effects that can substitute antibiotics and other toxic disinfectants in the battle with bacteria. Interaction between metal nanoparticles and microorganisms is a broad field of investigation. The silver salts were used for a long time ago and now silver nanoparticles are applied in less concentration and have the same or better antibacterial effect. Silver nanoparticles coating may be used in dental medicine, as a coating of medical devices and burn wounds. The silver ions are toxic to more than 12 species of bacteria including *E.coli* [4].

The mechanism of interaction is not completely understood but Sondy [6] prove the damage of cell wall structure with attack to phospholipids and membrane permeability and activity of signal proteins. The metal nanoparticles could damage the DNA and stop its replication, or bind to bacterial ribosome [3]. It is suggested that Ag ions and free radicals derived from the Ag nanoparticles are responsible for antimicrobial activity [3, 6].

MATERIALS AND METHODS

The most popular method to obtain Ag and Au nanoparticles is reduction from the solution. Different stabilizing agent could be used as poly-lysine, starch or 3-mercaptopropionic acid that could not afford the aggregation of metal nanoparticles. Ascorbic acid could be used as a reducing agent. The nanocomposites obtained under hydrothermal conditions exhibited different morphology depending on the type of carbohydrate used. In sucrose nanocomposites, spherical agglomerates are seen. Starch system consists of some spherical particles along with some ribbon-like structures. But the waxy starch-composites exhibit channelized growth of the particles. The nanoparticles formed in waxy corn starch matrix were isolated by centrifugation and were observed to have self assembled into wire-like structures. Although the exact mechanism of the formation of the

nanostructures is difficult to know, we think that the chain-shaped structure of starch could serve as a directing template for the growth of silver nanoparticles Starch is composed of a linear component, amylose and a branched component, amylopectin. The branching is due to 1,6 acetal linkage in amylopectin which is absent in amylose. It is assumed that as a result of the bond angles in the alpha acetal linkage, amylose forms a spiral structure which helps in stabilization. On the other hand, branched polymer might act as a morphology-directing agent facilitating the growth of silver nanowires [7].

The other method is to combine two different solutions of metal ions and to reduce them together to obtain bimetallic nanocomposites. They could be prepared by microwave assisted chemical reduction in aqueous medium, using the biopolymer as stabilizing agents [8]. Biosynthesis of gold, silver, gold-silver alloy, selenium, tellurium, platinum, palladium, silica, titania, zirconia, quantum dots, magnetite and uraninite nanoparticles by bacteria, actinomycetes, fungi, yeasts and viruses have been reported. However, despite the stability, biological nanoparticles are not monodispersed and the rate of synthesis is slow.Other investigators prove the synthesis of metal nanoparticles by different bacteria: *Klebsiella pneumoniae, Escherichia coli, Enterobacter cloacae,* and *Bacillus subtilis.* In other study, the bacteria *Rhodopseudomonas capsulata* was screened and found to successfully produce gold nanoparticles of different sizes and shapes. The important parameter, which controls the size and shape of gold nanoparticles, was pH value. The *R. capsulata* biomass and aqueous $HAuCl_4$ solution were incubated at pH values ranging from 7 to 4. The results demonstrated that spherical gold nanoparticles in the range of 10–20 nm were observed at pH value of 7 whereas a number of nanoplates were observed at pH 4 [5]. The nanoparticles formed are thoroughly characterized by UV-Vis, TEM, XRD, XPS and FTIR studies.

Toxicity tests are conducted with *E.coli*, *S. aureus,* yeasts and other strains pathogenic or nonpathogenic bacteria. Different methods are used in bacteria nanoparticles interaction investigations:

In a liquid medium:

- The growth curves are explored versus a control of bacterial suspension without nanoparticles. The prolongation of lag-phase is studied, decreasing of growth speed and increasing of generation time till 24 hours [3].
- Antibacterial activity of metal-NPs is investigated in liquid media to differentiate the minimal inhibition concentration (MIC) and minimal bactericidal concentration (MIB) [3,9].
- Bioluminescent test: the decreasing of emitted light of *Photobacterium phosphoreum*. The toxicity is inversely proportional to the intensity of light emitted after the contact with toxic substances. The measure is conducted after 5 and 15 minutes contact time [2].

In a solid medium:

- The direct inhibition method is applied to a different concentration of colony-forming units (CFU/ml) bacteria seeded in solid media on Petri dish or as a zone of inhibition in agar medium seeded with bacteria, or filter discs, soaked with nanoparticles[4,6,8].

RESULTS AND DISCUSSION

There are several physical and chemical methods for investigation of obtained nanoparticles: X-ray diffraction, Photoluminescence spectroscopy, Z- potential, UV-Visible and Infrared spectroscopy, Energy dispersive X-ray fluorescence, and Transmission and Scanning electron microscopy (TEM and SEM). Metallic nanoparticles- silver and gold, prepared by reduction of metallic ions in the presence of stabilizing capping agent shows one absorption band owing to surface plasmon resonance (SPR). The wavelength of SPR of metal NPs depends of particle size and dielectric constant of the metal. Spherical golden nanoparticles have absorption band round at

520nm, depending of their size and silver nanoparticles show one absorption band at 400 nm also depending of their size [1].

Antimicrobial activity of silver nanoparticles:

- The investigations were conducted with yeasts, *E. coli* and *S. aureus* on Muller Hinton agar (MHA) and Ag-NPs in different concentrations (from0.2 till 33 nM). The similar inhibition effect was observed at yeast treatment with a positive control- intraconazol, in comparison with the effect of Ag-NPs concentration of 33nM. Significant growth inhibition was observed from 13.2 nM. These results have shown, that MIC of Ag-NPs for yeasts is between 6.6 and 13.2 nM at these conditions. It is proved, that Ag-NPs are more effective to *E. coli.* MIC of metal particles is between 3.3 and 6.6 nM and growth inhibition changes and depends on their concentration. For *S. aureus* Ag-NPs have shown weak inhibition effect even in high concentration of 33 nM. There was no statistically significant effect compared with the positive control (Gentamycin) in this condition. MIC of Ag-NPs against *S. aureus* was estimated to be more than 33 nM. Also, there is no antimicrobial activity in solution devoid of Ag-NPs, reflecting that antimicrobial activity was directly related to the Ag nanoparticles. To determine whether the growth inhibitory effect of Ag nanoparticles is a specific effect or not, gold nanoparticles were used (~30 nM) as another control of nanosized metals. Au-NPs showed no growth inhibitory effect against various microorganisms in those experimental conditions [4].
- The growth curve of *S. capitis* (as a model of Gram-positive bacteria) from the other experiment shows that with increasing concentration of Ag-NPs, growth is increasingly reduced with the control value. The survival curve decreases until at concentration of 150 μM up to 60% of bacterial cell are killed. In contrast with that growth curve of *E. coli* shows that with increasing concentration growth is accelerated until 30 μM and then increasingly reduced compared with the control.

As a consequence, the survival curve increases up to 30 μM and decreases thereafter until at concentration of 150 μM up to 30 % of bacterial cell are killed. The results shows that increase in the concentration of NPs reduced the survival of bacteria [1].

The analyze of growth curve of *S. capitis* shows that increasing of concentration of Au-NPs growth of bacterial cell decrease until concentration of 70 μM in comparisson with control values where it then becomes relatively constant. The survival curve decreases until at a concentration of 70 μM – 20% of bacterial cells are killed. By increasing the concentration up to 150 μM, the percentage of survival remains almost the same, i.e. the toxic effect of gold NPs on the bacterial cell is no longer observed. The growth curve of E. *coli* shows that with increasing concentration of Au-NPs, growth moderately decreases until 70 μM, but after that concentration increases until 130 μM. The survival curve remains nearly the same with slight decrease of only 10 % up to concentration of 70 μM. Between 70 and 130 μM the survival curve increase. At still higher concentrations the survival curve decreases again to almost 10 % bacterial cell death. It is observed significant inhibition effect on *S. capitis* in comparisson with effect on E. *coli* which is given to differences of cell wall [1].

Other report shows growth curve of *E. coli* exposed to different concentrations of Ag-NPs:

The growth curve of *E. coli* treated with silver NPs is representing by measuring optical density at 600 nm. In presence of 0, 1.25, 2.5 and 5 μg/ml the growth curve included three phases: lag phase, exponential phase, and stabilization phase. Under absence of nanoparticles, *E.* coli reached exponential phase rapidly. But exposed to 1.25, 2.5 and 5 μg/ml of NPs, *E.* coli cells were lagged to 12, 36 and 48 h. With increasing concentration of silver nanoparticles, the delay ware more evident. When the concentration of silver NPs was 10 μg/ml , no growth of *E.* coli could be detected within 7 days, indicating the minimum inhibitory concentration (MIC) of silver NPs to *E. coli* was 10 μg/ml [9].

Action of silver NPs on the structure of *E. coli* cells :

The micrograph by SEM showed the surface of *E. coli* cell untreated with nanoparticles was smooth and showed typical characters of rod shape, while cells treated with 50 μg/ml Ag-NPs were damaged severely. Some cells showed large leakage, others misshapen and fragmentary. Micrographs by TEM showed the surface of native cells was smooth and intact, while the membrane of treated cell was damaged and fragmented. Electron-dense particles and precipitates were also observed around damaged bacterial cells [9].

Ag-NPs attach to phosphate and sulfur groups that are part of the phospholipid cell membrane or to membrane proteins and severely damage the cell and its major functions, such as permeability, regulation of enzymatic signaling activity, and cellular oxidation and respiratory processes [3].

Electron spin resonance (ESR) study of Ag nanoparticles:

The mechanism of growth-inhibitory effects of Ag-NPs on microorganisms has not been well understood. One possibility is that the growth inhibition may be related to the formation of free radicals from the surface of Ag-NPs. Uncontrolled generation of free radicals can attack membrane lipids and then lead to a breakdown of membrane function. To obtain insight into this possibility, it have to be measured the ESR spectra of Ag nanoparticles. Ag samples were prepared in powder form by stirring the Ag nanoparticles solution with a Zn bar, causing the Ag nanoparticles to aggregate. The ESR spectrum shows existence of free radicals from Ag nanoparticles, thus supporting that free-radical generation of Ag nanoparticles may be responsible for the antimicrobial effects [4].

Antioxidant effect of Ag nanoparticles and silver nitrate in antimicrobial activity:

To determine the relationship between free radicals and antimicrobial activity the antioxidant N-acetylcysteine (NAC) was use to test whether the antioxidant could influence antimicrobial activity induced by Ag nanoparticles. Ag nanoparticles and silver nitrate showed similar growth-inhibitory effect against *E.coli*. Inhibitory effect was abolished by the addition of NAC. NAC alone did not affect the antimicrobial activity [4].

Bioluminescent test

The bioluminescent test is widely used to evaluate the potential harmful effects of effluents discharged into surface water. Under the experimental conditions nor solutions, neither suspension of nanoparticles show toxic effect. In any case the toxic concentration is higher than the highest tested concentration [2].

CONCLUSIONS

The silver ion and silver based compounds have strong antimicrobial effects. These inorganic particles have a distinct advantage over conventional chemical antimicrobial agents. The most important problem caused by the chemical antimicrobial agents is multidrug resistance. The antimicrobial mechanism of chemical agents depends on the specific binding with surface and metabolism of agents into the microorganism. Various microorganisms have evolved drug resistance over many generations. Thus far, these antimicrobial agents based on chemicals have been effective for therapy; they have been limited to use for medical devices and in prophylaxis in antimicrobial facilities [4].

There are observed differences between inhibition effects of metal nanoparticles on gram-positive and gram-negative microorganisms.

It is necessary to make more research investigations to understand the mechanisms of antibacterial action of Ag nanoparticles and to use their properties completely.

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