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Effect of Ag nanoparticles on membrane properties of *E.coli*

Saricheva A.S. and Parshina E.Yu

Over the past few decades, inorganic nanoparticles, whose structures exhibit significantly novel and improved physical, chemical, and biological properties and functionalities due to their nanoscale size, have elicited much interest. Noble metal nanoparticles find application in detecting Raman spectra from large organic molecules. Low concentration of investigated molecules gives no chance to detect Raman spectra, but noble metal nanoparticles induce enhancement of Raman surface spectra from the molecule. This effect is called surface enhanced Raman scattering. In this work silver nanoparticles (Ag NPs) were studied. While developing SERS method scientists came across with the problem of silver solution toxicity, as silver is the most common antibiotic. A mechanism of the influence on cells is probably different for NPs and ions. Our recently investigation showed, that silver ions in Ag NPs solution are responsible for erythrocytes destruction and buffer containing chloride ions can slower this process. Not only red blood cells can be studied with SERS, but other organisms, especially some bacteria. Hemolisys is suitable for the toxicity detection to erythrocytes, but to microbial cells such method as bioluminescence will fit as a widely spread ecological analysis to register the range of toxicity of the environment. And the most common bacteria for such studies is E.Coli. So the aim of our work was to study the toxic effect of Ag NP on cells of E.coli. Our scopes were to synthesize Ag NP solution, to find the minimum concentration of AgNP and $AgNO_3$ inducing cell damage, to study cell breathing in presence of Ag NP, to study bioluminescence in the presence of Ag NPs and to study shape changes of E.Coli cells in presence of Ag NP .

We found out that AgNPs are less toxic in the comprising with silver nitrate with the same concentration of Ag. It can be explained by the fact that in the AgNPs solution silver is mostly in the metal form, rather than in ion, which is extremely toxic. To study the mechanisms of the influence of NP on cells we should find the minimum concentration of both AgNPs and $AgNO_3$ that would not kill cells in a moment, but causes significant toxicity in time. These concentrations were found in bioluminescence test.

Another common bacteria membrane property is breathing. It is known, that silver ion make the membrane more permeable for H^+ ions, so that breathing is accelerated. Free radicals are formed and the cell dies quickly. This process was studied by polarography method using Clark's electrode that is sensible to the O_2 concentration. The amount of absorbed oxygen by cells in the presence of $AgNO_3$ decrease more rapidly than in case of Ag NPs. That also confirms that $AgNO_3$ is more toxic agent. Also we investigated cell morphology in different media. The AFM can give us information about cell size, volume and shape. To study time dependence of the damage we incubated cells with Ag NPs and $AgNO_3$ for an hour and for 24 hours. The cells, incubated in distilled water looked as normal even after 24 hours of incubation. After incubation E.coli with $AgNO_3$ dramatically changes in morphology could be seen: cells became more round and rough. In case of Ag NPs the changes are the same and not so significant, but also pronounced.

Using AFM pictures we calculated such parameters as area, length, height and the relation between length and width (aspect). The area and length are decreased. In case of aspect we can see changes in time. After 24h incubation the control cells become more extended, as literature reports. But in presence of silver ions or NP cells after 24h become more compact. Interesting phenomena could be observed in case of height: the dependence on time is not the same for NP and Ag ions. In case of NPs the height is increased, while in case of silver ions it first increased but then decreased with time. To study this in details lower concentrations were taken. Under action of minimal concentration caused significant toxicity, area, length and aspect didn't change. The same could be seen in height with AgNP. But lower concentration of $AgNO_3$ causes the same changes as high concentration. So that we can conclude, that silver ions causes cell damage and this damaging process has 2 steps: firstly the height is reliably increased, and decreased in presence of more amounts of silver ions. The second conclusion could be made: the cell morphology measurements revealed that AgNPs of low toxic concentration causes no damage in the comprising with AgNO3. The same concentrations of these compounds behave themselves in the different way during bioluminescence investigation. That means the presence of another mechanism of influence on bioluminescence of E.Coli in case of NPs. The conclusions made by this work are:

- The decrease of bioluminescence and cell breathing was found. Minimum toxic concentration of $AgNO_3$ and AgNPs was obtained
- In comparing with $AgNO_3$, Ag NPs solutions with the same concentration of Ag has less effect of toxicity in about 103
- Reliable differences between cell morphology parameters were found
- The main mechanism of damaging cells with Ag NP might be connected with the presence of Ag^+, but in case of bioluminescence not only Ag ions may cause toxicity
- The process of shape change might proceed in two stages

assergevna@gmail.com