A novel mechanism for the synthesis of ultra-purified silver nanoparticles for the use in biomedical materials.

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ABSTRACT

Biomedical products are regulated by high standards of purity and quality compared to those that are not going to be linked to the human body. These requirements are crucial in order to guarantee biocompatibility. For this reason, the price of the final product is going to increase significantly [1]. So it is a real challenge to incorporate a nanotechnological product to be used in the biomedical industry without affecting its cost. Previous research has shown the antibacterial properties of nanoproducts such as silver nanoparticles (AgNPs) and single-walled carbon nanotubes (SWNTs) against infectious agents like bacteria and fungi. However, as mentioned before purity in those products has to reach higher levels than for other applications. Available reagents for the synthesis of AgNPs contain metal impurities such as iron that cannot only reduce the antimicrobial properties but even promote the growth of microbiological agents according to the level of purity of the reagent.

In this study, we describe a novel and more affordable mechanism for the synthesis and bio-purification of AgNPs that contain metal impurities. The nanoparticles were synthetized by the use of silver nitrate with different levels of purity. After tests with some species of fungi and bacteria we have found *Pseudomonas aeruginosa* as the proper specie to the bio-purification and thus obtaining a higher purity of AgNPs . With this novel mechanism, not only we were able to use a reagent with a higher quantity of impurities and reduce the synthesis cost, but also were able to achieve a higher purity in the synthetized nanoparticles. By synthetizing AgNPs this way, we not only obtained nanoparticles with a higher purity than the ones produced by using a higher purity reagent, but also reduced the cost around 50 percent. This method is important because AgNPs synthesized with these properties can be incorporated to polymers for products such as catheters, and alloys such as scalpels with better antimicrobial properties.

Keywords: AgNPs, biopurification, biocompatibility, purification, synthesis.

2. INTRODUCTION

Cross-disciplinary studies from the last years have advanced in the development of new materials for biomedical devices that will not allow infections. Inert surfaces such as orthopedic devices and catheters are surfaces where biofilms can occur. Bacterial biofilms consist of colonies on a surface in which there is a organization of communities. Those colonies are formed when bacteria attachs to surfaces and is aggregated in a hydrated polymeric matrix synthtethized by the microorganism. Because biofilms are very resistant to treatment it is often necessary the removal of the infected device [3]. Because of their large surface area and reactivity compared with the bulk material, solid metal nanoparticles are generating a growing interest for research.

The antibacterial effects of AgNPs are extremely vinculated to their size and to the properties of the suspensions such like pH, oxidation and, purity. Smaller particles show higher activities compared to the equivalent silver content. Oxidation of zero valent AgNPs and aggregation of AgNPs can result in a loss of antimicrobial properties. [4-5]. Some of the impurities that can be present on AgNPs may not be toxic to pathogenic organisms. Iron for example is an essential mineral that many enzymes require in order to trap oxygen molecules and it can be found as an impurity on many reagents, like silver nitrate. Bacteria in our bodies will need to find the way to obtain their iron supply , one of the major way for those organisms is the building of siderophores. Siderophores will play also an important role in this study, in order to prevent the growth of patogenic microoganisms in biomedical devices [6-7]

3. SYNTHESIS PROCESS OF ULTRA PURIFIED SILVER NANOPARTICLES

Among all known antibacterial nanoparticles, silver has the highest bactericidal activity and biocompatibility and does not require photocatalytic agent. In medical applications, AgNPs are a promising alternative to silver salts and bulk metal [7-8].

The antimicrobial properties of bulk materials containing silver are studied in terms of the concentration of Ag^+. It has been reported that low concentrations of Ag^+ can produce a proton leakage through the membrane of *Vibrio cholerae*. Ag+ interacts with nucleic acids and cytoplasm components, inhibit respiratory chain enzymes and interferes with membrane permeability [9].

A chemical reduction was performed for the AgNP synthesis.

$$4\,AgNO_3 + NaBH_4 + 3\,H_2O \rightarrow 4\,Ag + H_3BO_3 + 3HNO_3 + NaNO_3$$

Figure 1. Reaction for the synthesis of AgNPS.

Two 10 mL solutions of AgNO3 1 mM were prepared, with variations in the purity of the reagents. One was 99.99% trace metal basis and the other ACS reagent ≥99.0%. *Pseudomonas aeruginosa* were grown on TS media for 12 hours at 37,5 ºC. After the chemical synthesis process the bacteria were added to the solutions of AgNPs. At the end of the process the solution with the 99.99% trace metal basis had a darker color (Fig. 1B) and the solution with the ACS reagent was lighter. (Fig. 1A) The solutions of AgNPs stayed with the bacteria for 48 hrs at 25 ºC. After the 48 hours, the solutions were centrifugated for 15 minutes at 5000 r/min and decanted.

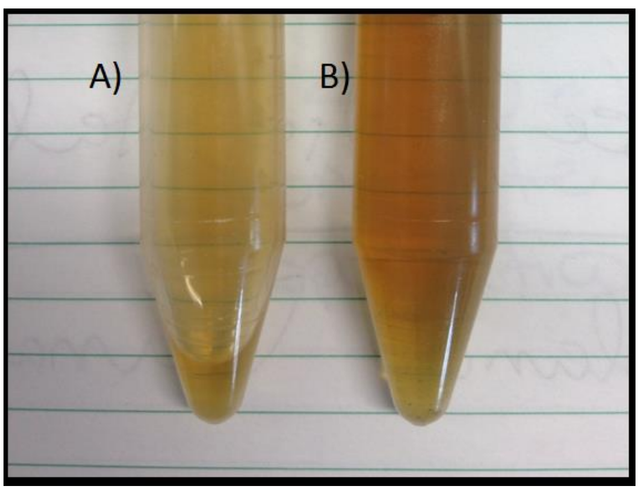

Figure 1. Comparison of synthetized silver nanoparticles previous centrifugation.

3.1 SILVER NANOPARTICLES FORMATION

By the usage of a Jeol JM-2100 Transmission Electron Microscope (TEM) the formation of AgNPs was confirmed. In general terms the nanoparticles are spherical and with an average size of 8 nm. In case of the nanoparticles with the ACS reagent the average size is 10 nm, it was found that the AgNPs are spherical but may form aggregates (fig. 2) that can encapsulate a group of AgNPs.

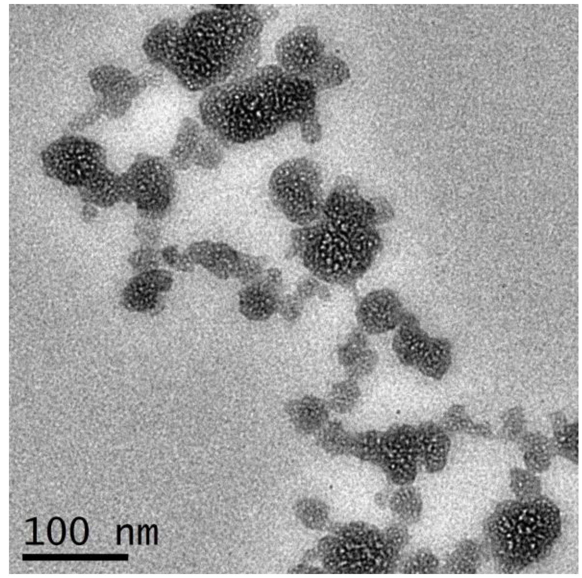

Figure 2. Spherical silver nanoparticles synthetized with the less pure reagent previous interaction with *P. aeruginosa*.

In case of the solution with $AgNO_3$ 99.99% trace metal the average size in the spherical nanoparticles is 6 nm (fig. 3). No evidence of encapsulations due to AgNPs aggregation was found in this solution.

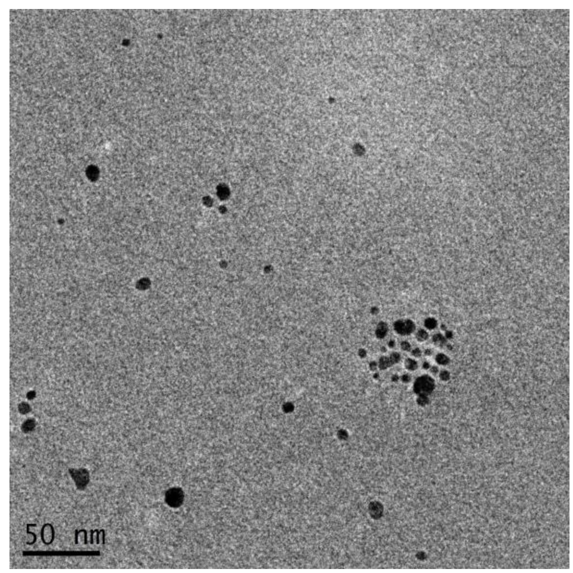

Figure 3. Spherical silver nanoparticles synthetized with the high pure reagent previousinteraction with *P. aeruginosa*.

3.2 EFFECT OF BACTERIA IN THE BIO-SYNTHESIS PROCESS

The presence of metal impurities such as iron in the silver salt cannot only reduce the antimicrobial properties but can even promote the growth of microbiological agents. [9] In this particular case, siderophores will be sent by the bacteria removing the impurities from the solution (Fig. 4)

NSTI-Nanotech 2013, www.nsti.org, ISBN 978-1-4822-0586-2 Vol. 3, 2013

At the same time will allow the cell ingestion of the AgNPs (Fig. 5).

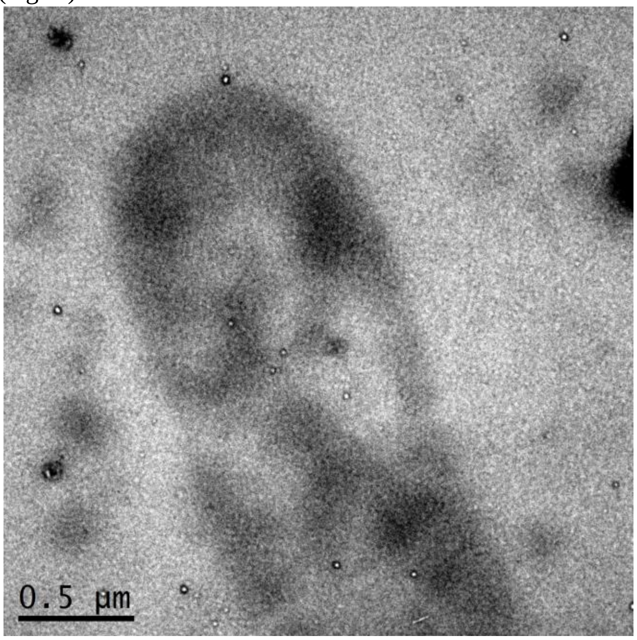

Figure 4. Siderophores synthethized by the bacterium.

Silver nanoparticles ingestion causes a weakness in the bacteria membrane afecting its permeability. A comparison of Energy Dispersive Spectroscopy (EDS) analysis was done in containing bacteria solutions and 0.20 µm membrane filtered solutions. It probed that iron impurities are catched by bacteria, specifically by the siderophores.

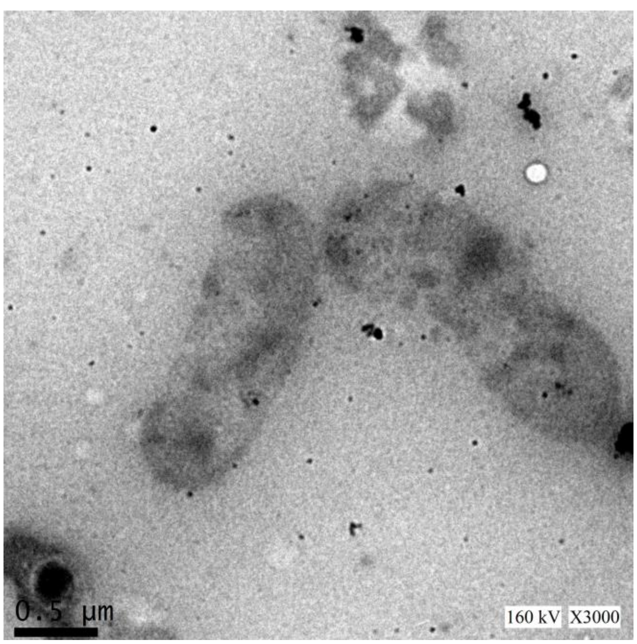

Figure 5. Ultra-purified AgNPs solution with weakened *P. aeruginosa*.

The antimicrobial properties were proved by the growth of *Staphylococcus aureus* on TSA plates at 37,5°C during 14 hours. At the time of the initial inoculation 0,5 mL of a solutions of ultra-purified AgNPs (Fig 6A) and a control growth. (Fig 6B) .

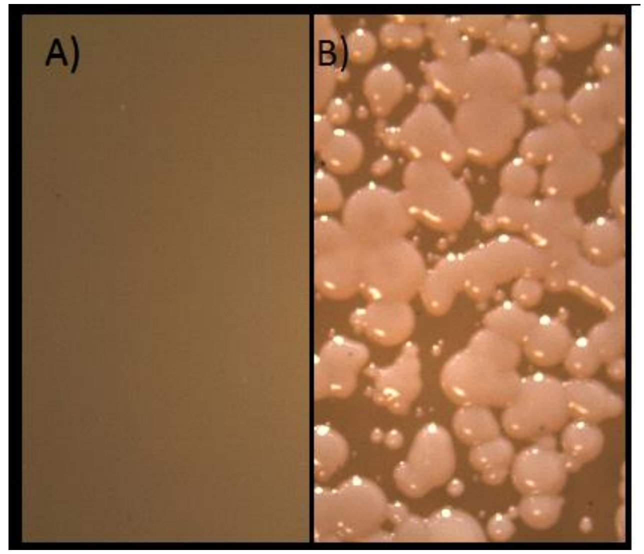

Figure 6. Antibacterial assesment of synthetized AgNPs.

4. CONCLUSIONS

Shown results bring us closer to the possibility of incorporating ultra purified silver nanoparticles as potential antimicrobial agent with lower cost. The influence of iron impurities on the loss of the antimicrobial properties of silver nanoparticles was demostrated. There is a dedicated system for the synthesis of this low molecular weight iron gathering molecules [5]. It was observed and succesfully incorporated in this biological purification synthesis method. The advantage of using this AgNPs is the enhanced biocompatibility achieved by the implementation of biological process during the synthesis. More experiments are being conducted currently that will explain more about the interaction of the synthetized nanoparticles in polymers for its usage in biomedical devices.

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