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Study of Impact of Silver Nano Particle on Biomembrane

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Abstract – Nanotechnology, utilising atomic scale substance tailoring, is anticipated to open new ways to tackle and avoid disease. Metallic nanoparticles, which exhibit enhanced chemical activity due to their broad surface to volume ratios and crystallographic surface structure, are among the most promising nanomaterials with antibacterial properties. Taking into account the recent rise in new resistant strains of bacteria to the most active antibiotics, the analysis of bactericidal nanomaterials is especially timely. Study into the well-known action of silver ions and silver-based materials, like silver nanoparticles, has been urged. The present research studies the impact of silver nanoparticles in the range of 1-100 nm on Gram-negative bacteria utilising Scanning Transmission Electron Microscopy (STEM) high angle annular dark field (HAADF). Our observations suggest that the nanoparticles' bactericidal properties are size-dependent, since the only nanoparticles that have a direct contact with the bacteria preferentially have a diameter of around 1-10 nm.

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INTRODUCTION

The specific mechanism used by silver nanoparticles to cause antimicrobial effect is not well understood and is a matter of debate. However, there are different hypotheses regarding the microbial impact of the action of silver nanoparticles on microbes. Silver nanoparticles have the potential to anchor and eventually infiltrate the bacterial cell layer, inducing structural modifications in the cell membrane, such as cell membrane permeability and cell death. Pits are created on the surface of the cell, and nanoparticles collect on the surface of the cell. Another process by which the cells die can be thought to be the creation of free radicals by silver nanoparticles. When in contact with the bacteria, the creation of free radicals by the silver nanoparticles, and these free radicals have the potential to damage and render the cell membrane brittle, which will inevitably contribute to cell death.

The silver ions that are emitted will interfere with and inactivate several essential enzymes in the thiol groups. Silver-contact bacterial cells carry in silver ions, which disrupt many functions in the cell and kill the cells. And there is the production of reactive oxygen species that are actually created by silver ions inhibiting a respiratory enzyme and attacking the cell itself. Silver is a soft acid, and an acid has a natural propensity to react with a foundation, where a soft acid interacts with a soft foundation. The cells are primarily made up of soft foundations of sulphur and phosphorus. The activity on the cell of these nanoparticles will trigger the reaction to take place and contribute to cell death subsequently. Another truth is that as its key ingredients, the DNA has sulphur and phosphorus; nanoparticles will operate on these soft bases and break the DNA that will certainly contribute to cell death. Silver nanoparticles' association with the DNA's sulphur and phosphorus will contribute to bacterial DNA replication problems and thus terminate the microbes. The nanoparticles were also shown to be able to modulate signal transduction in bacteria. It is a proven reality that the phosphorylation of protein substrates in bacteria affects the transduction of bacterial signals. Only in the tyrosine residues of gram-negative bacteria is dephosphorylation observed. The bacterial peptides' phospho tyrosine profile is changed by the nanoparticles.

The nanoparticles have been found to dephosphorylate the peptide substrates on the residues of tyrosine, leading to inhibition of signal transduction and thus stopping development. The blossoming sector of nanotechnology research is the development of nanoparticles of unique size and composition. The progress in the field of nanotechnology primarily relies on the capacity to synthesise and effectively combine nanoparticles of different material sizes and shapes into complex architecture. However, the production of nanoparticles is a newly proven area, as submicron

or nano sized particles have been synthesised for centuries. In the synthesis protocol, there is an increasing need to produce environmentally benign nanoparticles that do not use harmful chemicals. While microorganisms such as bacteria and yeast are used in many biotechnological applications such as toxic metal remediation, such microorganisms have recently been discovered as potential eco-friendly nanofactories. For the development of metallic nanoparticles, there are two alternate approaches: the "bottom-up" and the "top-down" method.

Bottom-up, or self-assembly, refers to the atom-by-atom, molecule-by-molecule, or cluster-by-cluster creation of a structure. In this method, the building blocks of nanostructures (i.e., nanoparticles) are initially formed and then assembled into the final material utilising chemical or biological methods for synthesis. The increased probability of obtaining metallic nanoparticles with comparatively smaller defects and more homogeneous chemical compositions is a distinct value of the bottom-up method. A appropriate starting content is decreased in size in the top-down method using physical (eg, mechanical) or chemical means. The imperfection of the surface framework is a key downside of the top-down solution. Because of the high aspect ratio, certain defects in the surface structure may have a major influence on the physical properties and surface chemistry of metallic nanoparticles (Kawshik et al., 2009). Due to their possible uses in different areas, such as biotechnology, chemistry , physics and medicine, the production and explanation of noble metal nanoparticles is attracting growing attention in the field of nanotechnology.

Metallic nanoparticles such as electro-chemical, sonochemical and microwave-assisted processes are produced by several techniques in operation, but most of these methods suffer from the use of high energy, dangerous chemicals and purification difficulties (Vijaykumar et al., 2013). Methods of chemical synthesis contribute to the existence of some harmful substances absorbed on the skin that could have a detrimental impact on medical applications (Manisha et al., 2013). In addition, the use of harmful chemicals in the synthesis can not be prevented through chemical processes. Biological standards have recently been developed to override these technological hitches. Currently, the value of biological synthesis is illustrated globally because chemical processes are capital expensive, harmful, non-ecological and poor efficiency (Kowshik et al., 2003). One of the most important areas of study is the production of the nanoparticle. The process used is a micellar process that can be quickly scaled up and enables nanoparticles to be regulated by their structure and size. The particle size of the nanoparticles differs from 1 to 50 nanometers, but relies heavily on the surfactant used. The greatest value of this approach is the multiple formulations and sizes that can be obtained. The metal nanoparticle, both in pure and composite form, has been thoroughly studied among the wide varieties of nanoparticles (Sun et al., 2002,) because of their peculiar optical and electronic properties (Schmid et al., 1998).

There are certain microorganisms that can tolerate concentrations of metal ions and can even expand under certain circumstances, and their tolerance to the metal is the explanation for this phenomena. Efflux processes, modification of solubility and toxicity by reduction or oxidation, biosorption, bioaccumulation, extracellular complex forming or precipitation of metals, and the absence of unique metal delivery systems are the pathways involved in resistance. The involvement of the nitrate reductase enzyme is the most commonly known silver biosynthesis process. Nitrate is transformed by the enzyme into nitrite. The existence of alpha-nicotinamide adenine dinucleotide phosphate reduced type (NADPH)-dependent nitrate reductase in in vitro synthesis of silver using bacteria will eliminate the downstream processing stage that is needed in other situations. Nitrate is converted into nitrite after the decline and the electron is passed to the silver ion; the silver ion is then reduced to silver (Ag+ to Ag0).

The proliferation of new resistant bacterial strains to conventional antibiotics has been a major public health issue, so there is a strong desire to produce new bactericides. This makes current studies especially timely for bactericidal nanomaterials. Bacteria have numerous membrane structures that cause them to be categorised as Gram-negative or Grampositive in a general way. Structural variations lie in the arrangement of the peptidoglycan, a central component of the membrane. In comparison, Gram-positive bacteria skip the outer membrane, but have a peptidoglycan layer of around 30 nm thick. Gram-positive bacteria show only a thin peptidoglycan layer (around 2-3 nm) between the cytoplasmic membrane and the outer membrane. For this purpose, silver-based substances have been commonly employed in many bactericidal applications because silver has long been considered to show a high toxicity to a broad variety of micro-organisms. Few instances are worth noting, such as inorganic composites with a slow silver release rate that are widely used in a number of goods as preservatives; another recent use involves modern compounds consisting of silica gel microspheres containing a silver thiosulfate complex that are blended into plastics for long-lasting antibacterial safety.

In the medical sector, silver compounds have often been used to treat wounds and a number of infections. It is very well known that silver ions have a bactericidal impact on micro-organisms, but the bactericidal process is only partly understood. It has been indicated that ionic silver heavily associates with and inactivates essential enzyme groups of thiol. Experimental data shows that DNA lacks its capacity to reproduce after silver ions

have handled the bacteria. Other experiments have shown signs of structural modifications in the cell membrane as well as the development of silver and sulphur-formed tiny electron-dense granules. Silver ions have been shown to be useful and efficient in bactericidal applications, but nanotechnology offers a fair solution to the production of new bactericides owing to the special properties of nanoparticles.

Metal particles display physical characteristics that vary from both the ion and the bulk content in the nanometer scale range. This makes them show extraordinary properties owing to morphologies with extremely active facets, such as enhanced catalytic activity. We checked silver nanoparticles for four forms of Gram-negative bacteria in this study: E. V. cholera, coli, P. aeruginosa and S. Typhoidism. To research the process by which silver nanoparticles associate with these bacteria, we implemented multiple electron microscopy techniques. We used scanning transmission electron microscopy (STEM) with high angle annular dark field (HAADF) and produced a novel sample preparation that prevents the use of compounds dependent on heavy metals such as OsO4. High resolutions have been reached and x-ray microanalysis has been more detailed.

EXPERIMENTAL PROCEDURE

Nanotechnologies, Inc. synthesised the silver nanoparticles used in this work. Inside a carbon matrix, the final product is a powder of silver nanoparticles that prevents coalescence during synthesis. In order to carry out the association of silver nanoparticles with bacteria, the silver nanoparticle powder is dissolved in water; a Cole-Parmer 8891 ultrasonic cleaner (UC) is used for suspension homogenisation. Solution particles are described by inserting a decrease of the homogeneous suspension in a lacy carbon film copper grid transmission electron microscope (TEM) and then utilising a JEOL 2010-F TEM at an accelerating voltage of 200 kV. Several concentrations of silver nanoparticles (0, 25, 50, 75 and 100 µg ml−1) have been evaluated against each form of bacteria as a first phase. Agar plates were prepared from agar solution, Luria-Bertani (LB) medium broth and the various silver nanoparticles concentrations, accompanied by plating a 10 µl log phase culture sample with an optical density of 0.5 at 595 nm and 37 ⁇ C. By increasing each of the bacteria to a log phase at an optical density at 595 nm of approximately 0.5 at 37 ⁇ C in LB culture medium, the association with silver nanoparticles was studied. Silver nanoparticles were then applied to the solution, creating a homogeneous 100 µg ml-1 suspension and allowing the bacteria to expand for 30 minutes. By centrifugation, the cells are extracted (3000 rpm, 5 min, 4 ⁇ C), washed and then resuspended with a PBS buffer solution. On TEM copper grids with a lacy carbon film, a 10 µl sample drop was deposited and the grid was then subjected to glutaraldehyde vapours for 3 h in order to correct the bacterial sample.

In order to establish the distribution and position of silver nanoparticles, as well as the morphology of the bacteria after treatment with silver nanoparticles, bacteria were examined in a JEOL 2010-F TEM fitted with an Oxford EDS device at an accelerating voltage of 200 kV in scanning mode using the HAADF detector. We used a particular sample preparation method in order to provide a more profound view of the bactericidal function of the silver nanoparticles. E. Samples of coli, previously exposed to silver nanoparticles using the same contact protocol mentioned above, were then calculated by exposure to 2.5% glutaraldehyde solution in PBS for 30 minutes, accompanied by cell dehydration utilising a sequence of 50, 60, 80, 90 and 100% ethanol / PBS solutions and exposure to each solution for 10 minutes in increasing order of e Finally, the cells were embedded into Spurr resin and left for 24 h to polymerize in an oven at 60 ⁇ C. The polymerized samples were sliced into slices with a thickness of approximately 60 nm. In STEM mode, we were then able to study the interior of the bacteria in the TEM. The same experiment was conducted to compare the results of silver in ionic and nanoparticle shape with 100 µg ml−1 of ionic silver from a 1 mM solution of AgNO3. TEM research was also carried out using sample staining.

The sample preparation adopted the same protocol as the cross-sectioned sample slices, but the cells were tinted with a 2 percent OsO4 /cacodylate buffer for 1 h before the dehydration phase. These samples were analysed at an accelerating voltage of 100 kV in a JEOL 2000. Often studied was the electrochemical behaviour of silver nanoparticles in water solution. Using a 25 µm diameter platinum ultramicroelectrode, the stripping voltammetry of silver nanoparticles immersed in an electrolyte solution was done. To detect silver (I) electrochemically at low concentrations, it is necessary to electro-deposit silver onto the electrode surface in a pre-concentration step by holding the potential of the electrode at −0.3 V versus Ag/AgCl for 60 s .This procedure reduces Ag+ to Ag0, which plates onto the electrode surface. The accumulated silver is oxidised to Ag+ and removed from the electrode as the potential is positively swept from −0.3 to +0.35 V, providing a typical stripping peak with a height equal to the Ag+ concentration in the solution.

RESULTS AND DISCUSSIONS

TEM examination of the silver nanoparticles used in this work shows that inside the carbon matrix, the particles appear to be agglomerated (inset figure 1(a)). However, a large amount of nanoparticles that have been released from the

carbon matrix are found owing to the porosity of the carbon and probably the energy supplied by the UC (figure 1(a)). A mean size of 16 nm with a standard deviation of 8 nm was seen by the study of the released particles. Since these nanoparticles have been extracted from the carbon matrix, they may be regarded as healthy.

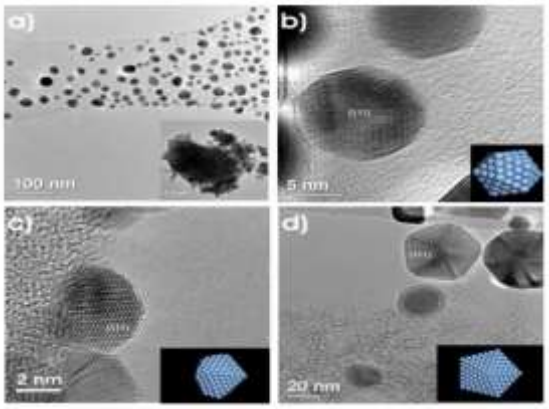

Figure 1: Figure 1 Nanoparticles of silver. (a) TEM graphic of silver nanoparticles released from the carbon matrix; example of agglomerated particles in the carbon matrix in the insert. (b)-(d) The most predominant morphologies of utilised particles. The facets of {111} are named and their respective versions are displayed as insets: (b) icosahedral particle, (c) twin particle, and (d) decahedral particle in the direction

Compared to the nanoparticles left within the carbon matrix, surface particles can improve their reactivity. As the TEM electron beam is condensed in the nanoparticle agglomerates, a fascinating phenomenon occurs; adequate energy is given to free the nanoparticles remaining in the carbon matrix, and the general size distribution of the nanoparticles is obtained: a mean size of 21 nm and a standard deviation of 18 nm. High resolution transmission electron microscopy (HRTEM) reveals that there are cuboctahedral and multiple-twinned icosahedral and decahedral morphologies in around 95 percent of the particles (Figures 1(b)-(d)). There are primarily {111} surfaces in both of these morphologies. Different analysis carried out on silver reactivity has shown that large atom density facets such as {111} support reactivity. A high reactivity of the nanoparticles used in this research is predicted relative to other particles having less {111} facet percentages. In order to observe the impact on bacterial growth, each of the bacteria was checked with varying concentrations of silver nanoparticles.

The findings revealed that with each form, the concentration of silver nanoparticles preventing the growth of bacteria is different, with P. aeruginosa and V. cholera becoming more immune than E. S. and coli. Typhoidism. However, no substantial development for any of the bacteria occurred at concentrations above 75 µg ml−1 (Figure 2(a)). The findings shown in Figures 2(b) and (c) imply that HAADF is useful without the use of heavy-metal staining to assess the existence of even very tiny (about 1 nm) silver nanoparticles on bacteria. This is primarily due to the assumption that electrons that have been dispersed at high angles due mainly to Rutherford-like scattering are produced by HAADF pictures. As a consequence, the picture contrast is related to the atomic number (Z) variations in the sample with strength varying as ⁇ Z2. A broad contrast in the images is provided by the difference in the atomic number of metal nanoparticles (silver) and the organic material (bacteria).

STEM examination of the polymerized slices revealed the inside of the bacteria and revealed that not just on the surface of the cell membrane but even inside the bacteria are the nanoparticles contained (Figures 3(a)-(c)). An elemental mapping study using the x-ray energy dispersive spectrometer (EDS) in the TEM (Figure 3(a)) confirmed this. All over the organism, the nanoparticles were found distributed; they were bound to the membrane and were able to enter the bacteria as well. It was found that only individual particles were bound to the membrane surface and no strong association of the bacterial membrane with the particle agglomerates in the carbon matrix was found. This gives ample proof that only the particles that were able to leave the carbon matrix were able to claim that.

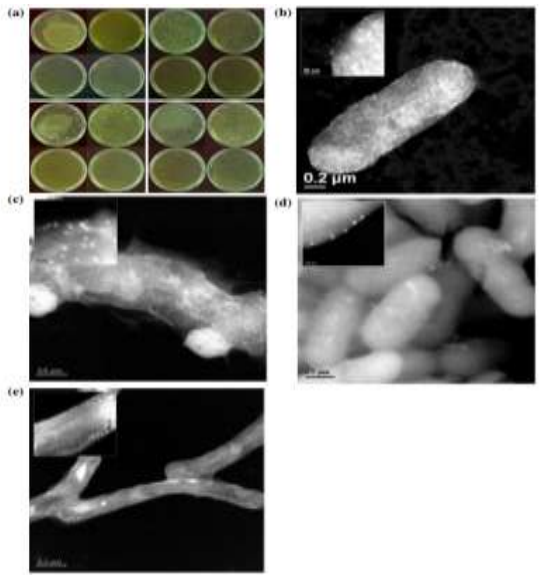

Figure 2. (a) Bacteria that develop at various concentrations of silver nanoparticles on agar plates. Upside-left, E. Coli; upper right hand, S. Typhus; P. aeruginosa, bottom left, and V. cholerae, bottom right. 0 µg ml−1 (above left), 25 µg ml−1 (above right), 50 µg ml−1 (below left) and 75 µg ml−1 (below right). HAADF STEM photos that display the association of silver nanoparticles with bacteria: (b) E. (c) coli, (c) S. Typhus, (d) P. aeruginosa and (e) V. cholerae, respectively. Insets refer to pictures with larger magnification.

Bacteria associate with them. Furthermore, the nanoparticles present within the cells are of identical sizes to those interfering with the membrane (figures 3(b)-(c)); this suggests that the bacteria will only get inside the particles that communicate with the membrane. Higher magnification photos indicate that it is very possible that the nanoparticles located on the surface of the membrane are faceted (Figure 4(a)). Figure 4(b) is a surface plot utilising the region's strength profiles enclosed in Figure 4(a). Figure 4(b) was built by the National Institute of Health with Picture J programme. The contrast of the STEM images is, as stated before, primarily proportional to Z2. The picture amplitude is related to the amount of electrons dispersed, whereas the chance of an electron interfering with an atom's nucleus is directly proportional to the sample's thickness. As the silver particles on the membrane surface are analysed, the atomic weight may be assumed stable, thus the intensities are purely attributable to the particle thickness. The particle's thickness profile shows faceting and a planar face. This implies a decahedral particle interaction, which has only {111} truth.

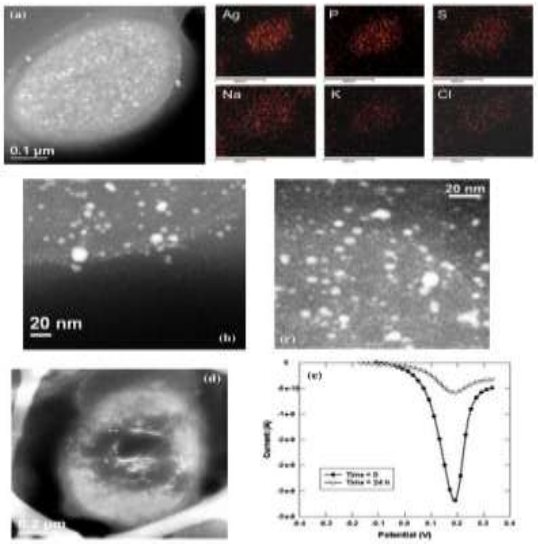

Figure 3: (a) Left: the presence of silver nanoparticles in the membrane and inside of an E is considerable. Sample coli. Right: Elemental mapping from EDS. It can be found that across the study, silver is well dispersed. (b) Amplifying the E. Coli membrane, which specifically observes the existence of silver nanoparticles. (c) A close-up of an E 's interior. Silver nanoparticles-treated coli study. Again, the occurrence of nanoparticles of silver is noted. (d) A image of an E. Silver nitrate treated coli sample, where a strong distinction is found when opposed to the nanoparticle treated sample. As previously reported (3), a centre region of low molecular weight is observed. (e) Stripping of the effects of voltammetry collected for freshly dissolved silver nanoparticles in 0.2 M NaNO3 and the curve determined 24 h later for the same solution.

From the HAADF photos, the size distribution of the nanoparticles interacting with each bacteria form was obtained. With a standard deviation of 2 nm, the mean size of these silver nanoparticles was ~5 nm. The distribution of the size of particles observed to communicate directly with E. In figure 4(d), coli is seen. The lower end of the size range for the published silver nanoparticles correlates to this range (mean size of 16 nm with a standard deviation of 8 nm). The bactericidal impact of the silver nanoparticles is clearly size based. Using the general size distribution mentioned in the manuscript (mean size of 21 nm and a standard deviation of 18 nm), the effective silver concentration was calculated using three hypotheses: (1) all nanoparticles smaller than 10 nm associate with the bacteria; (2) the nanoparticles are spherical and (3) it is necessary to discard the volume of carbon in the sample. If we use the general size distribution to consider the weight of the nanoparticles, the findings show that the ratio of nanoparticles between 1 and 10 nm correlates to the weight.

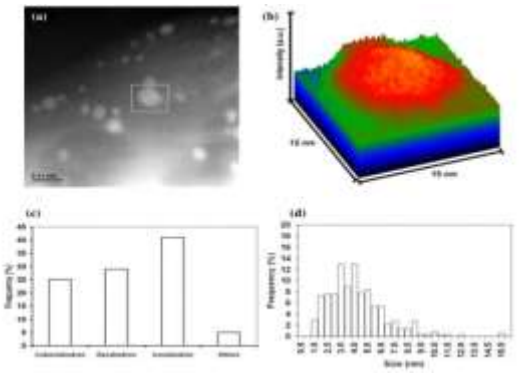

Figure 4. (a) Z-picture comparison of S. Typhus, where we can see silver nanoparticles faceted in the bacterial membrane. (b) The localised region's severity profile in (a). (c) Morphological distribution of nanoparticles used with diameters varying from 1 to 10 nm. (d) Size distribution of nanoparticles that are shown to have contact with E from many HAADF pictures. From coli.

To 0.093% of the survey. Even if this value appears to be minimal, provided the silver nanoparticle concentration of 75 µg ml-1 observed to be effective for all bacteria, it correlates to a large number of nanoparticles per millilitre. A mean diameter of ⁇ 5 nm and a silver density of 1.05 ⁇ 10−14 µg nm−3 is used to measure the amount of particles between 1 and 10 nm ml−11, ⁇ 9.8 ⁇ 1010. Therefore, as there was an OD of 0.5 for the bacterial culture used in our work, which corresponds to ⁇ 5 ⁇ 107 colony forming units (cfu) per ml of solution, the ratio between the amount of silver nanoparticles and cells is ⁇ 2000. A statistical analysis of particle morphologies between 1 and 10 nm found that octahedral and multiple-twinned icosahedral and decahedral forms

are around 98 percent of the particles. The strong reactivity of high density silver {111} facets as seen by many studies. The faceting of the particles as well as the direct association of the {111} facets is corroborated by these previous studies and our study of the thickness map of the nanoparticles contained on the bacterial surface. Electronic effects are found in metal particles of small sizes (about 5 nm), and are characterised as adjustments in the local electronic surface structure due to scale. These effects are stated to increase the reactivity of the surfaces of the nanoparticle. Furthermore, it is rational to propose that the binding power of the particles to the bacteria depends on the contact field of the surface. A higher percentage of the surface can associate specifically with smaller particles than larger particles; the existence of only particles of around 1-10 nm may be clarified by these two factors described before. TEM and staining with OsO4 is used to compare the findings obtained for the bacteria using HAADF.

In TEM mode, the morphologies of the bacteria and the impact of the particles on the bacteria (Figure 5) were quite close to those of STEM (Figures 3(a)-(c). It is found that the silver nanoparticles are distributed in the bacterial membrane as well as in its interior. This corroborates the utility of the approach used in this article, TEM analysis in STEM mode using HAADF. It is not completely known the process by which the nanoparticles are able to infiltrate the bacteria, but Salopek 's previous study indicates that in the case of E. The changes made in the membrane morphology of coli treated with silver nanoparticles can significantly increase its permeability and affect proper transport through the plasma membrane. In our case, the substantial amount of silver nanoparticles contained within the bacteria could be clarified by this process (Figure 3(c)). In studying the bactericidal function, the detection of silver nanoparticles connected to the cell membrane (figures 2(b)-(e)) and within the bacteria (figures 3(a)-(c) is fundamental. Silver would appear to have a stronger preference for interacting with phosphorus and sulphur compounds, as established by the principle of hard and soft acids and bases.

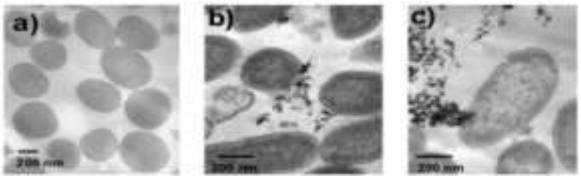

Figure 5: TEM photographs of a study of P. aeruginosa at varying magnifications are displayed. (a) Control samples, i.e. no silver nanoparticles have been used; (b) and (c) samples previously handled with silver nanoparticles, respectively. Silver nanoparticles can be observed within the bacteria, and as opposed to the test sample, noticeable disruption can be seen in the cell membrane.

The bacterial membrane is well known to include several proteins that include sulfur[28]; these could be preferential locations for silver nanoparticles. Nanoparticles located within, on the other hand, would also appear to react within the cell with other proteins containing sulphur, as well as compounds containing phosphorus, such as DNA. To conclude, in processes such as the respiratory chain and cell division, the modifications in morphology presented in the membrane of the bacteria, as well as the potential harm induced by the nanoparticles interacting with the DNA, would influence the bacteria, eventually triggering cell death. The probability of a contribution to the bactericidal impact of the nanoparticles by the silver ions that might be present in the nanoparticle solution has been checked. To do so, using stripping voltammetry, we studied the electrochemical activity of the nanoparticles.

As can be seen in figure 3(e), for silver nanoparticles freshly dissolved in 0.2 M NaNO3, a stripping peak is obtained, along with a peak obtained 24 h later for the same solution. It can be shown that Ag+ is automatically published at a concentration of ~1 μM as comparing with peak heights obtained from solutions with known concentration. After 24 h, the solution was retested, where it was observed that the Ag+ concentration had decreased dramatically (~0.2 μM). The results show that when the nanoparticles are first dissolved, rapid release of Ag+ happens, but only at levels of < 5 μM. There is no more dissolution and the free concentration of Ag+ reduces, probably because of reduction processes to form clusters comprising Ag0 or reassociation with the initial nanoparticles. The existence of micro-molar silver ion concentrations, which will lead to the biocidal activity of silver nanoparticles, was corroborated by this study.

A control experiment was carried out utilising silver nitrate (AgNO3) as a biocide to more precisely demonstrate the disparity between the effects of silver nanoparticles and pure ionic silver. Figures 3(a) and (d) demonstrate the results; the cumulative impact of the silver nanoparticles varies from the impact of just silver ions. In the centre of the bacteria, the silver ions produce the development of a low molecular weight area. This creation of the low density area is a defence mechanism by which the bacteria conglomerate their DNA to defend it from toxic compounds when a membrane disruption is detected by the bacteria. However, as stated by Feng and collaborators, when nanoparticles are used, we have not found proof of the development of a low density area rich in agglomerated DNA; instead, the bacteria present a large number of small silver nanoparticles within the bacteria. An additional reason for the nanoparticles' contact with the bacteria may be electrostatic forces. The literature has stated that, because of the dissociation of an excess amount of carboxylic and other groups in the membrane, the total surface of the bacteria is negatively charged

at biological pH values. On the other hand, the nanoparticles are trapped in a carbon matrix (insulator) where, due to their movement within the matrix, there is undoubtedly agitation of the nanoparticles; this may produce a surface charge. For these factors, the electrostatic attraction of nanoparticles and bacteria can be anticipated. An fascinating research for our future work introduces this form of interaction.

CONCLUSIONS

A large size distribution and morphologies with highly reactive facets are exhibited by silver nanoparticles used in this work, {111}. We have observed that silver nanoparticles work predominantly against Gram-negative bacteria in three ways: (1) nanoparticles bind to the surface of the cell membrane mainly in the range of 1-10 nm and dramatically disrupt its proper operation, such as permeability and respiration; (2) they are able to infiltrate the bacteria within and inflict more harm by interfering with sulphur and phosphate. In this research, we have applied HAADF-STEM and found it to be very useful in the study of silver particle bactericidal effects, and it can be generalised to other similar experiments.

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