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NANOTECHNOLOGY IN MEDICINE AND ANTIBACTERIAL EFFECT OF SILVER NANOPARTICLES

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Nanotechnology is expected to open some new aspects to fight and prevent diseases using atomic scale tailoring of materials. The ability to uncover the structure and function of biosystems at the nanoscale, stimulates research leading to improvement in biology, biotechnology, medicine and healthcare. The size of nanomaterials is similar to that of most biological molecules and structures; therefore, nanomaterials can be useful for both in vivo and in vitro biomedical research and applications. The integration of nanomaterials with biology has led to the development of diagnostic devices, contrast agents, analytical tools, physical therapy applications, and drug delivery vehicles**.** In all the nanomaterials with antibacterial properties, metallic nanoparticles are the best. Nanoparticles increase chemical activity due to crystallographic surface structure with their large surface to volume ratio. The importance of bactericidal nanomaterials study is because of the increase in new resistant strains of bacteria against most potent antibiotics. This has promoted research in the well known activity of silver ions and silver-based compounds, including silver nanoparticles. This effect was size and dose dependent and was more pronounced against gram-negative bacteria than gram-positive organisms.

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1. Introduction

Most of the natural processes also take place in the nanometer scale regime. Therefore, a confluence of nanotechnology and biology can address several biomedical problems, and can revolutionize the field of health and medicine [1]. Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences in several ways like imaging [2], sensing [3], targeted drug delivery [4] and gene delivery systems [5] and artificial implants [6]. The new age drugs are nanoparticles of polymers, metals or ceramics, which can combat conditions like cancer [7] and fight human pathogens like bacteria [8-12].

The development of new resistant strains of bacteria to current antibiotics [13] has become a serious problem in public health; therefore, there is a strong incentive to develop new bactericides [10]. Bacteria have different membrane structures which allow a general classification of them as Gram-negative or Gram positive. The structural differences lie in the organization of a key component of the membrane, peptidoglycan. Gram negative bacteria exhibit only a thin peptidoglycan layer (~2–3 nm) between the cytoplasmic membrane and the outer membrane [14]; in contrast, Gram-positive bacteria lack the outer membrane but have a peptidoglycan layer of about 30 nm thick [15]. Silver has long been known to exhibit a strong toxicity to a wide range of micro-organisms [16]; for this reason silver-based compounds have been used extensively in many bactericidal applications [17, 18]. Silver compounds have also been used in the medical field to treat burns and a variety of infections [19]. Several salts of silver and their derivatives are commercially employed as antimicrobial agents [20]. Commendable efforts have been made to explore this property using electron microscopy, which has revealed size dependent interaction of silver nanoparticles with bacteria [11]. Nanoparticles of silver have thus been studied as a medium for antibiotic delivery [21], and to synthesize composites for use as disinfecting filters [22] and coating materials [23]. However, the bactericidal property of these nanoparticles depends on their stability in the growth medium, since this imparts greater retention time for bacterium–nanoparticle interaction. There lies a strong challenge in preparing nanoparticles of silver stable enough to significantly restrict bacterial growth.

Studies were carried out on both antibiotic resistant (ampicillin- resistant) and non-resistant strains of gram-negative (*Escherichia coli*) and a non-resistant strain of gram-positive bacteria (*Staphylococcus aureus*). A multi-drug resistant strain of gram-negative (*Salmonella typhus*, resistant to chloramphenicol, amoxycilin and trimethoprim) bacteria was also subjected to analysis to examine the antibacterial effect of the nanoparticles [24]. Efforts have been made to understand the underlying molecular mechanism of such antimicrobial actions. The effect of the nanoparticles was found to be significantly more pronounced on the gram-negative strains, irrespective of whether the strains were resistant or not, than on the gram-positive organisms. We attribute this enhanced antibacterial effect of the nanoparticles to their stability in the medium as a colloid, which modulates the phosphotyrosine profile of the bacterial proteins and arrests bacterial growth.

The bactericidal effect of silver ions on micro-organisms is very well known; however, the bactericidal mechanism is only partially understood. It has been proposed that ionic silver strongly interacts with thiol groups of vital enzymes and inactivates them [25, 26]. Experimental evidence suggests that DNA loses its replication ability once the bacteria have been treated with silver ions [19]. Other studies have shown evidence of structural changes in the cell membrane as well as the formation of small electron-dense granules formed by silver and sulfur [19, 27]. Silver ions have been demonstrated to be useful and effective in bactericidal applications, but due to the unique properties of nanoparticles nanotechnology presents a reasonable alternative for development of new bactericides. Metal particles in the nanometer size range exhibit physical properties that are different from both the ion and the bulk material. This makes them exhibit remarkable properties such as increased catalytic activity due to morphologies with highly active facets [28–33]. We can apply several electron microscopy techniques to study the mechanism by which silver nanoparticles interact with these bacteria. We can use high angle annular dark field (HAADF) scanning transmission electron microscopy (STEM), and developed a novel sample preparation that avoids the use of heavy metal based compounds such as OsO4. High resolutions and more accurate x-ray microanalysis were obtained.

2. Nanotechnology

The term "nanotechnology" was first defined by Tokyo Science University, Norio Taniguchi in a 1974 paper [34] as follows: "'Nano-technology' mainly consists of the processing of, separation, consolidation, and deformation of materials by one atom or one molecule. Nanotechnology and nanoscience got a boost in the early 1980s with two major developments: the birth of cluster science and the invention of the scanning tunneling microscope (STM). This development led to the discovery of fullerenes in 1985.

As practiced at Rice University, there are three distinct nanotechnologies:

- **"Wet" nanotechnology** is the study of biological systems that exist primarily in a water environment. The functional nanometer-scale structures of interest here are genetic material, membranes, enzymes and other cellular components. The success of this nanotechnology is amply demonstrated by the existence of living organisms whose form, function, and evolution are governed by the interactions of nanometer-scale structures.

- **"Dry" nanotechnology** derived from surface science and physical chemistry, focuses on fabrication of structures in carbon (for example, fullerenes and nanotubes), silicon, and other inorganic materials. Unlike the "wet" technology, "dry" techniques admit use of metals and semiconductors. The active conduction electrons of these materials make them too reactive to operate in a "wet" environment, but these same electrons provide the physical properties that make "dry" nanostructures promising as electronic, magnetic, and optical devices. Another objective is to develop "dry" structures that possess some of the same attributes of the self-assembly that the wet ones exhibit.
- **Computational nanotechnology** permits the modeling and simulation of complex nanometer-scale structures. The predictive and analytical power of computation is critical to success in nanotechnology: nature required several hundred million years to evolve a functional "wet" nanotechnology; the insight provided by computation should allow us to reduce the development time of a working "dry" nanotechnology to a few decades, and it will have a major impact on the "wet" side as well.

These three nanotechnologies are highly interdependent. The major advances in each have often come from application of techniques or adaptation of information from one or both of the others.

3. Nanotechnology in the area of Medicine

Applying nanotechnology for treatment, diagnosis, monitoring, and control of diseases has been referred to as “nanomedicine”. Although the application of nanotechnology to medicine appears to be a relatively recent trend, the basic nanotechnology approaches for medical application date back several decades. The first example of lipid vesicles which later became known as **liposomes** were described in 1965 [35]; the first controlled **release polymer system** of macromolecules was described in 1976 [36]; the first long circulating stealth polymeric **nanoparticle** was described in 1994 [37]; the first **quantum dot** bioconjugate was described in 1998 [38, 39]; and the first **nanowire nanosenser** dates back to 2001 [40]. Recent Studies on new targeted nanoparticle contrast agents for early characterization of atherosclerosis and cardiovascular pathology at the cellular and molecular levels that might represent the next frontier for combining imaging and rational drug delivery to facilitate personalized medicine [41]. Nanotechnology-based highly efficient markers and precise, quantitative detection devices for early diagnosis and for therapy monitoring will have a wide influence in patient management, in improving patient’s quality of life and in lowering mortality rates, in diseases like cancer and Alzheimer’s disease.

- **Multi-functional nanoparticles for cancer therapy**

Biodegradable chitosan nanoparticles encapsulating quantum dots were prepared by D. K. Chatterjee and Y. Zhang, with suitable surface modification to immobilize both tumor targeting agent and chemokine on their surfaces. The interactions between immune cells and tumor cells were visualized using optical microscope. Use of Quantum dots in the treatment of cancer is a great advancement in this area. Quantum dots glow when exposed to UV light. When injected they seep into cancer tumour. The surgeon can see the glowing tumour. Nanotechnology could be very helpful in regenerating the injured nerves. During the last decade, however, developments in the areas of surface microscopy, silicon fabrication, biochemistry, physical chemistry, and computational engineering have converged to provide remarkable capabilities for understanding, fabricating and manipulating structures at the atomic level. The rapid evolution of this new science and the opportunities for its application promise that nanotechnology will become one of the dominant technologies of the 21st century.

- **Nanoscience enables early detection of Alzheimer’s Disease**

Brain represents one of the most complex systems in biomedicine. With an improved understanding of brain functioning, better diagnosis and treatment for neurodegenerative diseases like Alzheimer’s is offered by nanotechnology [42]. Presently, the prevailing problem is early detection for effective treatment of the disease. An ideal diagnostic tool for Alzheimer’s disease (AD) must have specificity & sensitivity more than 80% for its early diagnosis & excluding other causes. Since the neurodegeneration process begins well before AD becomes symptomatic the potential for early detection is another important characteristic of an ideal diagnostic tool. Nanotechnology can be the basis of new tools for very early detection of AD. Nanotechnology in the diagnosis of AD came into light after two articles were published in February 2005. The two detection approaches proposed in those papers were the Bio-barcode assay (BCA) & Localized surface plasmon resonance (LSPR) technology [43-44].

4. Health and safety implications from nanoparticles

Nanopollution is a generic name for all waste generated by nanodevices or during the nanomaterials manufacturing process. This kind of waste may be very dangerous because of its size. It can float in air the and might easily penetrate animal and plant cells causing unknown effects. Most human-made nanoparticles do not appear in nature, so living organisms may not have appropriate means to deal with nanowaste.

The smaller a particle, the greater its surface area to volume ratio and the higher its chemical reactivity and biological activity. The greater chemical reactivity of nanomaterials results in increased production of reactive oxygen species (ROS), including free radicals [45]. ROS production has been found in a diverse range of nanomaterials including carbon fullerenes, carbon nanotubes and nanoparticle metal oxides. ROS and free radical production is one of the primary mechanisms of nanoparticle toxicity; it may result in oxidative stress, inflammation, and consequent damage to proteins, membranes and DNA [45].

Nanomaterials have proved toxic to human tissue and cell cultures, resulting in increased oxidative stress, inflammatory cytokine production and cell death [46]. Unlike larger particles, nanomaterials may be taken up by cell mitochondria [47] and the cell nucleus [48, 49]. Studies demonstrate the potential for nanomaterials to cause DNA mutation [49] and induce major structural damage to mitochondria, even resulting in cell death [47, 50]. Size is therefore a key factor in determining the potential toxicity of a particle. However it is not the only important factor.Other properties of nanomaterials that influence toxicity include: chemical composition, shape, surface structure, surface charge, aggregation and solubility,[45] and the presence or absence of functional groups of other chemicals [51].

Significant environmental, health, and safety issues might arise with development in nanotechnology since some negative effects of nanoparticles in our environment might be overlooked. However nature itself creates all kinds of nano objects, so probable dangers are not due to the nanoscale alone, but due to the fact that previously non-toxic materials can become harmful when ingested or inhaled as nanoparticles. Social risks related to nanotechnology development include the possibility of military applications of nanotechnology in biological warfare, chemical warfare, ammunitions and armaments and even as implants for soldier "enhancement." Enhanced surveillance capabilities through nano-sensors are also of concern to privacy rights advocates.In discussing issues related to nanotechnology, the acronym NELSI is used to signify nanotechnology's ethical, legal, and social implications.

5. Silver as a biocide

Silver (Ag) is a transition metal element having atomic number-47 and atomic mass-107.87. The medicinal uses of silver have been documented since 1000 B.C. Silver is a health additive in traditional Chinese and Indian Ayurvedic medicine [52]. Its action as an antibiotic comes from the fact that it is a non-selective toxic "biocide." Silver based antimicrobial biocides are used as wood preservatives. In water usage, silver and copper based disinfectants are used in hospital and hotel distribution systems to control infectious agents (for example, *Legionella*). Silver together with copper, is commonly used to inhibit bacterial and fungal growth in chicken farms and in post harvested cleaning of oysters. Silver used to sterilize recycled water aboard the MIR space station and on the NASA space shuttle [53]. Microdyn (colloidal silver in gelatin) is sold in supermarkets to disinfect salad vegetables and drinking water. Johnson Mathey Chemicals (Nottingham, UK) developed an inorganic composite (immobilized slow-release silver product) for use as a preservative in cosmetics, toiletries, and similar retail hygine-sensitive products [54]. In Japan, a new compound (Amenitop, silica gel microspheres containing a silver-thiosulfate complex) is mixed into plastics for lasting antibacterial protection [18]. Silver halide is often incorporated into prescription eye glasses for reversible “photochromatic” protection, as it decreases transmitted visible light. Silver resistance is important to monitor because modern technology has developed a wide range of products that depend on silver as a key microbial component. In the late 1970s, Robert O. Becker discovered that silver ions promote bone growth and kill surrounding bacteria. Silver kills some 650 different disease organisms. Silver based topical dressing has been widely used as a treatment for infections in burns, open wounds and chronic ulcers. The Silver nanoparticles and Ag^+ carriers can be beneficial in delayed diabetic wound healing as diabetic wounds are affected by many secondary infections. These nanoparticles can help the diabetic patients in early wound healing with minimal scars [55]. Silver nitrate is still a common antimicrobial used in the treatment of chronic wounds [56].

6. Colloidal Silver

Scientists have discovered that the body's most important fluids are colloidal in nature: suspended ultra-fine particles. Blood, for example, carries nutrition and oxygen to the body cells. This led to studies with colloidal silver (electrical silver atoms). An electro-colloidal process, which is known to be the best method, is used for manufacturing the Colloidal silver. Colloidal silver appears to be a powerful, natural antibiotic and preventative against infections. Acting as a catalyst, it reportedly disables the enzyme that one-celled bacteria, viruses and fungi need for their oxygen metabolism. They suffocate without corresponding harm occurring to human enzymes or parts of the human body chemistry. The result is the destruction of disease-causing organisms in the body and in the food.

7. Synthesis of silver nanoparticles

Nanometer sized silver particles were synthesized by inert gas condensation and co-condensation techniques. Both techniques are based on the evaporation of a metal into an inert atmosphere with the subsequent cooling for the nucleation and growth of the nanoparticles. The size and morphology of the nanoparticles were analysed with a transmission electron microscope (TEM). The stability of nanoparticles was examined by exposing them to ambient conditions for one month. The antibacterial efficiency of the nanoparticles was investigated by introducing the particles into a media containing bacteria. The antibacterial investigations were performed in solution and on petri dishes. The silver nanoparticles were found to exhibit antibacterial effects at low concentrations. The antibacterial properties were related to the total surface area of the nanoparticles. Smaller particles with a larger surface to volume ratio provided a more efficient means for antibacterial activity. The nanoparticles were found to be completely cytotoxic to E. coli for surface concentrations as low as 8 μg of Ag/cm2 [12].

8. Effect of silver nanoparticles on the phosphotyrosine profile of bacterial proteins

By the examination of the phosphotyrosine content of proteins derived from gram positive and negative bacteria using a specific monoclonal antibody, the possible effect of nanoparticles on bacterial signal transduction affecting growth can be studied. Phosphorylation of various protein substrates is now well established in bacterial species [57] and is found to influence bacterial signal transduction [58]. Hardly any change in the profile of tyrosine phosphorylated proteins was observed for *S. aureus* upon treatment with silver nanoparticles; however, there was noticeable dephosphorylation of two peptides of relative masses 150 and 110 kDa in *E. coli* and *S. typhi* exposed to nanoparticles [24]. As tyrosine phosphorylation in bacteria would lead to activation of various protein substrates like RNA polymerase sigma factors and UDPglucose dehydrogenases [59], decreased phosphorylation may reflect inhibition of activity of these enzymes with critical implications on bacterial growth. A recent report has described tyrosine phosphorylation of bacterial single-stranded DNA-binding proteins (BsSSB), ubiquitous molecules binding DNA in various functional stages like replication and recombination [60]. A phospho-signalling pathway has also been shown to be critical for bacterial cell cycle progression [61]. The identity of the substrate peptides and that of the putative tyrosine phosphatases responsible for observed dephosphorylation in gram-negative bacteria, as described by Siddhartha et al., are yet to be established and can be the subject for future research. The present findings, along with reported interactions of silver nanoparticles with thiol rich enzymes and bacterial genomic DNA [11], can explain the inhibitory effect of the nanoparticles on growth of gram negative bacteria. Interestingly, phosphorylation of protein tyrosine kinases involved in exopolysaccharide and capsular polysaccharide biosynthesis and transport has been reported in a number of gram-negative and gram-positive bacteria [62].

9. Conclusions

Silver nanoparticles exhibit a broad size distribution and morphologies with highly reactive facets. The major mechanism through which silver nanoparticles manifested antibacterial properties is by anchoring to and penetrating the bacterial cell wall, and modulating cellular signalling by dephosphorylating putative key peptide substrates on tyrosine residues. Silver nanoparticles act primarily in three ways against Gram-negative bacteria: **(1)** nanoparticles mainly in the range of 1–10 nm attach to the surface of the cell membrane and drastically disturb its proper function, like permeability and respiration; **(2)** they are able to penetrate inside the bacteria and cause further damage by possibly interacting with sulfur- and phosphorus-containing compounds such as DNA;

(3) nanoparticles release silver ions, which have an additional contribution to the bactericidal effect of the silver nanoparticles [19]. Although bacterial cell lysis could be one of the reasons for the observed antibacterial property, nanoparticles also modulate the phosphotyrosine profile of putative bacterial peptides, which could thus affect bacterial signal transduction and inhibit the growth of the organisms. The effect is dose dependent and is more pronounced against gram negative organisms than gram-positive ones. The antibacterial effect of nanoparticles is independent of acquisition of resistance by the bacteria against antibiotics. However, further studies must be conducted to verify if the bacteria develop resistance towards the nanoparticles and to examine cytotoxicity [63] of nanoparticles towards human cells before proposing their therapeutic use.

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