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BIOMEDICAL APPLICATIONS AND TOXICITY OF NANOSILVER: A REVIEW

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ABSTRACT

Nanotechnology is a promising arena for generating new applications in medicine.

It is advancing rapidly due to the great progress achieved in various fileds including electronics, machanics, cosmetics, food, etc. In order to successfully bifunctionalise nanoparticles for a given biomedical application, a wide range of chemical, physical and biological factors have to be taken into account. Silver nanoparticles (AgNPs) exhibit strong antibacterial activity owing to their large surface to volume ratios and crystallographic surface structure. Nanosilver particles have been widely used in a range of biomedical applications including diagnosis, treatment, medical device coatings, drug delivery and personal health care products. With the growing application of nanosilver particles in medical contexts, it is becoming necessary for a better understanding the mechanisms of action, biological interactions and their potential toxicity on exposure. This review aims to provide critical assessment of the current understanding of antibacterial activity, biomedical applications and toxicity of silver nanoparticles.

KEYWORDS

Antibacterial, antiviral , diagnosis, toxicity, therapeutics.

INTRODUCTION

Nanotechnology is defined as the design, characterization and application of structures, devices and systems by controlling shape and size at nanometer scale level (ranging from 1 to 100nm).[1, 2] A nanometer is one billionth of a meter (10 - 9m). Nano-size particles of less than 100nm in diameter are currently attracting increasing attention for the wide range of new applications in various fields of industry. Silver nanoparticles (nanosilver or AgNPs) have attracted increasing interest due to their unique physical, chemical and biological properties compared to their macro-scaled counterparts.[3] AgNPs have distinctive physico-chemical properties, including a high electrical and thermal conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity and non linear optical behaviour.[4, 5] These properties are made use in production of inks, microelectronics, and medical imaging devices.[6, 7] Also, silver nanoparticles exhibit broad spectrum bactericidal and fungicidal activity that has made them extremely popular in a diverse range of consumer products including plastics, soaps, pastes, food and textiles, increasing their market value.[8] Furthermore, silver nanoparticles are being used as antimicrobial agents in many public places such as railway stations and elevators in China, and they are said to show good antimicrobial action.[9] According to the Project on Emerging Nanotechnologies PEN, over 1628 manufacturer-identified, nanotechnology-enabled products have entered the commercial market place around the world from 30 different countries. Among them, there are 383 products utilizing nanosilver (24% of products listed), this has made nanosilver the largest and fastest growing class of nanoparticles (Fig 1) in consumer products applications (http://www.nanotechproject.org).

The broadest definition of an antibacterial is an agent that interferes with the growth and reproduction of bacteria and antibacterial activity is related to compounds that locally kill bacteria or slow down their growth without being in general toxic to target tissue.[10] In general, agents that can slow down bacterial growth are classified as bactericidal or bacteriostatic. Antibacterial agents are paramount to fight infectious diseases. However, with their extensive use and abuse, the emergence of bacterial resistance to antibacterial drugs has become a common phenomenon, which is a major problem leading to drug resistance.[11] Resistance is an evolutionary process taking place during, for example, antibiotic therapy, and leads to inheritable resistance. In addition, horizontal gene transfer by conjugation, transduction or transformation can be a possible way for resistance to build up.[12] Antibiotics represent one of the most successful forms of therapy in medicine. But the efficiency of antibiotics is compromised by a growing number of antibiotic-resistant pathogens. Antibiotic-resistant bacteria that are difficult or impossible to treat are becoming increasingly common and are causing a global health crisis. In addition, drawbacks for conventional antimicrobial agents are not only the development of multiple drug resistance, but also adverse side effects. Furthermore, drug resistance enforces high-dose administration of antibiotics, often generating intolerable toxicity. This has prompted the development of alternative strategies to treat bacterial diseases. Among them, nanoscale materials have emerged as novel antimicrobial agents. Especially, several classes of antimicrobial NPs and nanosized carriers for antibiotics delivery have proven their effectiveness for treating infectious diseases, including antibiotic-resistant ones, in vitro as well as in animal models.[13] This review in particular discuss the recent developments, current perceptive, biological actions and toxicity of silver nanoparticles.

MECHANISMS OF ACTION OF NANOSILVER

The utilization of silver as a disinfecting agent is not new, and silver compounds were shown to be effective against both aerobic and anaerobic bacteria by precipitating bacterial cellular proteins and by blocking the microbial respiratory chain system.[14, 15] The chief structural differences lie in the organization of a key component of the membrane, peptidoglycan. Gram-negative bacteria exhibit only a thin peptidoglycan layer (~2-3nm) between the cytoplasmic membrane and the outer membrane ; in contrast, Gram-positive bacteria lack the outer membrane but have a peptidoglycan layer of about 30 nm thick.[16, 17] Nanoparticles show good antibacterial properties arising from their large surface area to volume ratio providing desirable contact with bacterial cell membranes[18] Several studies propose that AgNPs may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell.[19]

The possible mechanisms of action are

1. Better contact with the microorganism-nanometer scale silver provides an extremely large surface area for contact with bacteria. The nanoparticles get attached to the cell membrane and also penetrate inside the bacteria.[20]
2. Bacterial membranes contain sulfur-containing proteins and AgNPs, like Ag+, can interact with them as well as with phosphorus-containing compounds like DNA, perhaps to inhibit the function.[21, 22]
3. Silver (nanoparticles or Ag+) can attack the respiratory chain in bacterial mitochondria and lead to cell death.[23]
4. AgNPs can have a sustained release of Ag+ once inside the bacterial cells (in an environment with lower pH), which may create free radicals and induce oxidative stress, thus further enhancing their bactericidal activity.[21] Such interactions in the cell membrane would prevent DNA replications, which would lead to bacterial death (Fig 2).

BIOMEDICAL APPLICATIONS OF SILVER NANOPARTICLES

Nanomedicine, the application of nanotechnology to healthcare holds great promise for revolutionising medical treatments and therapies in areas such as imaging, faster diagnosis, drug delivery and tissue regeneration, as well as the development of new medical products. Indeed, materials and devices of nanometric dimensions are already approved for clinical use and numerous products are being evaluated in clinical trials.[24] Nanosilver particles are generally smaller than 100nm and contain 20-15,000 silver atoms. At nanoscale, silver exhibits remarkably unusual physical, chemical and biological properties. Due to its strong antibacterial activity, nanosilver coatings are used on various textiles as well as coatings on certain implants. Bacterial adhesion to the surface of implants in surgical procedures represents a major problem in surgeries, as it incurs high medical costs and could lead to postoperative infections. Different strategies have been developed to decrease the incidence of bacterial infections related medical devices failure. One approach is the modification of the surface of the devices using antibacterial coatings designed to be non-fouling, thus minimizing microbial adhesion. The ability of silver nanoparticles to destroy infectious micro-organisms makes them an attractive candidate for use against "super-bugs" resistant to antibiotics. Further, nanosilver is used for treatment of wounds and burns or as a contraceptive and marketed as a water disinfectant and room spray. Thus, use of nanosilver is becoming more and more widespread in medicine (Fig 3) and related applications.[25]

Nanosilver in diagnosis and imaging

In the last decade, the field of molecular diagnostics has witnessed an explosion of interest in the use of nanomaterials in assays for gases, metal ions, DNA and protein markers for many diseases. Intense research has been fuelled by the need for practical, robust, and highly sensitive and selective detection agents that can address the deficiencies of conventional technologies.

Chemists are playing an important role in designing and fabricating new materials for application in diagnostic assays.[26] Nanomaterials offer significant advantages over conventional diagnostic systems with regard to sensitivity, selectivity and practicality. Zhou et al.[27] developed a silver nanoparticle array biosensor for clinical detection of serum p53 in head and neck squamous cell carcinoma. This is the first clinical application of localized surface plasmon resonance (LPSR) using triangular silver nanoparticle array in head and neck squamous cell

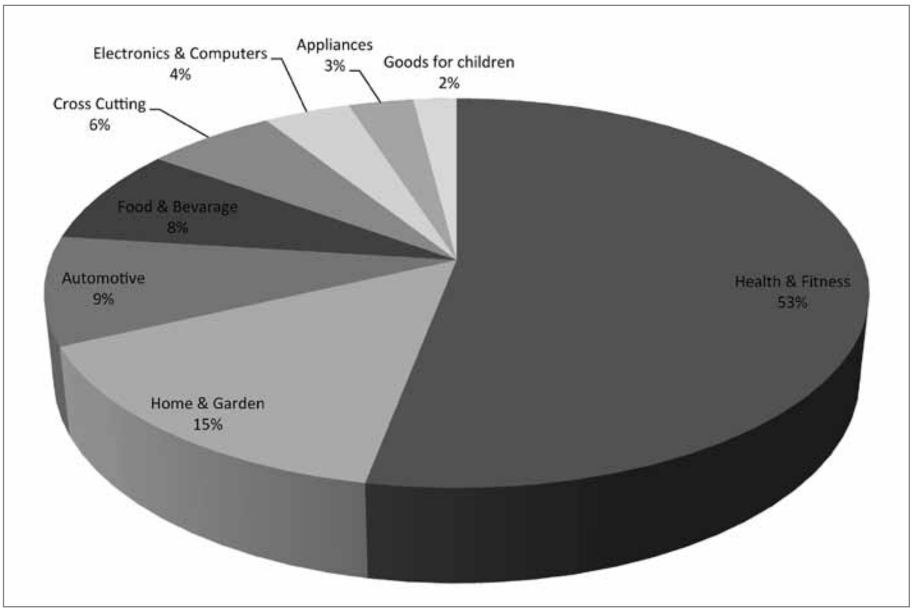

Figure 1: Nanoparticles production in different catogeries.

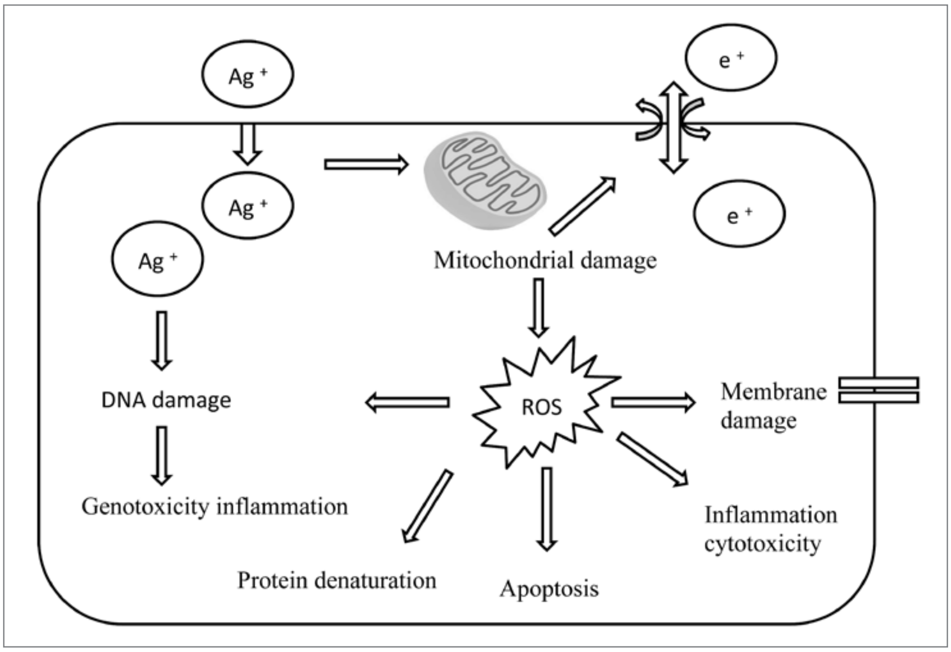

Figure 2: Illustration of toxicity mechanism of nanoparticles mediated by ROS response.

carcinoma (HNSCC). AgNPs are also employed to produce dual-imaging/therapy-immunotargeted nanoshells to locate cancer cells which can absorb light and selectively destroy targeted cancer cells through photothermal therapy.[28] In addition, AgNPs can detect the interaction between amyloid β-derived diffusible ligands (ADDL) and the anti-ADDL antibody, which are related to the development of Alzheimer's disease.[29] Furthermore, nanosilver is used in treatment of variety of clinical conditions including epilepsy, venereal infections, acnes, leg ulcers, and orthopaedics. The list is vast and due to space limitation and the immense area of the subject, the readers are advised to consult the latest review papers.[30, 31]

In early cancer detection, Au-Ag nanorods were used in a recent study as a nanoplatform for multivalent binding by multiple aptamers, so as to increase both the signal and binding strengths of the aptamers in cancer cell recognition. The molecular assembly of aptamers on the nanorods was shown to lead to a 26-fold higher affinity than the original aptamer probes.[32] Thus, these nanorod-aptamer conjugates are highly promising for use in specific cell targeting, as well as having the detection and targeting ability needed for cell studies, disease diagnosis, and therapy.

Nanosilver in therapeutics

Wound Dressing

Wound healing is regarded as a complex and multiple-step process involving integration of activities of many different tissues and cell lineages.[33] The most well documented and commonly used application of silver nanoparticles for this is in the use of wound dressings. Acticoat® is the first commercial wound dressing made up of two layers of polyamide ester membranes covered with nanocrystalline silver.[18] Silver in the form of nanoparticles seems to promote healing and achieve better cosmetic results (compared with the commonly used silver compounds that have been in use for many years). The proposed mechanism is that silver nanoparticles facilitate the proliferation and migration of keratinocytes, reduce the formation of collagen by fibroblasts and modulate the number of cytokines produced.[1] Interestingly, nanosilver is a very effective fungicide as well as having antiviral properties.[34] In the study of[35] the nanocrystalline silver-based dressing, Acticoat® Antimicrobial Barrier dressing was confirmed to provide the fastest and broadest-spectrum fungicidal activity among all tested wound dressings (including those containing silver nitrate or silver sulfadiazine). It also overcomes several problems associated with previously used wound-dressings, like tissue irritation and insufficiently wide spectrum of antifungal properties. Sibbald et al.[36] conducted a prospective study to evaluate the use of silver nanoparticles dressing on a variety of chronic non-healing wounds. The study concluded that silver nanoparticles dressing has a beneficial effect of protecting the wound site from bacterial contamination. Compared with other silver compounds, AgNPs seem also to promote healing and achieve better cosmetics after healing. Taken together, the use of silver nanoparticles in the aspects of wound healing seems to hold the greatest promise.

Silver impregnated catheters

The central venous catheter (CVC) is a commonly used device in managing acutely ill patients in the hospital. They can become colonised with viable micro-organisms within 24 hours of insertion, which can rapidly form biofilm. This colonisation is a precursor of catheter-related bloodstream infections (CR-BSI), which are associated with substantial morbidity, mortality, prolonged hospital stay and increased cost. Antimicrobials have been incorporated into the bulk material of CVC or applied to their surfaces as a coating in an attempt to reduce the incidence of CVC colonisation and infection. Bloodstream infections are major complications in patients who require a CVC. Several infection control measures have been developed to reduce bloodstream infections, one of which is CVC impregnated with various forms of antimicrobials (either with an antiseptic or with antibiotics).[37] It was shown that the overall rate of catheter-related blood stream infections was significantly lower when silver impregnated central venous catheters (CVC) than in the conventional format.[1] Nonetheless, there is a risk that the increasing use of antibiotic-impregnated catheters could lead to eventual bacterial resistance. A new generation of silver-

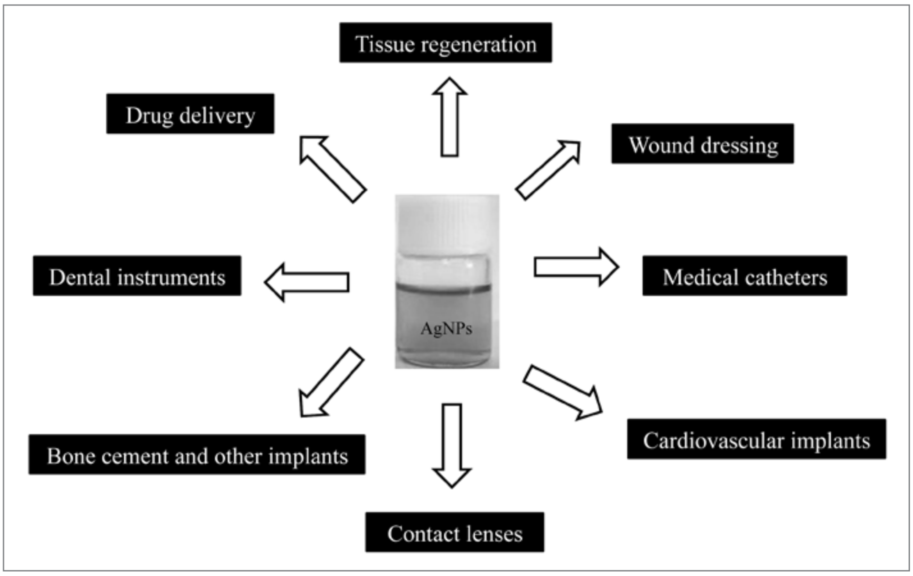

Figure 3: Biomedical applications of silver nanoparticles.

impregnated catheters based on the use of an inorganic silver powder, on which silver ions are bonded with an inert ceramic zeolite, has become available for clinical use. In a recent study comparing these silver-impregnated catheters with standard catheters in terms of incidence of catheter-related blood stream infections, it was shown that overall colonization rate was significantly lower in the silver-impregnated CVC tips. In addition, tip colonization by coagulase-negative staphylococci in the silver-impregnated CVC was lower. It would therefore appear that silver-impregnated catheters are destined for increasing use.[38]

Recently, antibacterial coatings on catheters for acute dialysis were obtained by an innovative and patented silver deposition technique[39] achieved for acute haemodialysis. The surface of catheter was decorated with AgNPs by photoreduction deposition. As-obtained material was characterized by good stability and high activity against E. coli after 30 days of soaking in water flow.

Surgical Mesh

Surgical meshes are used to bridge large wounds and for tissue repairs. Though these meshes are effective, they are susceptible to microbial infections. One million nosocomial infections occur each year in patients with prosthetic devices. To decrease the prosthetic infection rate, multiple antibacterial coatings have been developed for use on medical devices such as urinary catheters, endotracheal tubes and central venous catheters. Silver nanoparticle-coated polypropylene mesh is said to have good antimicrobial activity and can be considered an ideal candidate for surgical meshes.[40] Furthermore, coating devices with an antibacterial barrier would provide another layer of protection to help decrease the iatrogenic infection rate. Darouiche[41] reported that nanocrystalline silver has tremendous potential as an antibacterial coating to decrease the one million nosocomial infections a year seen in patients with implanted prosthetic materials. Moreover,[42] demonstrated that topical formulation of nanocrystalline silver particles obligate anti-inflammatory properties in a guinea pig model of atopic dermatitis with efficacy comparable to that of high potency steroids, tacrolimus and pimecrolimus. The potential anti-inflammatory mechanism of nanocrystalline silver particles is secondary to suppression of tumour necrosis factor-α and Interleukin (IL)-12 and induction of inflammatory cell apoptosis.

Recently, Nanolabs based in United States (http://nanolabs.us/news/viewstory/55/) announced the development of innovative hemostatic mesh, which creates a mechanical barrier stopping blood flow in wounds and integrates both physical and chemical protection, and antibacterial properties. The surgical mesh material is biocompatible, durable, and flexible enough to fit complex wounds; is stable and functional at extreme temperatures, has a long shelf life, and possesses antibacterial properties.

Antibacterial coatings

Infections arising from bacterial adhesion and colonization on medical device surfaces are a significant healthcare problem. Silver based antibacterial coatings have attracted a great deal of attention as a potential solution.[43] Medical device-associated infections are mainly caused by bacterial attachment. For this reason, it is well accepted that preventing bacterial adhesion to the device surface through the application of an antibacterial coating is a potential solution.[44] Presently, there is substantial research in silver coated medical devices.[45] This is mainly driven by the fact that silver is active against both Gram-positive and Gram-negative bacteria and resistance has not been yet been convincingly demonstrated for clinically-relevant pathogens.[2, 18] For more on the extensive efforts on development of silver based antibacterial coatings, we refer the reader to several instructive recent reviews on the topic.[46-48]

Recently, Taheri et al.,[43] reported on the development of a silver nanoparticles based antibacterial surface that can be applied to any type of material surface. The silver nanoparticles were surface engineered with a monolayer of 2-mercaptosuccinic acid, which facilitates the immobilization of the nanoparticles to the solid surface, and also reduces the rate of oxidation of the nanoparticles, extending the lifetime of the coatings. The coatings had excellent antibacterial efficacy against three clinically significant pathogenic bacteria i.e. *Staphylococcus epidermidis, Staphylococcus aureus* and *Pseudomonas aeruginosa*. Besides, studies with primary human fibroblast cells showed that the coatings had no cytotoxicity in vitro. Collectively, these coatings have an optimal combination of properties that make them attractive for deposition.

Furthermore, Ho et al.,[49] described the application of AgNPs incorporated into amphiphilically-modified hyperbranched polylysine (HPL) as controlled releasing antimicrobial coatings for surgical poly (glycolic acid) (PGA) suture. These coatings showed a constant release of silver ions over more than 30 days. After this period of washing, the sutures retained their high efficacies against bacterial adhesion. Cytotoxicity tests using L929 mouse fibroblast cells showed that the materials are basically non-cytotoxic and can be applied in preparation of antimicrobial equipment of medical for surgical suture.

Nanosilver as antiviral agents

Viruses represent one of the leading causes of disease and death worldwide.[34] Although the principal mechanism underlying the viral inhibitory activity of nanosilver particles is not yet fully understood, AgNPs are considered to be a broad-spectrum agent against a variety of viral strains including HIV-1, hepatitis B virus, respiratory syncytial virus, herpes simplex virus type 1, and monkeypox virus.[2] It has been observed that AgNPs have higher antiviral activity than silver ions, due to species difference as they dissolve to release Ag0 (atomic) and Ag+ (ionic) clusters, whereas silver salts release Ag+ only.[50] Infact[51] were the first to describe the antiviral activity of silver nanoparticles and they found that nanoparticles undergo size-dependent interactions with HIV-1. The most probable sites for interaction were found to be the sulfur-bearing residues of the gp120 glycoprotein knobs, which being limited in number, may also explain the inability of larger nanoparticles to bind the virus. Besides, silver ions can form complexes with electron donor groups containing sulfur, oxygen, or nitrogen that are normally present as thiols or phosphates on amino acids and nucleic acids and inhibit post-entry stages of infection by blocking HIV-1 proteins other than gp120, or reducing reverse transcription or proviral transcription rates by directly binding to the RNA or DNA molecules. Furthermore, silver nanoparticles are proved to be virucidal to cell-free and cell-associated HIV-1 as judged by viral infectivity assays.[52] HIV infectivity was effectively eliminated following short exposure of isolated virus to silver nanoparticles. These properties make silver nanoparticles a potential broad-spectrum agent not prone to inducing resistance that could be used preventively against a wide variety of circulating HIV-1 strains. Further in vitro and in vivo studies are warranted to elucidate the mechanisms of action which may render possible antiviral development of AgNPs as broad spectrum in treatment of infectious diseases to humans.

TOXICITY OF NANOSILVER PARTICLES

The biocidal activity of metal nanoparticles in general and silver nanoparticles (AgNPs) depends on several morphological and physicochemical characteristics of the particles. Many of the interactions of the AgNPs with the human body are still poorly understood; consequently, the most desirable characteristics for the AgNPs are not yet well established. Therefore, the development of nanoparticles with well-controlled morphological and physicochemical features for application in human body is still an active area of interdisciplinary research.[53] Silver nanoparticles at some concentrations cause health associated problems if improperly used, thus it is very important to address the biosafety concerns of AgNPs in human health.

Silver nanoparticles are reported to be cytotoxic to several types of cells including human peripheral blood mononuclear cells, human alveolar epithelial cell line (A549), murine and human alveolar macrophage cell line, neuroendocrine cells, rat liver cell line and mouse germline cells.[54, 55] Although the details of the toxic mechanism are unclear, it suggests that silver nanoparticles are ionized in the cells, which leads to activate ion channels and changes the permeability of the cell membrane to both potassium and sodium, interaction with mitochondria and induction of the apoptosis pathway via the production of reactive oxygen species which leads to cell death (56). Recently, Poirier et al.[57] examined the role of AgNPs with a starting size of 20nm (AgNP20) in human neutrophils and concluded that AgNP20 induced apoptosis and can act as potent inhibitors of protein synthesis. Especially, the cytotoxicity of nanosilver with respect to mammalian cells remains unclear, because such investigations can be biased by the nanosilver coatings and the lack of particle size control.[58] Wang et al.[59] demonstrated that nanosilver on erythroid cells exhibited a robust inhibition on RNA polymerase activity and overall RNA transcription through direct Ag binding to RNA polymerase, which is separated from the cytotoxicity pathway induced by Ag ions. Moreover, results set forth that “particle-specific” effects could be the predominant mediator in eliciting biological influences on erythroid cells under relatively low concentrations of nanosilver exposure.

The common routes of entry of nanosilver particles into human are respiratory tract, gastrointestinal tract, skin and female genital tract through direct substance exchange with the environment. Additionally, systemic administration is also a potential route of entry, since colloidal silver nanoparticles have been exploited for diagnostic imaging or therapeutic purposes. On the subject of biodistribution, organ accumulation, degradation, possible adverse effects and toxicity associated with medical use of nanosilver the readers are suggested to refer review by Chen and Schluesener.[60] Majority of animal and human studies indicate that it is difficult to remove silver completely once it has been deposited in the body; however nanosilver can be excreted through the hair, urine, and faeces.[61] There is no consensus on nanosilver’s toxicity to humans, and most toxicity investigations of silver nanoparticles are based on in vitro cellular experiments and relatively short-term animal experiments.

OTHER MEDICAL APPLICATIONS

Nanosilver find application in the diagnosis and treatment of cancer, joint replacement compounds (bone cement), dental materials, cardiovascular implants, anti inflammatory agents, antifungal agents, contact lenses and can act as drug carriers that can deliver therapeutic agents.[62-67] The use of nanosilver in combination with vanadium oxide in battery cell components is one example of advanced silver nanotechnology improving battery performance in next-generation active implantable medical devices.[68] Since the shape, size, and composition of AgNPs can have significant effects on their function and possible risks to human health, extensive research is needed to fully understand their synthesis, characterization, and possible toxicity.

CONCLUSION

Silver nanoparticles are unavoidable as they are of substantial scientific and economic potential. The major contributing factors comprise shape, size and composition and can have significant effect on their function and possible risks to human health. Significant research has to be focused on this new emerging and exciting field to address the safety issues of *in vivo* applications as discussed. In the next decade, it is anticipated that new materials, methods and technologies promote further development and use of silver nanoparticles in particular to serve more effectively in human health care system. In this review, we first gave an overview of mechanism of action of silver nanoparticles, then reviewed biomedical and clinical applications of silver nano particles.

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DECLARATION OF INTEREST

The authors declare no conflict of interest.

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