Silver nanoparticles—the real "silver bullet" in clinical medicine?

The use of silver nanoparticles has become more widespread in our society. While many believe that silver can be extremely useful in clinical medicine, firm evidence is still lacking. Thus, we present here a review of their current use in clinical medicine.

Dr Kenneth Wong is currently clinical assistant professor in the Department of Surgery, University of Hong Kong. A medical graduate of the University of Edinburgh, UK, he received his clinical training in UK before embarking on lab-based research. He obtained his Ph.D in immunology from Imperial College, UK. Dr Wong's research interests include genetic basis of paediatric surgical diseases and tumor immunobiology. For the past few years, he has been focusing his basic research on nanomedicine, in particular the use and mechanisms of nano-metals in wound healing and inflammation and also targeted chemotherapy against childhood neuroblastoma using conjugated nano-composite molecules.

Kenneth Wong

Introduction

In recent years nanotechnology has been emerging as a rapidly growing field with numerous applications in science and technology for the purpose of manufacturing new materials. This technology is defined as the design, characterization and application of structures, devices and systems by controlling shape and size at nanometre scale level (1 nm to 100 nm) and has already found practical applications in health and daily life,[1,2] such as better drug delivery methods,[3,4] chemical deposition for environmental pollution cleanup,[5,6] medical imaging,[7,8] as well as military purposes.[9,10]

Out of all kinds of nanoparticles, the metallic nanoparticles, including gold, silver, iron, zinc and metal oxide nanoparticles, have shown great promise in terms of biomedical applications, not only due to their large surface area to volume ratio,[11,12] but also because they exhibit different biomedical activities.[13] These have been demonstrated in experiments using gold and cerium oxide nanoparticles for the treatment of tumors and for anti-inflammation, respectively.[12–14]

For silver, this precious metal was originally used as an effective antimicrobial agent and as a disinfectant, as it was relatively free of adverse effects.[15] However, with the development of modern antibiotics for the treatment of infectious diseases, the use of silver agents in the clinical setting had been restricted mainly to topical silver sulfadiazine cream in the treatment of burn wounds.[6–18]

From the 1990's, there has been a resurgence of the promotion of silver (as colloidal silver) as an alternative medicine treatment. It has been marketed with claims of it being an essential mineral supplement, or that it can treat various diseases.[19,20] Although colloidal silver products are legally available as health supplements, it is illegal in the U.S. to make such claims of medical effectiveness for colloidal silver.

The commercial product referred to as "colloidal silver" includes solutions that contain various concentrations of ionic silver compounds, silver colloids or silver compounds bound to proteins. Unlike clinical drug production, the manufacturing of such products is not standardized and thus results in various concentrations and also particle sizes.

At present, there are no evidence-based medical uses for ingested colloidal silver. Indeed, the U.S. National Center for Complimentary and Alternative Medicine has issued an advisory indicating that the marketing claims made about colloidal silver are scientifically unsupported.[19]

Despite this, interest in the clinical use of silver has been rekindled due to the availability of silver nanoparticles (AgNPs). The diameters of AgNPs are generally smaller than 100 nm and contain 20–15 000 silver atoms (Fig. 1).[21] In the case of exposing cells or tissue to AgNPs, the active surface of AgNPs would be significantly large compared to silver compounds, and thereby exhibiting remarkably unusual physicochemical properties and biological activities.[22] Despite the fact that AgNPs have been increasingly applied in the biomedical or pharmacological fields, relatively little research has been done in clinical medicine. This review will discuss the current understanding of the biological actions of the silver nanoparticles. Furthermore, the various uses of silver nanoparticles in the field of clinical medicine will be described.

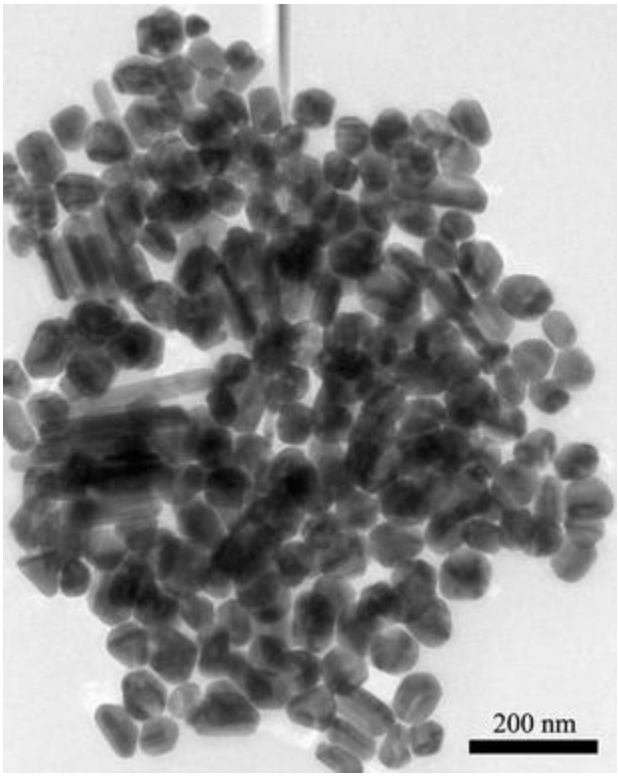

Fig. 1 Transmission electron micrograph of silver nanoparticles (averaging 15 nm)

produced by the reduction method.

Synthesis of silver nanoparticles

a. Chemical and physical synthesis methods of AgNPs. For biological use, the main aim of making AgNPs will be for them to be stable in solution, so that each silver nanoparticle can thoroughly be exposed to the cells in tissue and exert their maximal bio-effects. Since Turkevich *et al.* first reported their preparation of AgNPs based on the reduction of silver nitrate with citrate, similar updated methods have also been reported.[23–25] Nowadays, AgNPs of different sizes and shapes can be made.

In addition to chemical synthesis of AgNPs, Yen *et al.* reported the production of AgNPs by physical manufacturing. First, silver bulk material was ground into the silver target materials. Then they were vaporized to the atomic level by an electrically gasified method under vacuum then further condensed in the presence of inert gas, and piled up to form AgNPs. The sizes of AgNPs could be effectively managed depending on the evaporation time and electric current used. The AgNPs were collected in a cold trap and centrifuged to obtain the final product.[22]

b. Biosynthesis of AgNPs from staphylococcus aureus and fungi. Apart from chemical and physical methods, AgNPs can also be synthesized using a reduction of aqueous Ag ions with the culture supernatants of *Staphylococcus aureus*.[23,24] The supernatant was added separately to the reaction vessel containing silver nitrate. The bioreduction of the silver ions in solution was monitored and the spectra measured in a UV-vis spectrophotometer at a resolution of 1 nm. Furthermore, Gajbhiye even reported the use of fungus *Alternaria alternata* to produce AgNPs.[25]

Biological properties of silver nanoparticles

a. Anti-bacterial properties of silver nanoparticles. 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.[26–32] Before the advent of silver nanoparticles, silver nitrate was an effective antibacterial agent used clinically.[33–39] Afterwards, the use of silver agents decreased as antibiotics came into prominence during the last century. Nonetheless, the combination of silver and sulfonamide to form silver sulfadiazine, has remained useful in the treatment of burns, even to this day.[40–42] Silver returned to prominence recently due to the emergence of antibiotic-resistant bacteria as a result of the overuse of antibiotics.[43,44] With the advancement of nanotechnology, the interest in the use of the anti-bacterial efficiency of silver nanoparticles has been rekindled. Compared with silver compounds, the mechanism for the antimicrobial action of AgNPs may be similar, although neither is properly understood. However, because of the larger surface area to volume ratio, AgNPs may have much better efficiency.[21,45] 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;[44,46]

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;[47,48]

3. Silver (nanoparticles or Ag^+) can attack the respiratory chain in bacterial mitochondria and lead to cell death;[49]

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 (Fig. 2).[50,51] Furthermore, a recent study showed that yeast and *E. coli* were inhibited at a low concentration of AgNPs, study of mechanisms revealed that free radicals and oxidative stress were responsible for the antibacterial activities.[52]

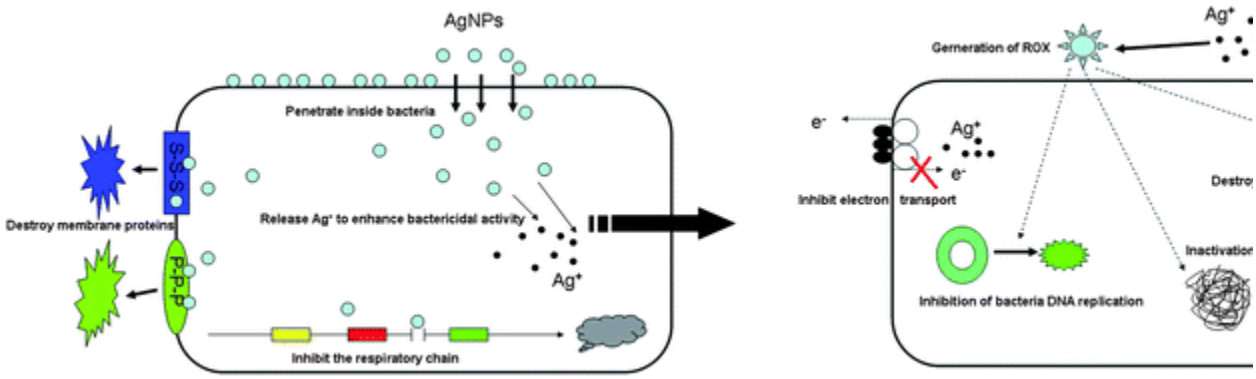

Fig. 2 A schematic drawing showing the various mechanisms of antibacterial activities exerted by silver nanoparticles.

b. Anti-inflammatory properties of silver nanoparticles. Apart from being an excellent anti-bacterial agent, we were also able to show, in the burn wound model, as well as in a peritoneal adhesion model in mice, that AgNPs had anti-inflammatory properties. In the burn model, significantly lower levels of the pro-inflammatory cytokine IL-6 were found in animals treated with AgNPs using quantitative real-time RT-PCR. Conversely, mRNA levels of IL-10, an anti-inflammatory cytokine, stayed higher in the AgNPs group in comparison with other silver compounds at all time points monitored during healing.[53,54]

Polymorphonuclear cells (PMNs) and fibroblasts produced IL-6, which has been recognized as an initiator of events in the physiological alterations of inflammation;[55] decreased expression of IL-6 may result in fewer neutrophils and macrophages recruited to the wound and less cytokines being released in the wound with subsequently lower paracrine stimulation of cellular proliferation, fibroblast and keratinocyte migration, and extracellular matrix production.

IL-10 could inhibit the synthesis of pro-inflammatory cytokines,[56,57] and also inhibits leukocyte migration toward the site of inflammation, in part by inhibiting the synthesis of several chemokines, including monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1 α (MIP-1 α).[58] The differences found in mRNA levels of various cytokines further confirmed that AgNPs can effectively modulate cytokine expression during suppressing inflammation.

Apart from our group, others have also demonstrated the anti-inflammatory effects of silver nanoparticles. Nadworny *et al.* explored the effect of AgNPs using a porcine model of contact dermatitis, while Bhol and Schechter utilized AgNPs in a rat model of ulcerative colitis.[59,60] In both models, although the set of pro-inflammatory cytokines measured were different from ours (IL-1; TNF-α), the findings did confirm that AgNPs had direct anti-inflammatory effects and improved the healing process significantly when compared with controls.

Nonetheless, in a peritoneal adhesion model, we provided further evidence for, and contributed to the understanding of, anti-inflammation properties of AgNPs.[54] Here, the mechanisms of the anti-inflammation effects were suggested to be through a reduction of IFN-γ and TNF-α *via* macrophages.

Applications of silver nanoparticles in medicine

In the past, silver was used for a variety of clinical conditions including epilepsy, venereal infections, acnes and leg ulcers. Silver foil was applied to surgical wounds for improved healing and reduced post-operative infections, while silver and 'lunar caustic' (pencil containing silver nitrate mitigated with potassium nitrate) was used for wart removal and ulcer debridement.[61] Although some centers still use these solutions, they have been shown to be very impractical to use on large wounds or for extended time periods due to instability. With nanotechnology, the availability of silver nanoparticles has enabled the use of pure silver to achieve a rapid growth in medical practice. Since the size, shape and composition of silver nanoparticles can have a significant effect on their efficacy, extensive research has gone into synthesizing and characterizing silver nanoparticles. The application of nanosilver can be broadly divided into diagnostic and therapeutic uses.

a. Nanosilver in diagnosis and imaging. Early diagnosis of any disease condition is vital to ensure that early treatment is started and perhaps resulting in a better chance of cure. For example, in patients undergoing general anesthesia for surgery, the risk of developing pulmonary complications will be lowered if any sub-clinical upper respiratory tract viral infections can be detected prior to surgery. Surface-enhanced Raman spectroscopy (SERS) has emerged as a powerful analytical tool that extends the possibilities of vibrational spectroscopy. SERS differs from standard Raman scattering in that the incoming laser beam interacts with the oscillations of plasmonic electrons in metallic nanostructures to enhance the vibrational spectra of molecules adsorbed to the surface. In a recent study, SERS was used to obtain the Raman spectra of the respiratory syncytial virus (RSV), using substrates composed of silver nanorods. It was shown in this study that the four virus strains tested were readily detected at very low detection limits.[62]

In terms of detecting cancer, 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.[63] 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.

b. Nanosilver in therapeutics. *(i) Wound dressing.* Wound healing is regarded as a complex and multiple-step process involving integration of activities of different tissues and cell lineages.[64] Perhaps the most well documented and commonly used application of silver nanoparticles for this is in the use of wound dressings.[27,65] In this regard, Acticoat®, which is the first commercial dressing made up of two layers of polyamide ester membranes covered with nanocrystalline silver ions, has been studied extensively. Acticoat® has been shown to have the lowest MIC and MBC values, and the fastest Kill kinetics against the five bacteria tested in *in vitro* studies.[66,67] Further, the sustained release of silver particles should minimize the likelihood of bacteria developing resistance to silver. In a randomized prospective clinical study involving 30 patients with each group of patients having comparable burn wound size, depth and location, the wounds were either treated with silver nanoparticles dressing or a gauze soaked in 0.5%

silver nitrate solution. The frequency of burn wound sepsis, as well as secondary bacteraemia, were found to be less in patients treated with silver nanoparticles than in those treated with the control.[68] As well as burn wounds, there is now increasing evidence for the use of silver nanoparticles in the treatment of chronic wounds, such as leg ulcers, diabetic foot ulcers and pressure ulcers. Sibbald *et al.* 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.[69] Compared with other silver compounds, AgNPs seem also to promote healing and achieve better cosmetics after healing.

The biological effects of AgNPs on wound healing appear to be manifols. When we performed experiments using an excisional wound model, we were able to show that AgNPs could exert differential effects on keratinocytes and fibroblasts during healing.[70] AgNPs, on the one hand, could promote wound healing through facilitating the proliferation and migration of keratinocyte, on the other hand, they could reduce the formation of collagen by fibroblasts by driving their differentiation into myofibroblasts.

In addition to this significant finding, AgNPs were also shown to facilitate wound healing through modulation of various cytokines. Using a contaminated wound model in pigs, Wright *et al.* found accelerated healing was characterized by rapid development of well vascularized granulation tissue that supported the tissue grafting after injury; furthermore, the promoted healing was associated with reduced local matric metalloproteinase (MMP) levels and enhanced cellular apoptosis.[71] This finding was supported in other studies.[53,72] Taken together, the use of silver nanoparticles in the aspects of wound healing appears to hold the greatest promise.

(ii) Silver-impregnated catheters.

Central venous catheters. Central venous catheters (CVC) are widely used in hospital practice, with around 5 million being inserted in the United States alone each year.[73] However, the widespread use of CVCs is associated with potential infective complications, with the incidence of catheter-related bloodstream infection estimated at around 80 000 cases annually.[74,75]

Previous studies have suggested that impregnation of catheters with antibiotics could decrease the rates of colonization of catheters.[76–78] Nonetheless, there is a risk that the increasing use of antibiotic-impregnated catheters could lead to eventual bacterial resistance. A new generation of silver-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.[79] It would therefore appear that silver-impregnated catheters are destined for increasing use.

Vascular prosthesis. For vascular surgeons, much research in vascular surgery has focused on the development of infection-resistant prosthetic grafts over the years. Recently, the use of the InterGard Silver® bifurcated polyester graft coated with collagen and silver has been shown in a multi-centre study to achieve excellent patency rates over a long-term period with a low rate of graft infection.[80] Nonetheless, a randomized trial is still needed to validate this early promising result.

Ventricular drainage catheters. Insertion of temporary external ventricular drainage (EVD) is a commonly used procedure in intensive care patients for the management of acute occlusive hydrocephalus. However, an important complication of external cerebrospinal fluid (CSF)

drainage is bacterial colonization of the catheter, resulting in ventriculomeningitis and encephalitis. The availability of silver-impregnated ventricular catheters since 2004 resulted in a pilot study addressing their clinical efficacy in neurological and neurosurgical patients requiring external CSF drainage. The authors found that CSF cultures performed at least three times a week yielded 25% more positive cultures in the control group compared to 0% in the treatment group using silver catheters. Furthermore, aseptic meningitis due to inflammation was not seen in patients with the silver-impregnated biomedical material.[81]

(iii) Silver in orthopaedics. Artificial joint replacements have become the gold standard treatment for many arthritic diseases. Like all biomaterials, bone cement based on polymethylmetacrylate (PMMA) has an elevated risk of infection when implanted into the human body.[82] Indeed, an increasing number of joint infections with multi-resistant bacteria mean that an adequate prophylaxis against these organisms is necessary. Recent studies have been carried out to evaluate bone cement loaded with nanosilver.[83] Here, nanosilver-loaded bone cement could be shown to have high antibacterial activity against all tested strains including methicillin-resistant *Staphylococcus aureus* (MRSA). Furthermore, the nanoparticles did not seem to have cytotoxicity to osteoblasts grown *in vitro*.

As well as bone cement, the use of silver nanoparticles has been studied in artificial joints. For many years, ultra high molecular weight polyethylene (UHMWPE) has been the material of choice for fabrication of bearing inserts for joint replacement components. A major problem with the longevity of UHMWPE is wear and concomitant debris generation, which can activate macrophages, with subsequent inflammation, and eventual failure of the artificial joints. In one study, incorporation of silver nanoparticles was demonstrated to lead to both physical and chemical stabilization of the polymer surface layer toward friction oxidation and degradation.[84] This procedure was further shown to significantly decrease the process of polymer/metal tribochemical debris formation and at the same time enhances UHMWPE biocompatibility and antimicrobial activity.

Taken together, it would appear that silver nanoparticles could play a significant role in the next generation of biomaterials in orthopaedics.

(iv) Surgical mesh. For general surgery, surgical implants are often unavoidable. Surgical meshes are commonly used for bridging large wounds, as well as acting as reinforcements to tissue repair. However, being foreign material, they do carry a risk of infection. Indeed, it has been estimated that one million nocosomial infections are seen each year in patients with implanted prosthetic materials.[85] The use of silver nanoparticles polypropylene mesh has been studied recently. Similar to other studies using silver nanoparticles, the results showed that silver nanoparticles polypropylene mesh had significant bactericidal efficacy against *S. aureus*. Furthermore, it was shown that silver nanoparticles could continue to diffuse off the mesh and had sustained activity.[86] These results clearly warrant further *in vivo* studies to determine whether silver nanoparticles-coated polypropylene mesh can decrease the prosthetic infection rate and the host inflammatory response in the clinical setting.

Are silver nanoparticles harmful?

With the use of silver nanoparticles in medical appliances, exposure to silver in the body is therefore inevitable and increasing. In order to gain further widespread use in clinical medicine, the issue of the potential toxicity of AgNPs needs to be fully evaluated.

Although silver is believed traditionally to be relatively non-toxic to mammalian cells, from previous evidence taken from workers in the silver industry, it is not known if this is still the case

for silver nanoparticles. This is of particular concern because, due to the small size, silver nanoparticles can gain increasing access to tissues, cells and biological molecules within the human body.

In this regard, many *in vitro* studies have been performed. Hsin *et al.* provided evidence for molecular mechanism of AgNPs-induced cytotoxicity, showing that AgNPs acted through ROS and JNK to induce apoptosis *via* the mitochondrial pathway in NIH3T3 fibroblast cells.[87] Park *et al.* reported cytotoxicity using AgNPs prepared by dispersing them in fetal bovine serum, as a biocompatible material, on the cultured RAW264.7 macrophage cell line, which induced cellular apoptosis.[88] Furthermore, AgNPs decreased intracellular glutathione level, increased NO secretion, increased TNF-α in protein and gene levels, and increased the gene expression of matrix metalloproteinases, such as MMP-3, MMP-11, and MMP-19. Kim *et al.* demonstrated cytotoxicity induced by AgNPs in human hepatoma HepG2 cells and indicated that AgNPs agglomerated in the cytoplasm and nuclei of treated cells, and induced intracellular oxidative stress and was independent of the toxicity of Ag^+ ions.[89] On a similar note, Kawata *et al.* showed an upregulation of DNA repair-associated genes in hepatoma cells cultured with low dose silver nanoparticles, suggesting possible DNA damaging effects.[90] In HeLa cells, Miura and Shinohara reported that the expressions of ho-1 and mt-2A, well-known oxidative stress-related genes, were upregulated by AgNPs treatment, showing that AgNPs had the potential for cytotoxicity in the case of exposure at high concentrations.[91]

Despite the findings in these *in vitro* studies, the overall significance in the *in vivo* setting, and also the applicability to humans remain unknown. In the clinic, silver nanoparticle-based wound dressings are perhaps the most universally used. Since nanosilver wound dressings are applied to wounded skin where the strict barrier is broken, it is thus expected that the entry of the nanoparticles into the body would be easier. This, along with the observation that particles in the skin can be phagocytosed by macrophages and Langerhans cells, might theoretically lead to perturbations of the immune system. At the same time, nanoparticles entering capillaries could become circulatory and would soon encounter the liver and expose the liver to a high dose of silver nanoparticles. Nonetheless, systemic toxicity of ingested silver nanoparticles is scarcely seen. Supporting this, when we injected silver nanoparticles into experimental mice intravenously, we did not observe any overt systemic effects, despite the silver nanoparticles solution used being at a relatively high concentration of 100 mM (unpublished data). At the local level, although others showed that when cultured keratinocytes were exposed to extracts of silver-containing dressings, their proliferation was significantly inhibited, we did not observe any increase in cell death or inhibition of cell growth in our experiments using keratinocytes and fibroblasts.[70,92,93] In contrast, we found that silver nanoparticles increased the growth rate of keratinocytes. The differences between our data and others' might be attributable to the difference in laboratory conditions and techniques employed. The concentration of silver used in experiments might also be an important factor.

Nonetheless, the issue of “argyria”, the deposition of silver metal causing discoloration of the tissues, is another concern with chronic ingestion or inhalation of silver preparations. Although argyria is not a life-threatening condition, it is, however, cosmetically undesirable.[94] In wound care, Wang *et al.* reported that if silver dressing was topically applied to the porcine deep dermal partial thickness model, a larger amount of silver would deposit in cutaneous scar tissue (136 $\mu g\ g^{-1}$) than normal skin (less than 0.747 $\mu g\ g^{-1}$). The wound would have a slate-gray appearance.[95] Contrary to this finding, Jaya *et al.* reported that when compared with conventional silver agents, AgNPs were a safer alternative because of their sustained release dose regime.[96]

Taking into account the existence of silver nanoparticle-impregnated catheters for clinical use, hemo-compatibility is another safety concern. Previous reports suggested that nanoparticles present in blood were associated with thrombosis and activation of immunological reactions. Studies have provided evidence that exposure to ambient ultrafine particles elicits inflammatory responses in vascular endothelial cells and blood cells.[97,98] For silver, a recent study revealed that silver nanoparticles could greatly enhance the electron-transfer reactivity of myoglobin.[99] Further, the recent identification of the cytotoxicity of silver nanoparticles towards the spermatogonial stem cell line has aroused great concern over the biosafety of nanomaterials.[100] As discussed previously, the liver appears to be an eventual accumulation site of circulatory silver nanoparticles. Similar patterns of cytotoxicity of silver nanoparticles (decrease of mitochondria function, LDH leakage and abnormal cell morphologies) were observed in *in vitro* studies. Nonetheless, during our other experiments using silver nanoparticles, we routinely harvested organs (liver, spleen, lung, heart and kidney) and analyzed the silver content using inductively coupled plasma mass spectroscopy (ICP-MS) after trypsin digestion. Thus far, in experiments using therapeutic doses of silver nanoparticles, only very low levels of silver (below 0.5 $\mu g\ g^{-1}$ of organ) could be detected in the organs of the mice, suggesting that nanosilver was safe at these low concentrations. Indeed, in clinical situations, wound exudation and systemic proteins might also contribute towards silver nanoparticles' *in vivo* safety, as the high protein content probably neutralises nanosilver's tissue toxicity. Taken together, it may be fair to say that silver nanoparticles would be safe to use clinically at low doses.

Future therapeutic directions

a. Anti-inflammatory agent. The potential anti-inflammatory action of silver nanoparticles has been suggested in various studies described previously. On the other hand, inflammation has been noted to play a significant part in the formation of post-operative adhesions. In animal models, we showed that intra-peritoneal injection of silver nanoparticles significantly reduced the degree of post-operative fibrous adhesions. The anti-inflammatory effects have also been substantiated in other inflammatory disease models by others. Taken together, it would suggest that silver nanoparticles can indeed reduce inflammation and its use in other inflammatory conditions is eagerly anticipated.

b. Antiviral drug. The antiviral properties of metal nanoparticles are of significant medicinal interest. With finding a cure for human immunodeficiency virus (HIV) in mind, the post-infected anti-HIV-1 activities of silver nanoparticles toward Hut/CCR5 cells were evaluated in one study.[101] Here, silver nanoparticles were shown to have dose-dependent anti-retrovirus activities and exhibited high potency in inhibiting HIV-1 replication. Further, these nanoparticles did not show acute cytotoxicity to either the Hut/CCR5 cells or to normal peripheral blood mononuclear cells. It remains to be seen whether silver nanoparticles have activities against other types of viruses.

c. Anti-platelet agent. Thrombotic disorders have remained a significant problem in clinical medicine. Results thus far have shown that anticoagulant and thrombolytic therapy may sometimes lead to serious bleeding complications.[102] As platelets play a central role in thrombotic disorders, the focus has now shifted to regulating and maintaining these cells in an inactive state. Recently, Shrivastava *et al.* demonstrated that AgNPs could effectively inhibit integrin-mediated platelet functional responses like aggregation, secretion, adhesion to immobilized fibrinogen or collagen and retraction of fibrin clot in a dose-dependent manner.[103] Further, *in vivo* studies using mouse models also supported the anti-platelet properties of silver nanoparticles. The results,

significant inhibition of platelet functions with a relatively low dose of AgNPs, combined with the lack of cell lysis, raise the hope for its use as an anti-platelet therapeutic agent.

Future application of silver nanoparticles—effects on stem cells?

The epidermal stem cells, which reside in the dermal layer in the skin, play the most important roles for repairing the epidermis, regenerating hair and maintaining tissue homeostasis after injury. Stem cells have the remarkable capacity to both self-perpetuate and also give rise to the differentiating cells that constitute one or more tissues.[104] In recent years, many scientists have been exploring mysteries underlying their remarkable capacity to perform these feats.[105–111] In our ongoing study, we have also found proliferation of epidermal stem cells in skin promoted by silver nanoparticles at low concentrations (unpublished data). We are exploring the exact mechanism of this phenomenon.

Apart from epidermal stem cells, the use of mesenchymal stem cells (MSCs) is one promising modality for cell-based therapy applications due to their easy isolation and culture as well as the expansion capacity. Furthermore, MSCs can also provide pleuripotent potential and develop into various lineages such as skin, bone, tendon, ligament, muscle, fat and blood.[112–114] In the environment of a healing wound, the use of programmed MSCs can thus be an important tool to compensate for the tissue loss and recovery of function and structure. We are currently studying the possibility of enhanced proliferation and survival of hMSC by silver nanoparticles, and results are eagerly awaited.

Conclusion

The advance in nanotechnology has enabled us to utilize particles in the size of the nanoscale. This has created new therapeutic horizons, and in the case of silver, the currently available data only reveals the surface of the potential benefits and the wide range of applications. We have yet to elucidate the exact cellular pathway of silver nanoparticles. Furthermore, it remains to be seen whether any potential complications for the silver nanoparticles would surface after prolonged clinical use. Nonetheless, a bright future holds for this precious metal.

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