INSTRUCTIONAL REVIEW: GENERAL ORTHOPAEDICS Silver nanoparticles and their orthopaedic applications

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Implant-associated infection is a major source of morbidity in orthopaedic surgery. There has been extensive research into the development of materials that prevent biofilm formation, and hence, reduce the risk of infection. Silver nanoparticle technology is receiving much interest in the field of orthopaedics for its antimicrobial properties, and the results of studies to date are encouraging. Antimicrobial effects have been seen when silver nanoparticles are used in trauma implants, tumour prostheses, bone cement, and also when combined with hydroxyapatite coatings. Although there are promising results with in vitro and in vivo studies, the number of clinical studies remains small. Future studies will be required to explore further the possible side effects associated with silver nanoparticles, to ensure their use in an effective and biocompatible manner. Here we present a review of the current literature relating to the production of nanosilver for medical use, and its orthopaedic applications.

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Silver is a soft, white, lustrous transition metal which is recognised to have antimicrobial properties and has assumed an important role in the treatment of infections.[1-3] As a non-specific biocidal agent, in suitable doses silver is not toxic to mammalian cells and disinfects a broad spectrum of bacterial and fungal species, including antibiotic-resistant strains.[1-3] Silver and silver nanoparticles are used as antimicrobials in a variety of industrial, healthcare and domestic applications.[4,5] Silver has been incorporated into wound dressings, and as an antimicrobial coating on medical devices in order to prevent biofilm formation.[6] The clinical potential of silver nanotechnology is of particular interest to the field of orthopaedics, where infection of implanted devices represents a persistent threat. In this paper we provide a review of the literature on the use of silver nanoparticles and their application in the field of orthopaedics. In addition, we review the potential for toxicity if silver is not used with caution.

Mechanism of action

Silver has well-documented broad-spectrum activity against Gram-positive and Gram-negative bacteria, fungi, protozoa and viruses.[7] However, its mechanism of action has only recently been elucidated. *In vitro* studies have shown that silver releases biologically active ions from its surface. The released ions bind to a number of bacterial cell structures, including the peptidoglycan cell wall and plasma membrane, the bacterial DNA and bacterial proteins (Fig. 1).[8] This creates three different mechanisms by which silver exerts its toxic effects. The binding of ions to the cell wall damages the outer cell layers, causing loss of cell contents and creating structural abnormalities.[9] As Gram-positive bacteria have thicker cell walls, a higher concentration of silver is necessary to prevent bacterial growth than for Gram-negative bacteria.[10] Interaction with sulfhydryl (SH) groups in bacterial proteins and enzymes impairs many key cell functions, such as respiration and permeability. Binding to nucleic acids in DNA prevents cell reproduction.[11] A further mechanism of toxicity is through the production of reactive oxygen species (ROS) by silver ions. This generation of ROS probably acts in synergy with the SH group interaction mechanism. Evidence for this is the increased antimicrobial activity seen in aerobic versus anaerobic conditions.[12] These mechanisms of action provide for deconstitution of surface species, such as proteins, cells and resultant biofilms, which in turn promotes long-term exposure of the surface silver nanoparticles and their associated antimicrobial effects.

The multimodal activity outlined above has major clinical significance, as there is far less potential for bacterial resistance to silver than with traditional antibiotic agents. When bacterial resistance to silver nanoparticles does

occur, it develops more slowly than resistance to antibiotics.[13] Some of this resistance appears to be related to genes that control the pumping of silver out of the cell.[14] Taglietti et al[15] showed that a self-assembled monolayer of silver nanoparticles on glass was obtained via amino-silanisation of the glass surface. They found that there was prolonged release of silver ions, whereas the silver nanoparticles remained attached to the underlying substrate, and strong antibiofilm activity against the biofilm-producer *Staphylococcus epidermidis* was detected. The structural make-up of silver has consequences for its antimicrobial efficacy. Choi et al[16] conducted a comparative study of nanosilver, silver chloride and silver nitrate and concluded that nanosilver has greater efficacy against bacteria. This is thought to be because nanosilver has a secondary mechanism of action. Nanosilver particle size is another important factor that influences the level of antimicrobial activity. It has been shown that smaller particles (< 10 nm) are significantly more efficacious, as there is a greater surface area for the release of silver ions. Therefore, particle size may be of greater significance than concentration or mass.[17] The period of activity has also been shown to be related to nanoparticle size and, when present, the type of nanoparticle functionalising element (i.e. a nanoparticle that has had a chemical functional group added to its surface). It is reported that ion concentrations for the best antibiotic effects range between 10 nM and 10 μM.[18] A number of studies[19,20] have investigated the use of silver nanoparticles in combination with antibiotics and have found a synergistic effect. Fayaz et al[19] used *Trichoderma viride* for the biosynthesis of silver nanoparticles and also investigated the antimicrobial activity of these nanoparticles in the presence of antibiotics. They found that activity against Gram-positive and Gram-negative bacteria was increased for ampicillin, kanamycin, erythromycin and chloramphenicol. Dar, Ingle and Rai[20] investigated the properties of nanosilver produced using the fungus *Cryphonectria* sp. The fungally produced nanosilver was found to have higher antibacterial activity against *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* than streptomycin and amphotericin. It also displayed antifungal activity against *Candida albicans*, and enhanced the effects of other antibiotics.[20]
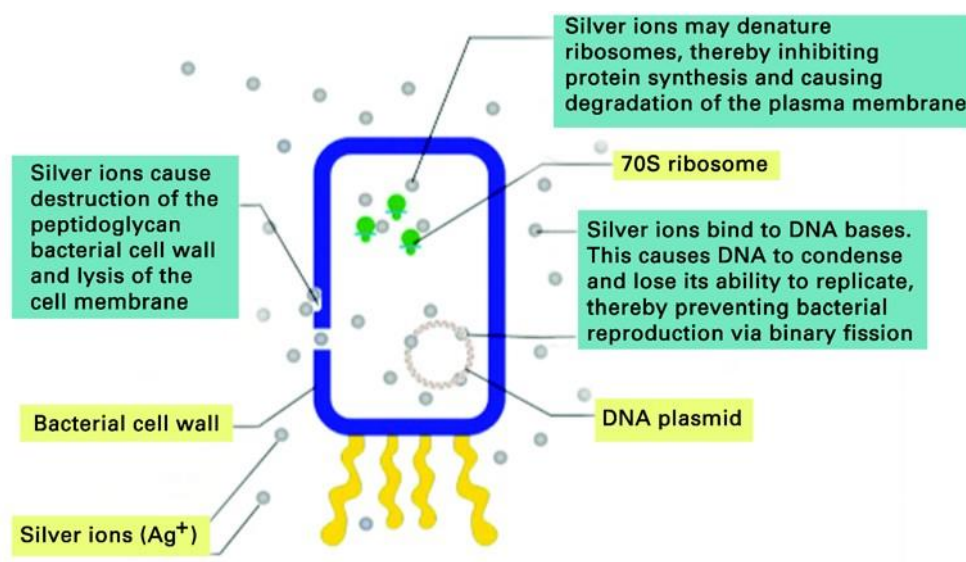

Fig. 1

Diagram showing mechanism of action
of silver ions.

Manufacturing processes

Silver has been used medically in mineral and compound forms, such as silver zeolite and silver nitrate, and the advance of nanotechnology has enabled the production of nanosilver. Nanosilver constitutes minute structures of silver atoms measuring between 1 nm and 100 nm in diameter, metallically bonded together. Silver nanoparticles have been produced using a wide variety of methods, but the two main methods of production are chemical reduction and photoreduction. In these processes, negatively charged electrons are donated to the positively charged silver ions, causing them to return to their metallic form.[21] A stabilising agent is usually added during production which prevents aggregation of the silver atoms, which could negatively affect the high antimicrobial activity associated with nanoscale dimensions.[22] More recently, biological methods of producing nanosilver have been explored,[23] which involve the use of organisms as reducing agents rather than as chemicals. This is an exciting development, as it eliminates the need for chemicals that may have added toxic effects, especially in clinical applications, thereby increasing the biocompatibility of the produced nanosilver. Multiple organisms have

been used, including bacteria, fungi and plants. Unlike chemical reduction, which requires separate reducing and stabilising agents, the micro-organism used for biological synthesis provides both agents. Each produces silver nanoparticles that vary in size and shape, and which may be found intracellularly or extracellularly on the cell wall.[23] A study involving the reduction of silver using *S. aureus* showed that the nanoparticles produced had high antimicrobial activity against methicillin-resistant *S. aureus* (MRSA), methicillin-resistant *S. epidermidis* (MRSE) and *Streptococcus pyogenes*.[24] Nanoparticles produced chemically can aggregate in liquid, reducing the surface area available for high antimicrobial activity. One of the advantages of using biogenic silver is that it has been found to be more stable over longer periods of time in a liquid environment.[25] Coating medical devices is the major use of silver nanoparticles, with the aim of preventing biofilm formation. A number of nanotechnology approaches to facilitate the adherence of silver to device surfaces, have been developed. These include vacuum-sputter coating and electrodeposition technologies, which fix vaporised silver on to the surface of the device.[26]

Tumour prostheses

Peri-prosthetic infection is a significant problem in orthopaedic oncology, with infection rates of 9% to 29%.[27-29] This patient group is at increased risk of infection compared with standard arthroplasty patients, because of immunosuppression related to their underlying disease, and also from adjuvant treatments such as chemotherapy and radiotherapy. Implant-related infection can result in amputation in these patients.[28,31] Gosheger et al[30] investigated the antimicrobial efficacy and possible side effects of a silver-coated mega-prosthesis in a rabbit model. They compared 15 titanium with 15 silver-coated MUTARS endoprostheses (Implantcast GmbH, Buxtehude, Germany), each of which had been inoculated with *S. aureus*. Rabbits in the silver group displayed significantly lower signs of inflammation as measured by C-reactive protein (CRP), neutrophil count and rectal temperature. The silver group also showed significantly lower rates of infection (7%) than the titanium group (47%), and no toxicological side effects were observed.[30] The effect of silver coatings on infection rate was prospectively assessed in 51 sarcoma patients using a silver coating applied to the implanted mega-prostheses by galvanic deposition of elementary silver.[31] The layer thickness ranged from 10 μm to 15 μm. No silver coating was applied at the articulating surfaces. The infection rate was compared with that of a historical control group of 74 patients who had undergone surgery using a non-silver-coated titanium mega-prosthesis and was found to be substantially reduced, from 17.6% in the titanium group to 5.6% in the silver group. A total of five patients out of 16 (38.5%) in the titanium group who developed a deep infection required amputation, but there were no amputations for infection in the silver-coated group.[31] At present, we indicate silver-coated prostheses in order to reduce the rate of infection in tumour patients, and also in patients with infection following extensive trauma. However, there have been no clinical studies comparing silver-coated revision arthroplasty with non-coated implants, nor have there been any clinical studies using silver nanoparticle technology.

External fixator pins

Pin tract infection is the most common complication associated with the use of external fixators and has been reported to be present in up to 42% of inserted pins.[32] Pin tract infection can result in loosening requiring removal or exchange, loss of fixation, fracture non-union and osteomyelitis.[33] An *in vitro* study has examined the antimicrobial efficacy of surface-coated external fixator pins. Stainless steel pins were coated with a continuous layer of polymer in which nanoparticulate silver was embedded. These test specimens were compared against uncoated stainless steel, titanium and copper pins. The test pins were incubated with *S. epidermidis* for 20 hours to initiate a biofilm, following which the test pins were analysed for highly adherent bacteria. It was demonstrated that fixation pins with a coating of nanoparticulate silver showed a 3-log step reduction in biofilm-forming bacteria compared with non-coated stainless steel or titanium implants.[34] These findings are supported by *in vitro* work by Wassall et al,[35] who examined the antimicrobial effect of conventional silver and showed a significant reduction in adhesion for *E. coli, Pseudomonas aeriginosa* and *S. aureus* when silver coated pins were compared with stainless steel pins. Collinge et al[36] reported on the potential benefit of silver coating on external fixator pins inoculated with *S. aureus* inserted into the iliac crest of sheep. After two and a half weeks, the pin sites were examined for movement, inflammation and infection. A reduction in the rate of infection from 84% to 62% was demonstrated when comparing silver coated and stainless steel pins. In addition, silver-coated pins were less frequently found to be loose than stainless steel pins. The authors postulated that bacterial adherence to the surface of the silver-coated pins was prevented by inhibition of the formation of a bacterial glycocalyx membrane on the surface of the pin, rather than by local leaching of silver ions.[36] Coester et al[37] performed a prospective randomised study in patients to compare the effect of silver coating on pin tract infection. The study design involved a monolateral fixator with stainless steel pins and silver-coated pins being used in the same construct, allowing for direct comparison of the effects of silver coating within the same environment. The results from 33 external fixators showed that there was no difference in the rate of pin tract

infection, torque to remove pins or radiographic lucency around the pins.[37] The difference in outcome between the results of Collinge et al[36]and those of Coester et al[37] may be explained by the lack of direct inoculation of bacteria into the pin tract in the latter.

Massè et al[38] prospectively studied 24 men who were treated with monolateral external fixators for tibial or femoral diaphyseal fractures. A total of 50 screws were silver coated and were compared with 56 uncoated stainless steel screws. The coated screws resulted in a lower rate of positive culture (30.0%) than the uncoated screws (42.9%), but this did not reach statistical significance (p = 0.243). The clinical behaviour of the pins did not differ between the groups, with both showing similar inflammation and mechanical anchorage scores. Because of an increase in the serum silver levels the study was discontinued on ethical grounds. However, the development of nanoparticle coatings should reduce the systemic absorption of silver and allow similar studies to be conducted safely.
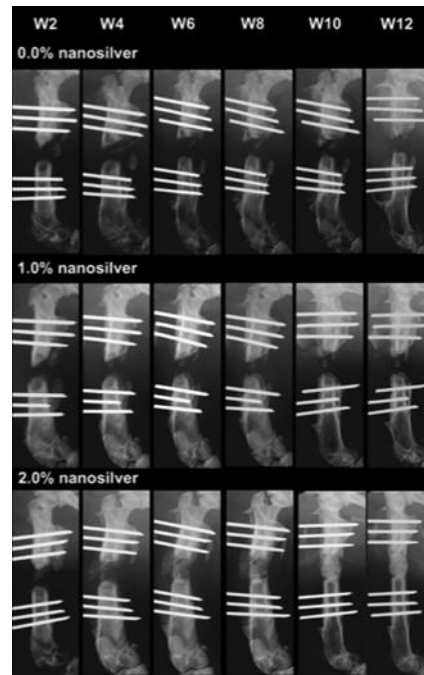

Fig. 2

Radiographic images of 108 CFUs*Staphylococcus aureus* Mu50-infected rat femoral segmental defects implanted with 0.0%, 1.0% and 2.0% nanosilver–polylactic-co-glycolic acid bone grafts coupled with 30 mg/ml bone morphogenetic protein-2. From left to right at weeks 2, 4, 6, 8, 10 and 12. Reproduced with permission from Zheng Z, Yin W, Zara JN, et al. The use of BMP2 coupled Nanosilver-PLGA composite grafts to induce bone repair in grossly infected segmental defects. Biomaterials 2010;31:9293-9300.

Osteomyelitis and infected nonunion

Electrically generated silver ions have been used to treat osteomyelitis and infected nonunions with variable success.[39-42] Concerns regarding toxicity and the ability to overcome large bone defects have limited the application of silver iontophoresis; a physical process by which ions flow diffusively in a medium driven by an applied electric field. The insertion of autogenous bone graft or allograft in the presence of active infection is contraindicated because the graft may act as a nidus for infection,[43] but a tissue-engineered graft that can control infection and promote bone regeneration would be advantageous. Zheng et al[44] have developed a composite bone graft consisting of bone morphogenetic protein 2 (BMP-2) coupled with nanosilver polylactic-co-glycolic acid (PLGA). A critical defect measuring 6 mm was created in the femoral diaphysis of rats, and bone grafts containing variable quantities of nanosilver were implanted into the defects. The grafts were injected with ten colony-forming units (CFU) of vancomycin-resistant MRSA. No antibiotic was administered post-operatively. Radiographic and histological analysis 12 weeks after implantation showed defect fusion from new bone formation in the BMP-2-2% nanosilver–PLGA graft (Fig. 2). In contrast, there was loss of bone and regression of the proximal and distal cut bone ends of the femur in the control group, which had a BMP-2-PLGA graft without nanosilver. Elimination of bacteria in the defect by the 2% nanosilver graft composite resulted in considerably more stimulation of new bone formation than the control graft, with resultant union.

Hydroxyapatite (HA) coating

HA is often used to coat trauma implants as it stimulates osseo-integration. It does not, however, contain any antibacterial properties unless it is combined with zinc, copper, titanium (Ti) silver or other antibiotic agents. A number of studies have investigated the efficacy of silver-containing hydroxyapatite coatings (Ag-HA) as potential agents for reducing the rate of implant-associated infections. In an *in vitro* study comparing the differences in bacterial adhesion on Ti, HA and Ag-HA surfaces, Chen et al[45] assessed the efficacy of co-sputtered silver-containing HA. There was significantly lower adhesion of *S. aureus* and *S. epidermidis* on the Ag-HA surfaces, indicating that these coatings were bactericidal.

There have been several studies investigating various methods for the incorporation of silver into HA coatings. These include ion exchange, thermal decomposition, sol-gel technology, magnetron sputtering, ion beam-assisted deposition and electrochemical deposition.[46-50] The goal is to achieve a uniform coating that produces an antibacterial effect without compromising the osteoconductive effect of HA. The results have been promising, with most studies concluding that silver can be added to HA coatings without affecting the mechanical properties of HA. A universal occurrence in all studies of silver coatings is the 'peak effect', whereby a large release of silver ions is seen in the initial period. This is because the ions lie on the surface of the coating. This large release of ions helps to protect against infection in the first few weeks, when the risk of peri-prosthetic infection is highest.[51] However, after this period there is minimal antimicrobial efficacy. A recent study[52] investigated the co-deposition of silver and HA by electrochemical means. It was found that this method gave a steady prolonged release of silver ions which the authors suggested was due to the release of silver as the HA dissolved (compared with a double coating, in which silver overlies the HA coating). Thus, co-deposition gives good long-term antimicrobial cover but with loss of the 'peak effect', as there is limited bactericidal effect in the initial period.[52] Shimazaki et al[53] conducted a study in which AgHA-coated titanium plates were implanted subcutaneously in the backs of Sprague–Dawley rats, comparing Ag-HAcoated plates with HA-coated plates in terms of their activity against MRSA. The results demonstrated significantly fewer MRSA CFUs on the Ag-HA-coated plates, showing promise for the use of such coatings clinically. Akiyama et al[54] investigated the bactericidal activity of Ag-HA-coated titanium in the medullary cavities of rat tibias. Again, the effects of Ag-HA coatings were compared with those of HA coatings and their relative activity against MRSA. The reduction in the numbers of viable MRSA in the tibiae with Ag-HA-coated implants compared with the HA-coated implants was statistically significant when measured at 24, 48 and 72 hours post-operatively (p = 0.002, p = 0.008 and p = 0.041, respectively). They also found significant differences on radiological assessment at four weeks, suggesting a prolonged inhibitory effect of the silver ions on bacterial growth.[54]

Bone cement

Alt et al[55] reported an *in vitro* study comparing nanosilverloaded bone cement with gentamicin-loaded and plain cement. Each cement type was tested against *S. epidermidis*, MRSE and MRSA. Only the nanosilver cement had high antimicrobial efficacy against all bacterial strains. Prokopovich et al[56] conducted a novel study using tiopronin (a thiol compound) as a stabilising agent, and encapsulating the produced nanosilver in bone cement. The tiopronin provided excellent stability to the nanosilver and the combination exhibited good antimicrobial efficacy without affecting the mechanics of the cement or producing cytotoxicity.

Dressings

Silver has been used in wound dressings for some time, and more recently dressings containing nanosilver have become available. These are designed to provide a sustained release of silver ions over a number of days, taking effect via delivery into the wound and through their action on the exudate released from the wound. Studies have shown that dressings containing nanosilver have a higher antimicrobial effect than bulk silver, although little is known about the possibility of increased toxic effects.[57]

Coating of trauma implants

Silver coatings on trauma implants have generally proved effective *in vitro*, but *in vivo* studies have shown mixed results.[58,59] In 2004, Sheehan et al[60] investigated the effect of Ti, stainless steel and silver-coated implants against biofilm formation in rabbits. A Kirschner wire (silver-coated titanium, silver-coated stainless steel, and titanium and stainless steel controls) 2 mm in diameter was implanted into each of the distal femoral canals of the rabbits, and *S. epidermidis* and *S. aureus* were introduced by direct inoculation. There was no statistically significant difference in bacterial adhesion when comparing the control groups with the silver-coated implants. More recent *in vivo* work by Kose et al[61] used a more sophisticated method of silver coating. In their study, instead of directly coating the implants with metallic silver, a silver ion-doped calcium phosphate-based ceramic nanopowder was developed, the idea being that ionic silver is more biologically active. This coating was applied to 2.5 mm

titanium alloy pins, which were implanted in the distal femoral canals of rabbits, similar to the study by Sheehan et al,[60] and MRSA was inoculated. Colonisation of the silver-coated implants was compared with that of the uncoated and HA-coated implants. In the silver-coated group, the antimicrobial outcomes were more favourable, with a lower proportion of positive cultures and lower rates of osteomyelitis.[61] Comparing the results of these two studies, it may be hypothesised that advances in the production of silver coatings have allowed for the manufacture of more biologically active models.

Toxicity

In addition to their use on orthopaedic implants, silver nanoparticles can be found in common consumer products as a result of their excellent antimicrobial properties.[62-64] With the recent concerns relating to the effect of metal ion exposure as a result of metal-on-metal arthroplasty, surgeons are apprehensive about the potential toxic effect of silver nanoparticles.[65] A cautionary note has been sounded elsewhere regarding the introduction of nanotechnology to medicine.[66] A number of studies have been performed exploring the effect of sliver nanoparticles on a variety of cell types. These investigations have demonstrated that silver nanoparticles have the potential to induce developmental abnormalities in zebra fish embryos,[67] disrupt the cell membrane,[68] and induce genotoxic and cytotoxic damage to human lung fibroblast and glioblastoma cells,[69] in addition to a system-wide suppression of the immune system.[70] One of the difficulties of using silver nanoparticles in orthopaedics is controlling the release of the silver ions. These particles can be released from the connection sites on the implants to which they were attached, under the biodegrading effects of body fluid during medical treatment, and can harm the surrounding tissue.[71] Considering that most patients into whom these implants have been inserted have undergone some bony procedure, the influence of the silver nanoparticles on bony metabolism is of critical importance. Pauksch et al[72] recently explored this by investigating the effect of silver nanoparticles on primary human mesenchymal stem cells and osteoblasts. Their study demonstrated silver nanoparticle-mediated cytotoxicity at higher concentrations (10 μg/g) and suggested that a therapeutic window for the application of these particles in medical products might exist. Greulich et al[73] also confirmed that silver nanoparticles were taken up by human mesenchymal stem cells and were found intracellularly in endolysosomal structures but not in the cell nuclei. A further study by Necula et al[74] focused on the *in vitro* cytotoxicity of silver nanoparticles of various concentrations using a human osteoblastic cell line and evaluated their bactericidal activity against MRSA. The results showed that high concentrations of silver nanoparticles (3.0 Ag) were extremely cytotoxic, but lower concentrations (0.3 Ag) demonstrated optimum cell growth of osteoblasts as well as good antibacterial properties. It appears that the cytotoxicity is not only dose dependent but also particle size dependent. Albers et al[75] demonstrated that the antibacterial effects of silver nanoparticles occurred at concentrations that were two to four times higher than those inducing cytotoxic effects. The authors stated that such adverse effects on osteoblast and osteoclast survival may have deleterious effects on the biocompatibility of orthopaedic implants.

Another area of concern with respect to toxicity is the effect that silver nanoparticles might have on DNA synthesis, which would be particularly relevant when implants coated with silver nanoparticles are used in women of childbearing age. Powers et al[76] showed that silver nanoparticles compromised neurodevelopment in PC12 cells, a model used for neuronal differentiation and neurosecretion. Their study revealed that exposure to silver nanoparticles was dependent on size and the coating to which it was attached, indicating that the effects are not due simply to the release of silver ions into the environment. Subsequent work from this group explored the effect of embryonic exposure of silver nanoparticles on zebra fish. The results indicate that the silver nanoparticles act as a developmental neurotoxicant, which can cause persistent neurobehavioral effects, which is also highly dependent on particle coating and size.[77,78] Kovvuru et al[79] explored the effect of oral ingestion of silver nanoparticles on inducing DNA damage and permanent genome alterations in a mouse model. Their results revealed that silver nanoparticles induced large DNA deletions in developing embryos, irreversible chromosomal damage in bone marrow, and double-strand breaks and oxidative DNA damage in peripheral blood. These issues highlight the health concerns about ions released from silver nanoparticles, particularly at high concentrations, although it should be noted that none of these issues have so far been reported in humans. A further concern relates to the effect of circulating silver nanoparticles, which inevitably end up in contact with vascular endothelium and have the potential to induce cardiovascular damage. In an *in vitro* study Ucciferri et al[80] demonstrated that silver nanoparticles have a toxic effect on primary human endothelial cells. Furthermore, endothelial cells were shown to be more susceptible when exposed to silver nanoparticles under flow conditions in a bioreactor. This study highlights the fact that although simple *in vitro* tests are useful to screen compounds and to identify the type of effect induced on cells, they may not be sufficient to define safe exposure limits. The authors contend that more physiologically relevant *in vitro* models need to be developed to understand better how nanomaterials can affect human health. With respect to nanosilver, particle size can be significant in terms of side effects. Martínez-Gutierrez et al[81] conducted a study which showed that the optimum particle size in terms of antimicrobial efficacy is in the region of 20 nm to 25 nm. They also studied the cytotoxic effects associated with nanoparticles of this size

and concluded that 24 nm particles of nanosilver had potent antimicrobial activity, but this size of nanosilver particle was cytotoxic to macrophages, causing a proinflammatory response and apoptosis. There are limited examples of toxic effects of silver in orthopaedic practice. Vik et al[82] reported a neuropathy, resulting in grave muscle paralysis, in a patient in whom silver-impregnated cement was used at revision total hip arthroplasty. Intra-operative analysis of the fluid drawn from the hip joint revealed that the concentration of silver was 1000 times the normal serum reference value. Following removal of the prosthesis and cement, the serum concentration of silver decreased over a period of two years from more than 60 times to 20 times normal, and the patient partially recovered from her muscle paralysis.[83] Gosheger et al,[84] in a rabbit model, also explored the infection rates and toxicological effects of silver-coated megaendoprostheses. Their study demonstrated that the concentration of silver in blood and organs was elevated with the use of silver-coated implants, although there were no pathological changes in laboratory parameters or histological changes of the organs.

In conclusion, the use of silver nanoparticle technology in orthopaedic devices has great potential as a means to reduce the incidence of implant infection. Whereas many of the early animal studies produced positive initial results, this area certainly warrants further research, especially to elucidate the potential harmful effects of circulating silver nanoparticles. Further development of silver nanoparticle technology in orthopaedics needs to focus on controlling the release of the nanoparticles from the implant so that it is not only bioactive in preventing infection but also biocompatible with the host.

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