Silver and nanoparticles of silver in wound dressings: A review of efficacy and safety

Article *in* Journal of Wound Care · November 2011

DOI: 10.12968/jowc.2011.20.11.543 · Source: PubMed

3 authors, including:

Richard White
University of Worcester
215 PUBLICATIONS **2,451** CITATIONS

SEE PROFILE

Silver and nanoparticles of silver in wound dressings: a review of efficacy and safety

Wound infections present a significant clinical challenge, impacting on patient morbidity and mortality, with significant economic implications. Silver-impregnated wound dressings have the potential to reduce both wound bioburden and healing time. The silver ion Ag^+ is the active antimicrobial entity; it can interfere with thiol (–SH) groups and provoke the generation of reactive oxygen species (ROS), a major contributor to its antibacterial efficacy. Recently, silver nanoparticles have gained considerable interest in wound bioburden reduction and in anti-inflammation, as they can release Ag^+ ions at a greater rate than bulk silver, by virtue of their large surface area. If released from dressings, they also have the potential to cross biological compartments. This review aims to consolidate recent findings as to the efficacy and safety of different formulations of silver used as an antiseptic agent in dressings, summarising the features of silver nanomaterials, with particular attention to the dose-dependencies for biological effects, highlighting the need for information on their uptake and potential biological effects.

silver nanoparticles; wound bioburden; antibacterial efficacy

Use of silver-based compounds in wound care dates back to the early 1970s, which saw the introduction of the antimicrobial-antibiotic combination silver sulphadiazine, providing an effective, broad-spectrum treatment.[1] This led to the incorporation of silver into wound dressings.

Silver is inert but ionises on contact with an aqueous environment, yielding Ag^+, which is believed to be the active antimicrobial agent.[2] The mechanisms of microbial cell death may be via a number of routes. First, the Ag^+ must penetrate the peptidoglycan cell wall, to gain access to the bacterial cell. Entry into the cell is followed by damage to DNA and bacterial proteins involved in key metabolic processes, resulting in lack of bacterial replication and, ultimately, cell death.[3]

The uptake of silver ions into *Escherichia coli* has been shown to be followed by the inhibition of phosphate uptake and exchange; a process that is reversible by the presence of thiols (–SH),[4] suggesting the role of protein thiol oxidation in toxicity. Eukaryotic cells are less susceptible to silver toxicity than prokaryotes, which is thought to be due to the increased level of structural and functional redundancy within eukaryotes and an increase in cell size, requiring an increased silver concentration for a toxic effect.[5]

Dressings incorporating silver preparations, including those with nanocrystalline material, have been commercially available for some time. The dressings are designed either to provide a sustained source of silver ions for delivery into the wound, over a period of up to 7 days, to maximise bacterial killing,[6] or the silver ions may be chemically attached to the dressing material and exert their antimicrobial effects on exudate being drawn out of the wound by the action of the dressing.

L.J. Wilkinson;[1]
R.J. White;[2]
J.K. Chipman;[1]
1 School of Biosciences, The University of Birmingham, UK;
2 Institute of Health and Society, University of Worcester, UK.
Email: j.k.chipman@bham.ac.uk

Declaration of interest: The authors have performed consultancy services for Mölnlycke Healthcare AB. This review was funded by an unrestricted research grant from Mölnlycke Healthcare.

A maximum concentration of ~1 part per million (1µg/ml) silver ions is thought to be achievable in wound exudate exposed to different silver-treated dressings.[7] This maximal concentration is thought to be due to the action (at least in part) of chloride ions in exudate, which bind Ag^+, forming an insoluble salt (AgCl) and limiting availability of silver ions to the wound bed.[8] Data from some *in vitro* microbiological studies suggest that 1ppm silver is sufficient to achieve bactericidal action,[9] whereas other *in vitro* data show that over 80% of the organisms tested in complex media have minimal inhibitory concentrations (MIC) that exceed 1ppm of silver.[10] The nature of the medium (salt and protein content) can have a marked influence on silver ion levels (lower levels were found in the presence of saline compared with water).[9]

The promotion of wound healing by silver agents is still very much theoretical, with no proof of efficacy. It is, nevertheless, worthy of conjecture based on the existing laboratory research. It has been established that, in acute murine wounds hydrogen peroxide and other reactive oxygen species (ROS) produced locally in wounds, principally through the NADPH oxidase activity of inflammatory cells, can stimulate cell proliferation, through induction of vascular endothelial growth factor.[11]

In chronic wounds, this natural process may be limited due to oxygen deficiency; reactive oxygen provision from silver ions or silver nanomaterials could potentially help restore this necessary repair mechanism. The inhibition of serine proteases by silver ions also has an anti-inflammatory potential, particularly in chronic wounds.[12]

In a porcine model of contaminated wounds, Wright et al. provided evidence that nanocrystalline silver may contribute to healing, through the ability to reduce local matrix metalloproteinase activity and to elevate cellular apoptosis, potentially reducing inflammation.[13] Indeed, it has been shown that erythema was reduced in a concentration-dependent manner by nanocrystalline silver in a guinea pig model of allergic contact dermatitis, suggesting a beneficial influence on skin inflammation.[14] Furthermore, in a mouse model of dermatitis, nanocrystalline silver suppressed the expression of the cytokines TNF-alpha and IL-12 and induced apoptosis of inflammatory cells.[15] Thus, these anti-inflammatory effects, which appear to be pH-dependent, are of great interest.[16] However, as silver nanomaterials have also been associated with anti-proliferative effects if the concentration is high enough, there is a need to ensure an optimal concentration.[17]

This review aims to consolidate recent findings on the efficacy and safety of different formulations of silver used as an antiseptic agent in dressings, summarising the features of silver nanomaterials, with particular attention to the dose-dependencies for biological effects, highlighting the need for information on their uptake and potential biological effects.

Silver nanoparticles

A nanoparticle, or nano-crystal, is defined as having diameter 10–100nm. While they are defined by their size, their versatility comes from their physical and chemical properties, which has resulted in their incorporation into an increasing array of consumer products.

The decrease in the size of a material is accompanied by a corresponding increase in its surface area to volume ratio and hence an increase in the number of atoms on the surface, compared with its larger equivalents.[18] The net effect of this is that more atoms are available for interaction with other materials; in practical terms, this gives rise to unique and frequently unpredictable properties associated with nanoparticle products.

While the unique physicochemical properties of nanoparticles have proven to be advantageous in many fields, they have also raised concern about the potential for novel and unanticipated interactions with biological systems, based on the possibility of increased reactivity and potential for toxicity.

The initial interest in the potential for silver nanoparticles as antimicrobial agents was based on the assumption that they have similar bactericidal effects to other silver preparations that release Ag^+. However, they may also have advantages due to their unique physicochemical characteristics, in particular their large surface area in relation to dose. Indeed, release of Ag^+ from nanosilver particles of diameter <20nm is more than 100-fold higher than that from larger silver particles.[2]

Properties related to nanostructure may give rise to intrinsic antimicrobial activity over and above the release of Ag^+ ions.[3] Early indications as to their efficacy have been promising and a number of studies have demonstrated nanosilver dressings to have higher bactericidal capability *in vitro* than those containing bulk silver, albeit through similar mechanisms.[19] To date, however, despite silver nanoparticles being highly efficacious in terms of antimicrobial action *in vitro*,[20] little is known about their capacity for toxicity in humans, or their potential to adversely affect ecosystems when released into the environment.

Silver uptake and distribution in the body

Normal concentrations of silver in humans are very low; serum concentration are typically less than 2µg/l, deriving from inhalation of particulate matter and dietary sources,[21] while silver has the potential to enter the human body via inhalation, dermal absorption and oral ingestion.[2]

Dermal uptake of xenobiotics (chemicals found in an organism, not normally expected to be present

in it) depends on various parameters, including the concentration of the material in question, the area exposed and the duration of exposure. Intact human skin was thought to be largely impermeable to silver nanoparticles, due to epidermal keratin and phospholipids, and protein thiol groups.[22] Studies devoted to absorption of nanoparticles from sunscreen, for example, have also demonstrated little, if any absorption beyond the stratum corneum.[23] However, as silver dressings involve direct contact with damaged skin (a breached dermal barrier), there is the potential for systemic absorption of silver and thus for associated toxicity.[24] Thus, one aspect still needing investigation is the extent to which silver nanoparticles can be released from the skin dressings in which they may be employed.

Chronic exposure to silver in humans can result in its deposition in the body, giving rise to a condition known as 'argyria', which is associated with blue-grey skin and eye discolouration.[20] This gives an indication as to the pattern of distribution of silver *in vivo*.

Two of the major determinants of the extent of passage of silver across biological membranes are the particulate size and degree of ionisation of the silver source.[25] Thus, soluble silver compounds are more readily absorbed than metallic or insoluble silver.[26] Silver ions (Ag^+) are highly reactive, and thus are not thought to undergo passive diffusion across biological membranes. Instead they are believed to predominantly traverse membranes by binding ion transporters, such as those responsible for cellular Na^+ and Cu^+ uptake.[27] Silver nanoparticles may enter the cell via pinocytosis and endocytosis.[4]

Particles on the nanoscale penetrate deeper into skin than larger particles; this finding has led to nanoparticles being further developed as novel drug delivery entities. Liu et al.[28] studied the impact of the size of nanosilver particles, ranging 5–50nm, on their uptake to human cells *in vitro*, concluding that smaller sized particles penetrated into the cells more readily. More evidence for the importance of particle size in absorption comes from studies on the gastrointestinal tract,[29] with evidence that smaller sized nanoparticles undergo increased intestinal uptake compared with their larger counterparts.

It is thought that silver nanoparticles undergo a unique pattern of distribution within the human body, compared with larger sized particles;[30] although, to date, their precise pattern of distribution is largely unknown. There is evidence of silver nanoparticles being able to bind plasma proteins,[31] including globulins,[32] and also to intracellular metal-binding proteins, such as metallothioneins.[2] This binding may impact on the potential for distribution and persistence of nanosilver and also affect the binding and transport of endogenous ligands.

The liver appears to be the main site of deposition of silver nanoparticles in the body following their uptake by macrophages;[24] however, their potential impact on normal liver function is currently unknown. A patient documented by Trop et al.,[33] as noted below, was revealed to have raised levels of liver enzymes in the blood and elevated plasma silver concentrations following dermal exposure to nanocrystalline silver. Although the observed raised level of liver enzymes was transient, this may indicate a potential for alterations in hepatic function following exposure to nanosilver.

The potential for silver to cross other biological barriers must also be considered. Theoretically, any compound that is present in the maternal circulation has the ability to cross the placenta, either by diffusion, or by utilising the specialised transport mechanisms that have evolved to facilitate the transport of nutrients and waste products to and from the foetus. To date, the capacity of silver in any form to cross the placenta is unknown, as is the potential for reproductive or teratogenic effects.[24]

Another main area of concern in considering the distribution of xenobiotics is the brain and central nervous system; however, studies have demonstrated a lack of silver localisation to neuronal tissue, thought to be due to lysosomal binding of silver within the blood brain barrier.[34] Silver nanoparticles, but not silver microparticles, have been found to cross through an *in vitro* blood brain barrier model system and accumulated within the rat brain microvessel vascular endothelial cells of this model.[34,35]

There are few studies on the ultimate fate of silver, following its absorption and distribution in humans. Estimates of silver's biological half-life in humans range from several days up to 50 days.[32] Biliary excretion of silver appears to be quantitatively the most significant route in humans and laboratory animals, renal excretion of silver occurring to a lesser extent.[2] The excretion pattern of silver nanoparticles in humans is largely undetermined.

Silver exposure from wound dressings

Skin that has been damaged via trauma or disease, whereby the stratum corneum is compromised, is more vulnerable to the passage of xenobiotics, potentially including nanoparticles.[36] Studies on wound dressings containing silver preparations have demonstrated that the majority of the Ag^+ ions released into the wound are not available for systemic absorption.[37] Silver was detected in urine, blood (maximum 120µg/l) and tissues of humans following treatment of serious skin burns with 0.5% silver nitrate.[38] In silver sulphadiazine (SSD)-treated burn patients, plasma concentrations of silver were reported up to 50µg/l within 6 hours, with a maximum recorded level of 310µg/l and silver deposits were also identified in the liver, kidney and corneal tissue.[21] Walker et al.[9] found that treatment of burn wounds with dressings containing nanocrystalline

References

1 Cooper, R., White, R.J. Silver sulfadiazine: a review of the evidence. Wounds-UK. 2005; 1: 2, 51–61.

2 Lansdown, A.B. A pharmacological and toxicological profile of silver as an antimicrobial agent in medical devices. Adv Pharmacol Sci. 2010; 2010:910686. Epub 2010.

silver resulted in deposition of the silver particles into the mid and deep dermis, leading to argyria in some cases. There is one report of a 17-year-old boy with 50% burns who displayed symptoms of argyria after treatment with a nanocrystalline silver dressing.[33] The plasma concentration reached was reported as 107µg/kg.

Potential toxicity of silver

The potential for toxicity of silver salts is well documented due to the varied history of use of silver as a therapeutic agent. Chronic ingestion, inhalation or dermal exposure to silver salts in sufficient quantities give rise to argyria and argyrosis: blue-grey discolouration of the skin and eyes, respectively, due to deposition of precipitates of silver. Argyria is thought to be irreversible in many cases and is not associated with toxicity, but is disfiguring and therefore generally considered undesirable.[2,20] Other than this, there is little in the literature to suggest significant toxicity following exposure to silver, with few studies on the intracellular mode of action. There are some historical studies, which associate high doses of silver nitrate with damage to the gastrointestinal tract and occasional fatality.[2]

There is no evidence to date for the ability of silver in any form to exert significant toxicity on the immune, cardiovascular, nervous or reproductive system in humans.[5,39] Furthermore, the lethal dose of silver nitrate to humans is thought to be in the region of 10g. There are reports of allergic reactions to silver, mostly following dermal exposure via cosmetic and occupational sources, and there is also some evidence of contact hypersensitivity arising from exposure to SSD and also to silver-containing dressings. The American Conference of Governmental Industrial Hygienists (ACGIH) set a threshold limit value (TLV) for airborne exposure of 0.1mg/m^3 for metallic silver and 0.01mg/m^3 for soluble silver (which is more readily absorbed). These limit values are based on protection against argyria. Other international organisations have the same or similar values set.[26]

Potential toxicity of silver nanoparticles

The site of cellular deposition of any substance has the potential to determine its mechanism of action and toxicity at the cellular (or indeed molecular) level. Nanoparticles have been observed to exist free in the cytoplasm of eukaryotic cells and to undergo distribution to cytoplasmic compartments and the nucleus.[41] Currently, there is little evidence of adverse effects to humans resulting from exposure to nanosilver in consumer products, but this requires further investigation.[27]

In order to predict the risk associated with exposure to silver nanoparticles, it is important to clarify the mechanisms by which they may undergo cellular uptake and their potential mechanisms of toxicity. A number of *in vitro* models have been developed to investigate potential cytotoxic effects that may result from exposure to nanosilver. Toxicity of silver ions to eukaryotic cells mirror those suggested to underlie their means of microbial killing and are believed to be primarily via damage to protein thiols and the production of ROS. The net effects of ROS production in eukaryotes are wide and varied, and may result in oxidative damage to DNA, cellular membranes and proteins, the net effect being cell death via apoptosis or necrosis.[17] However, these adverse effects occur only when the natural antioxidant defence mechanisms of the body are overwhelmed, as ROS production and inactivation occurs naturally. A number of cellular antioxidant mechanisms have evolved to protect the cell from oxidative damage, as oxidants are normal endogenous by-products. Ordinarily, such protective mechanisms would serve to protect the cell from oxidative damage and allow cell survival.

Observations of deposition of silver nanoparticles *in vivo* have led to studies on target organ toxicity in animal models. One such study involved 28 day repeat oral dosage of rats with either low, medium or high concentrations of nanosilver. The high dose (1000mg/kg) was associated with raised levels of alkaline phosphatase (ALP) and serum cholesterol, which are markers of hepatotoxic changes.[41] However, one criticism of this study, as with others, was the selection of dose ranges and the relevancy of the very high dose to that which may be encountered in humans.

Possible mechanisms of toxicity of silver nanoparticles *in vitro* have been investigated with the aid of a number of key cytotoxicity assays. Such assays have investigated parameters such as alterations in metabolic activity of the cell, oxidative stress and resulting perturbations to cellular morphology and viability.[42] Nanoparticles in general appear to exert their effects in a sequential manner, initiated by an increase in ROS production and followed by inflammation, genotoxicity and cytotoxicity.[43]

Cellular effects of silver nanoparticles could potentially occur as a result of extracellular release of Ag^+ at the cell surface, leading to oxidative damage to the cell membrane (Fig 1). Although cytotoxicity of silver nanoparticles in human hepatoma cells appeared to be mediated by oxidative stress independent of Ag^+ release.[41] As stated above and illustrated in Fig 1, silver nanoparticles may enter the call via pinocytosis or endocytosis and, once free in the cytoplasm, have the potential to initiate oxidative damage via a number of pathways. The dissolution of Ag^+ from the surface of the nanoparticle can result in the production of hydroxyl radicals ($^{\bullet}OH$), for example through interaction with hydrogen peroxide. Silver nanoparticles may also interact directly with NAPDH oxidase or with mitochondrial function, resulting in the production of ROS.[43]

3 Chaloupka, K., Malam, Y., Seifalian, A.M. Nanosilver as a new generation of nanoproduct in biomedical applications. Trends Biotechnol. 2010; 28: 11, 580–588.

4 AshaRani, P.V., Low Kah Mun, G., Hande, M.P., Valiyaveettil, S. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano. 2009; 24; 3: 2, 279–290.

5 Wijnhoven, S.W.P., Peijnenburg, W.J.G.M., Herberts, C.A. et al. Nano-silver — a review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicology. 2009; 3: 2, 109–138.

6 Silver, S., Phung le, T., Silver, G. Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds. J Ind Microbiol Biotechnol. 2006; 33: 7, 627–634.

7 Percival, S.L., Bowler, P.G., Russell, D. Bacterial resistance to silver in wound care. J Hosp Infect. 2005; 60: 1, 1–7.

8 Ip, M., Lui, S.L., Poon, V.K.M. et al. Antimicrobial activity of silver dressings: an *in vivo* comparison. J Med Microbiol. 2006; 55: 59–63.

9 Walker, M., Cochrane, C.A., Bowler, P.G., et al. Silver deposition and tissue staining associated with wound dressings containing silver. Ostomy Wound Manage. 2006; 52: 1, 42–50.

10 Nadworny, P.L., Burrell, R.E.A review of assessment techniques for silver technology in wound care. Part 1: *In vitro* methods for assessing antimicrobial activity. J Wound Technology. 2008; 2: 6–13.

11 Roy, S., Khanna, S., Nallu, K., et al. Dermal wound healing is subject to redox control. Mol Ther. 2006; 13: 1, 211–220.

12 Chambers, J.L., Christoph, G.G., Krieger, M., et al. Silver ion inhibition of serine proteases: crystallographic study of silver-trypsin. Biochem Biophys Res Commun. 1974; 59: 1, 70–74.

13 Wright, J.B., Lam, K., Buret, A.G. et al. Early healing events in a porcine model of contaminated wounds: effects of nanocrystalline silver on matrix metalloproteinases, cell apoptosis, and healing. Wound Repair Regen. 2002; 10: 3, 141–151.

Nanosilver has been demonstrated to target the mitochondria[44] and affect ATP production via disruption of the respiratory chain, resulting in further generation and release of ROS. More evidence for mitochondrial involvement in silver nanoparticle toxicity is provided by various research groups observing a decrease in the mitochondrial reduction of dimethyl thiazolyl diphenyl tetrazolium salt (MTT) in a dose-dependent manner in exposed cells.[45] Mitochondrial damage is followed by the release of both Ca^{2+} and Cytochrome c, an intermediate in the apoptotic pathway. The release of Cyt c into the cytoplasmic compartment results in the initiation of the so-called 'caspase cascade', the net effect of which is cell death by apoptosis.[46] A rise in intracellular calcium levels may also impact on Ca^{2+}/calmodulin dependent enzymes, leading to necrotic cell death.[47] However, we emphasise again that such effects are entirely dependent on a sufficient level of intracellular dosage.

A number of studies, including that by Hackenberg et al.,[40] report structural genetic aberrations including chromosome deletions and exchanges caused by nanomaterials. The ability of nanoparticles to bring about genetic instability has led to concern regarding their potential for mutagenicity and carcinogenicity.[4,48] However, there is little evidence to show that such a risk exists.

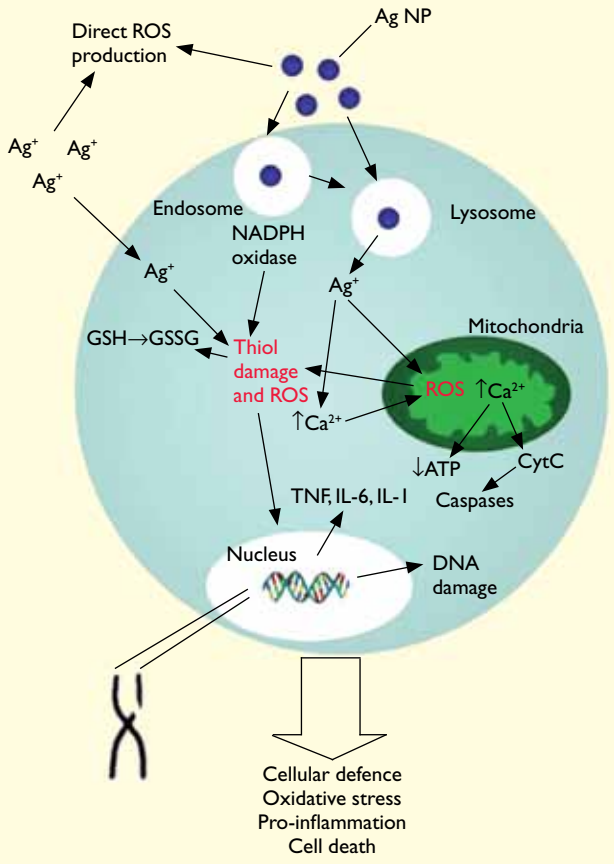
AshaRani et al.[4] conducted a study that involved exposing human lung fibroblast cells and human glioblastoma cells to silver nanoparticles at concentrations of up to 400µg/ml and observed an increase in production of ROS and a decrease in ATP production, both findings supporting the hypothesis for ROS-driven cell toxicity and disruption of cellular respiration. The authors also noted distribution of the particles to the cytoplasm, endosomes, mitochondria and nucleus. Evidence for genotoxicity, with a dependence on concentration, resulting from exposure to the nanoparticles was provided by increased DNA damage, as identified by the COMET assay and cell cycle arrest. However, studies point to genotoxicity resulting indirectly from ROS production,[49] and generally mutagenicity assays of silver have proved negative or at high concentrations only.[50,51] The potential for carcinogenic activity is therefore unlikely.

The issue of dose and extrapolation

The vast majority of studies dedicated to studying the effects of silver salts and nanosilver are conducted *in vitro*, due to practicalities, such as time, cost and ethical implications. One of the key criticisms levied against *in vitro* work on nanoparticles is the selection of dose ranges and the extent to which these reflect the concentrations to which individuals are likely to be exposed *in vivo*.[30] Hence, it is unclear in many cases as to the relevance of findings of toxicity studies to real life exposures. Indeed, we emphasise the fact that many of the potential biological effects of silver ions and silver nanomaterials noted above may well occur only at high concentrations, which would exceed those feasible for wound dressings. Nevertheless, an understanding of the potential effects and their dose dependency is important in safety assessment.

Any *in vitro* study is also subject to potential discrepancies between the doses introduced to the model system versus that available to the cells. This is further complicated when trying to extrapolate from *in vitro* to *in vivo* systems, with consideration of the impact of physiology and metabolism in an intact organism. This uncertainty is compounded when working with nanoparticles as their unique physicochemical properties give rise to differences in dissolution and surface reactivity, compared with larger particles. Furthermore, there is debate on the definition of dose and the relevance of mass, the traditional definition of dose versus the extent to which dose should be based on particulate surface area and crystalline structure.[27]

Fig 1. Proposed modes of action of silver ions and silver nanoparticles in biological cells at cytotoxic concentrations (Adapted from Li et al.)[43]



14 Bhol, K.C., Alroy, J., Schechter, P.J. Anti-inflammatory effect of topical nanocrystalline silver cream on allergic contact dermatitis in a guinea pig model. Clin Exp Dermatol. 2004; 29: 3, 282–287.

15 Bhol, K.C., Schechter, P.J. Topical nanocrystalline silver cream suppresses inflammatory cytokines and induces apoptosis of inflammatory cells in a murine model of allergic contact dermatitis. Br J Dermatol. 2005; 152: 6, 1235–1242.

16 Nadworny, P.L., Wang, J., Tredget, E.E., Burrell, R.E. Anti-inflammatory activity of nanocrystalline silver-derived solutions in porcine contact dermatitis. J Inflamm (Lond). 2010; 7: 13.

17 Asharani, P.V., Hande, M.P., Valiyaveettil, S. Anti-proliferative activity of silver nanoparticles. BMC Cell Biol. 2009; 10: 65.

Various studies have employed concentration ranges of nanoparticles that may better represent those encountered *in vivo*. Cha et al.[52] employed concentrations notably far lower than those in the previous study, exposing human hepatoma cells to concentrations of up to 2.4µg/ml silver particles of 15nm diameter. The investigators observed no effect on levels of reduced glutathione (GSH), an indicator of oxidative stress, or perturbations to mitochondrial function, but they did observe lower DNA levels in cells exposed to nanoparticles, suggestive of apoptosis.

Perhaps most relevant to dermal exposure of nanoparticles is the work of Arora et al.,[44] who investigated the effects of silver particles of 7–20nm on primary mouse fibroblasts and liver cells and observed an increase in levels of reduced GSH and superoxide dismutase (SOD), both indicators of oxidative stress. The authors hypothesised that, at lower concentrations of silver (up to 25µg/ml and 100µg/ml for the fibroblasts and liver cells, respectively), there was an enhancement of cellular protection mechanisms. In their earlier work on secondary skin cell lines, this enhancement was restricted to lower doses of silver, but it is noteworthy that the non-toxic concentration range is comparable to that encountered in topical applications of silver products, providing evidence for the safety of silver nanomaterials at such doses.[53]

Additional considerations when comparing studies on nanosilver toxicity and mode of action surround the characteristics of different batches of particles.[4] Ahamed et al.[48] compared the effects of polysaccharide-coated versus uncoated silver nanoparticles and concluded that the coated particles induced a greater degree of DNA damage than the native particles and that the two types may undergo different intracellular distribution patterns. This was hypothesised to be due to the increased tendency of uncoated particles to undergo agglomeration and so increase in mass.

Silver and silver nanoparticles in the environment

The use of silver preparations, including those containing nanoparticles, in medicinal products and an array of other consumables offers the potential for the metal to enter the environment via a number of routes. Worldwide production of silver as nanoparticles is estimated to be in the region of 500 tonnes per annum.[54] These may enter the environment via discharge during synthesis and manufacturing, but also during the disposal or recycling.[55] An estimated 270 tonnes of silver nanoparticles end up in the sewage system each year.[54] In the UK, USA and other countries, sewage sludge is utilised as an agricultural fertiliser,[56] so any nanoparticles present in sewage effluent have the potential to (re)enter the food chain.

Bacterial biofilms are widely utilised in sewage treatment[57] and, owing to the broad spectrum antimicrobial action of silver, there is concern as to the impact of silver ions on the denitrifying capacity of bacteria contained within such biofilms. Ultimately, this may result in problems for wastewater treatment.

Silver is also associated with toxicity to marine and freshwater fish, and other aquatic species, at concentrations as low as 1–5µg/l;[58] this has led to concerns about the impact of nanosilver on aquatic organisms. It raises the possibility that the concentration of nanosilver in this environment might enhance any toxic effects.[27] There is additional evidence of an increased uptake of silver nanoparticles compared with bulk silver and a different pattern of distribution.[59] The potential toxicity of nanosilver has also been found to depend on environmental conditions, such as constituents of organic matter and characteristics of water sources such as hardness and chloride levels, all of which might affect the physicochemical characteristics of the particles and their tendency towards bioaccumulation in the environment.[27]

The presence of silver in the environment is not the only concern, consideration must also be given as to the persistence of silver in the air, soil and in aquatic systems,[27,60] and the possibility of bioconcentration of silver in the food chain.

Conclusion

Wound infections are a common complication of surgery and trauma injuries and, owing to the increasing problem of antibacterial resistance, there is increasing pressure to source alternative anti-microbial agents. The antimicrobial potential of silver has been realised for centuries and recent developments in nanotechnology have led to the development of an array of consumer products, including wound dressings, containing silver as nanoparticles.

Silver salts, irrespective of their route of absorption, are associated with minimal toxicity to humans, hence silver's rich history as an antimicrobial agent. Manifestations of silver toxicity are limited to argyria, which, although considered unsightly, is harmless.[27] To date, there are significant gaps in our knowledge regarding the risk associated with exposure to nanosilver. Of concern are the potential unidentified and unpredictable effects from exposure to silver nanoparticles suggested by their unique surface chemistry and novel distribution patterns *in vivo* that are largely poorly understood.[61] There are an increasing number of studies dedicated to elucidating the mode of action of nanosilver species *in vitro* to better understand how they might exert their effects *in vivo*. Future studies must aim to fill in current knowledge gaps and enable a better understanding of the potential for toxicity of nanosilver *in vivo*. This should achieve better experimental design and standardised experimental procedures, together with more accurate characterisation of the nanoparticles in question.

18 Jones, C.F., Grainger, D.W. *In vitro* assessments of nanomaterial toxicity. Adv Drug Deliv Rev. 2009; 61: 438–456.

19 Lok, C.N., Ho, C.M., Chen, R., et al. Proteomic analysis of the mode of antibacterial action of silver nanoparticles. J Proteome Res. 2006; 5: 4, 916–924.

20 Samberg, M.E., Oldenburg, S.J., Monteiro-Riviere, N.A. Evaluation of silver nanoparticle toxicity in skin *in vivo* and keratinocytes *in vitro*. Environ Health Perspect. 2010; 118: 3, 407–413.

21 Wan, A.T., Conyers, R.A., Coombs, C.J., Masterton, J.P. Determination of silver in blood, urine, and tissues of volunteers and burn patients. Clin Chem. 1991; 37: (10 Pt 1), 1683–1687.

22 Lansdown, A.B. Physiological and toxicological changes in the skin resulting from the action and interaction of metal ions. Crit Rev Toxicol. 1995; 25: 5, 397–462.

23 Stern, S.T., McNeil, S.E. Nanotechnology safety concerns revisited. Toxicol Sci. 2008; 101: 1, 4–21.

24 Johnston, H.J., Hutchison, G., Christensen, F.M., et al. A review of the *in vivo* and *in vitro* toxicity of silver and gold particulates: Particle attributes and biological mechanisms responsible for the observed toxicity. Crit Rev Toxicol. 2010; 40: 4, 328–346.

25 Lansdown, A.B., Williams, A., Chandler, S., Benfield, S. Silver absorption and antibacterial efficacy of silver dressings. J Wound Care. 2005; 14: 4, 155–160.

26 Drake, P.L., Hazelwood, K.J. Exposure related health effects of silver and silver compounds: a review. Ann Occup Hyg. 2005; 49: 7, 557–585.

27 Fabrega, J., Luoma, S.N., Tyler, C.R., et al. Silver nanoparticles: Behaviour and effects in the aquatic environment. Environ Int. 2011; 37: 2, 517–531.

28 Liu, X., Lee, P.Y., Ho, C.M., et al. Silver nanoparticles mediate differential responses in keratinocytes and fibroblasts during skin wound healing. Chem Med Chem. 2010; 1; 3, 468–475.

The main questions that need to be considered in establishing the safety of nanomaterials in general are linked to the uncertainties around the dose response relationships for the various responses that might occur *in vivo* based on observations made largely in cell culture systems and whether the surface area is an appropriate dose metric. Of particular relevance are the questions about the mechanisms and extent of cellular uptake of silver nanomaterials from wounded skin; can nanomaterials be translocated to systemic sites and into the brain or across the placenta and how are they cleared from the body? Within cells is there access to DNA and ability to cause genetic damage? What surface characteristics are associated with potential toxicity and are the protein interactions that occur at the surface of the particles protective or adverse? It is emphasised that these questions do not imply that there might be a problem of safety in use of such materials in wound treatment but it highlights the areas of particular interest to ensure safety.

In summary, silver has a long history of use and silver dressings have shown great benefit in wound disinfection and healing. Silver has defined antimicrobial properties, is shown to be very effective in wound care treatments and is generally regarded as safe. Since the toxicity of silver is low, the benefit/risk ratio is favourable especially for moderate burns.[62] Nevertheless we recommend, as in White et al.,[63] that such dressings be used for no longer than 2 weeks, without sound clinical justification, and that failure to respond to treatment should result in careful reassessment and a potential change of topical antimicrobial agent. The comparative benefits of different silver compounds and different formulations require further study. In particular, nanoparticles provide considerably higher levels of silver than traditional formulations, and may potentially cross into cells with the potential for toxic effects. The potential environmental risks of nanoparticles must also be considered. ■

29 Ahamed, M., Alsalhi, M.S., Siddiqui, M.K. Silver nanoparticle applications and human health. Clin Chim Acta. 2010; 14: 411(23–24), 1841–1848.

30 Maynard, A.D., Warheit, D.B., Philbert, M.A. The new toxicology of sophisticated materials: nanotoxicology and beyond. Toxicol Sci. 2011; 120: (Suppl 1), S109–129.

31 Kunzmann, A., Andersson, B., Thurnherr, T., et al. Toxicology of engineered nanomaterials: focus on biocompatibility, biodistribution and biodegradation. Biochim Biophys Acta. 2011; 1810: 3, 361–373.

32 World Health Organisation (WHO). Guidelines for drinking-water quality. Vol 2. Health criteria and other supporting information (2nd edn). WHO, 1996.

33 Trop, M., Novak, M., Rodl, S. et al. Silver-coated dressing acticoat caused raised liver enzymes and argyria-like symptoms in burn patient. J Trauma. 2006; 60: 1, 648–652.

34 Lansdown, A.B. Critical observations on the neurotoxicity of silver. Crit Rev Toxicol. 2007; 37: 3, 237–250.

35 Tang, J., Xiong, L., Zhou, G. et al. Silver nanoparticles crossing through and distribution in the blood-brain barrier *in vitro*. J Nanosci Nanotechnol. 2010; 10: 10, 6313–6317.

36 Choksi, A.N., Poonawalla, T., Wilkerson, M.G. Nanoparticles: a closer look at their dermal effects. J Drugs Dermatol. 2010; 9: 5, 475–481.

37 Karlsmark, T., Agerslev, R.H., Bendz, S.H., et al. Clinical performance of a new silver dressing, Contreet Foam, for chronic exuding venous leg ulcers. J Wound Care. 2003; 12: 9, 351–354.

38 Bader, K.F. Organ deposition of silver following silver nitate therapy of burns. Plast Reconstr Surg. 1966; 37: 6, 550–551.

39 Chen, X., Schluesener, H.J. Nanosilver: a nanoproduct in medical application. Toxicol Lett. 2008; 176: 1, 1–12.

40 Hackenberg, S., Scherzed, A., Kessler, M. et al. Silver nanoparticles: evaluation of DNA damage, toxicity and functional impairment in human mesenchymal stem cells. Toxicol Lett. 2011; 201: 1, 27–33.

41 Kim, S., Choi, J.E., Choi, J. et al. Oxidative stress-dependent toxicity of silver naoparticles in human hepatoma cells. Toxicol In Vitro. 2009; 23: 6, 1076–1084.

42 Asharani, P.V., Low Kah Mun, G., Hande, M.P., Valiyaveettil, S. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano. 2009; 3: 2, 279–290.

43 Li, N., Xia, T., Nel, A.E. The role of oxidative stress in ambient particulate matter-induced lung diseases and its implications in the toxicity of engineered nanoparticles. Free Radic Biol Med. 2008; 44: 9, 1689–1699.

44 Arora, S., Jain, J., Rajwade, J.M., Paknikar, K.M. Interactions of silver nanoparticles with primary mouse fibroblasts and liver cells. Toxicol Appl Pharmacol. 2009; 236: 3, 310–318.

45 Ahamed, M., Posgai, R., Gorey, T.J. et al. Silver nanoparticles induced heat shock protein 70, oxidative stress and apoptosis in Drosophila melanogaster. Toxicol Appl Pharmacol. 2010; 242: 3, 263–269.

46 Galluzzi, L., Morselli, E., Kepp, O., Kroemer, G. Targeting post-mitochondrial effectors of apoptosis for neuroprotection. Biochim Biophys Acta. 2009; 1787: 5, 402–413.

47 Chipman, J.K. Mechanisms of cell toxicity. In: Bittar, E., Bittarm N. (eds). Principles of Medical Biology, Molecular and Cellular Pharmacology. Vol 8C. JAI Press, 1997.

48 Ahamed, M., Karns, M., Goodson, M., et al. DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. Toxicol Appl Pharmacol. 2008; 233: 3, 404–410.

49 Foldbjerg, R. Dang, D.A., Autrup, H. Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. Arch Toxicol. 2010; 85: 7, 743–750.

50 McCoy, E.C., Rosenkranz, H.S. Silver sulfadiazine: lack of mutagenic activity. Chemotherapy. 1978; 24: 87–91.

51 Denizeau, F., Marion, M.. Genotoxic effects of heavy metals in rat hepatocytes. Cell Biol Toxicol. 1989; 5: 1, 15–25.

52 Cha, K., Hong, H.W., Choi, Y.G. et al. Comparison of acute responses of mice livers to short-term exposure to nano-sized or micro-sized silver particles. Biotechnol Lett. 2008; 30: 11, 1893–1899.

53 Arora, S., Jain, J., Rajwade, J.M., Paknikar, K.M. Cellular responses induced by silver nanoparticles: *In vitro* studies. Toxicol Lett. 2008; 179: 2, 93–100.

54 Blaser, S.A., Scheringer, M., Macleod, M., Hungerbühler, K. Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles. Sci Total Environ. 2008; 390: 2–3, 396–409.

55 Köhler, A.R., Som, C., Helland, A., Gottscalk, F. Studying the potential release of carbon nanotubes throughout the application life cycle. J Cleaner Prod. 2008; 16: 927–937.

56 Nicholson, F.A., Smith, S.R., Alloway, B.J. et al. An inventory of heavy metals inputs to agricultural soils in England and Wales. Sci Total Environ. 2003; 311: 1–3, 205–219.

57 Khan, S., Mukherjee, A., Chandrasekaran, N. Silver nanoparticles tolerant bacteria from sewage environment. J Environ Sci (China). 2011; 23: 2, 346–352.

58 Farkas, J., Christian, P., Gallego-Urrea, J.A. et al. Uptake and effects of manufactured silver nanoparticles in rainbow trout (Oncorhynchus mykiss) gill cells. Aquat Toxicol. 2011; 101: 1, 117–125.

59 Yeo, M.K., Yoon, J.W. Comparison of the effects of nano-silver antibacterial coatings and silver ions on zebrafish embryogenesis. Mol Cell Toxicol. 2009; 5: 23–31.

60 Da Silva, B.F., Pérez, S., Gardinalli, P. et al. Analytical chemistry of metallic nanoparticles in natural environments. TrAC Trends in Analytical Chemistry. 2011; 30: 3, 528–540.

61 Ahamed, M., Karns, M., Goodson, M., et al. DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. Toxicol Appl Pharmacol. 2008; 233: 3, 404–410.

62 Coombs, C.J., Wan, A.T., Masterton, J.P. et al. Do burn patients have a silver lining? Burns. 1992; 18: 178–184.

63 White, R.J., Fumarola, S., Denyer, J. Interim advice on silver dressings in neonatal/paediatric wound and skin care. J Wound Care. 2011; 20: 4, 192.