Review

Nanosilver as a new generation of nanoproduct in biomedical applications

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Nanosilver (NS), comprising silver nanoparticles, is attracting interest for a range of biomedical applications owing to its potent antibacterial activity. It has recently been demonstrated that NS has useful anti-inflammatory effects and improves wound healing, which could be exploited in developing better dressings for wounds and burns. The key to its broad-acting and potent antibacterial activity is the multifaceted mechanism by which NS acts on microbes. This is utilized in antibacterial coatings on medical devices to reduce nosocomial infection rates. Many new synthesis methods have emerged and are being evaluated for NS production for medical applications. NS toxicity is also critically discussed to reflect on potential concerns before widespread application in the medical field.

Introduction

Silver nanoparticles (NPs), or nanosilver (NS), are clusters of silver atoms that range in diameter from 1 to 100 nm and are attracting interest as antibacterial and antimicrobial agents for applications in medicine. NS is a blossoming field of research and has been highly commercialized. Clothing manufacturers have incorporated NS into fabrics for socks and exploit the antibacterial activity for neutralization of odor-forming bacteria [1,2]. In addition, NS has been integrated into various food contact materials, such as plastics used to fabricate food containers, refrigerator surfaces, storage bags and chopping boards, under the pretext of preserving foods longer by inhibiting microorganism growth [3]. The medical industry has been slow to exploit the potential of NS in infection prophylaxis, but this field is now gaining momentum.

Recent evidence suggests that NS has a potent anti-inflammatory effect [4–6] and accelerates wound healing [7,8]. Silver has long been known to possess antibacterial activity and has been used throughout history, from Hippocrates' early treatment of ulcers to C.S.F. Crede's treatment for gonococcal infections in newborns. Silver is still used clinically and NS use is emerging as a valuable tool in the therapeutic armory (Figure 1). Silver sulfadiazine is the gold standard in the topical treatment of burn patients [9]. Resurgent interest in silver and NS has been motivated by the emergence of rampant antibiotic-resistant bacteria and the increasing prevalence of hospital-acquired bacterial infections. Silver use has been severely limited by the toxicity of silver ions to humans; however, nanotechnology has facilitated the production of smaller silver particles with increasingly large surface area-to-volume ratios, greater efficacy against bacteria [10,11] and, most importantly, lower toxicity to humans [12].

This review critically discusses the emerging use of NS in medicine, highlighting recent advances in the field and concerns regarding potential toxicity. We evaluate novel methods of NS synthesis and review current and emerging medical NS applications. For clarification, nanosilver and silver NPs are equivalent and serve as general terms for all silver nanostructures, whether nanocrystals, nanospheres [13] or colloidal NPs [14].

NS synthesis

Nanotechnology and modern synthetic chemistry have been used to establish a plethora of well-characterized methods for NS synthesis (Table 1). Each method has its own merits and limitations. Parameters that are influenced by the synthesis method used include the mean NP diameter and size, size distribution, NP shape, stability, inclusion of ligand shells and capping agents protecting the NS core, chemical yield of the reaction, and the presence of impurities.

An impressive range of extremely novel and ingenious methods for NS synthesis has been reported in the literature, although only a few have been adopted in mainstream production. Most commonly, NS is obtained by reduction of silver nitrate using either a reducing agent (e.g. sodium borohydride) or photoreduction via UV light [15,16]. However, a number of alternative 'green' chemistry synthesis routes have been reported [17]. Capping agents, such as citrate, are used to prevent aggregation and agglomeration of NPs. During chemical reduction, the reducing agent donates electrons to the silver ions (Ag^{+}), causing silver to revert to its metallic form (Ag^{0}). By controlling the experimental conditions (e.g. temperature, energy input, presence of capping agents), the reaction kinetics can be manipulated such that clustering silver atoms form NS of nanoscale dimensions.

NS biosynthesis has been achieved and many publications have documented the use of various species of bacteria and fungi [18,19]. These methods share the common methodology whereby silver nitrate solution is added to a microbial supernatant. Reducing agents, such as

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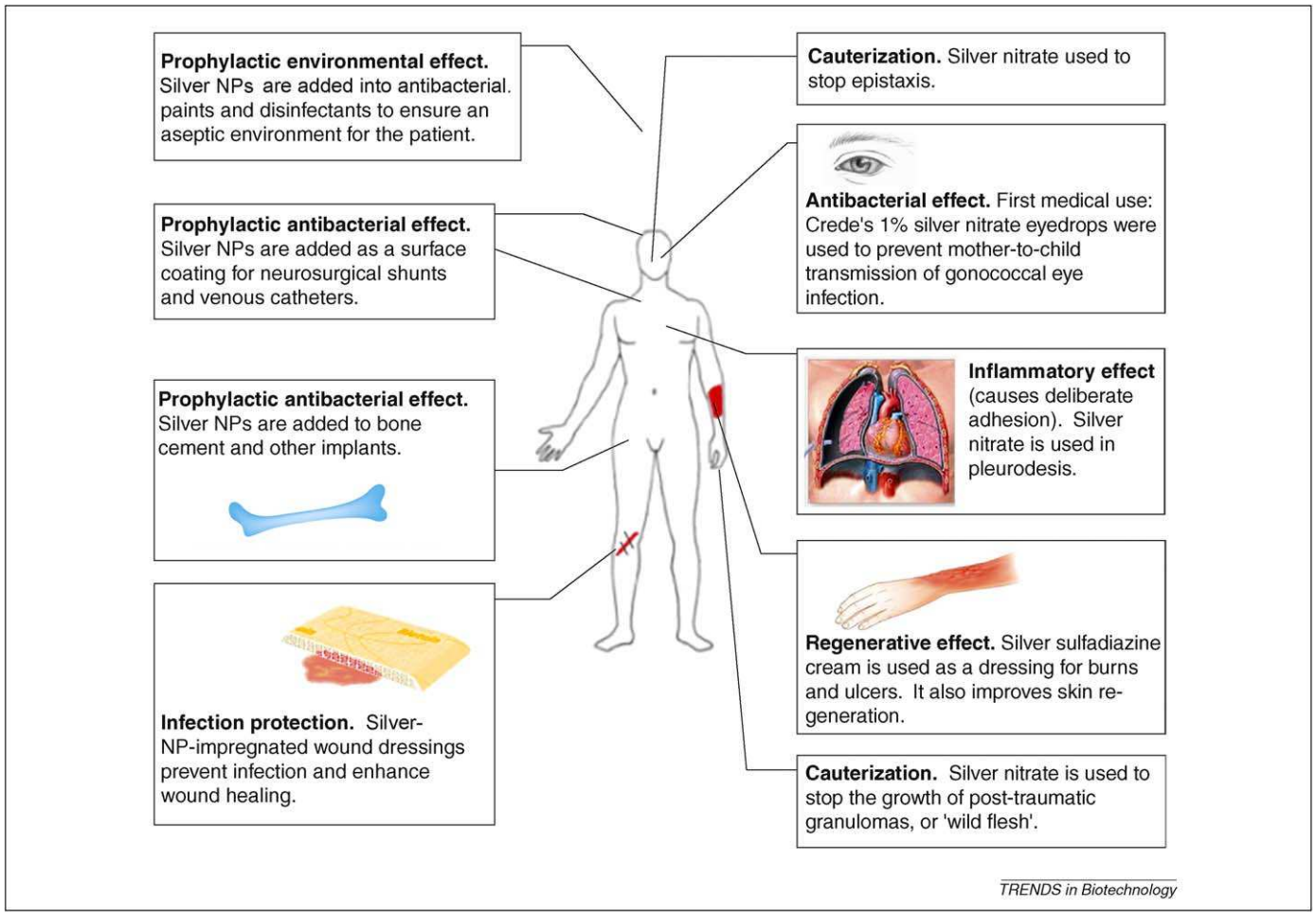

Figure 1. Uses of silver (right-hand side) and silver NPs (left-hand side) in medicine. Traditionally, silver nitrate is used in a number of clinical contexts, including stemming the flow of blood from nosebleeds, inducing pleurodesis when closing chest tube wounds and cauterization of granulomas. C.S.F. Crede's introduction of 1% silver nitrate eyedrops in 1881 prevented neonatal conjunctivitis and is still used clinically in developing countries. Silver sulfadiazine cream is used in the widespread treatment of burns [67], although argyria (discoloration of the skin) remains a prevalent side effect [8,49]. NS is emerging as a next-generation antibacterial agent [56], augmenting antibiotics and disinfectants for coating of medical devices. NS-based wound dressings are already commercially available (e.g. Acticoat^{TM}) and in current clinical use [53]. NS is used as an antibacterial additive or coating in a range of catheters [40–42] and in bone cement [46]. NS can also be used in hand gels [68] and paints [69] as a prolonged antibacterial disinfectant.

hydroquinones present in the microbial supernatant, reduce Ag^+ to NS under constant conditions (e.g. temperature). The obvious drawback of this method is the need to purify the sample and extract the NS because pathogenic bacteria might contaminate NS used in medical applications.

NS properties

NS comprises nano-sized structures formed from silver atoms that are metallically bonded together. At the nanoscale, particles exhibit different physical, optical and chemical properties owing to the dominance of quantum mechanics. These unique properties are exploited in biomedical imaging and sensing in applications such as surface-enhanced Raman scattering [10] and single-step immunoassays [20].

The antibacterial activity of silver is well known, as evidenced by its current clinical use in treating burns [9]. It is accepted that silver and NS in aqueous solution release silver ions, which are biologically active and actually mediate the bactericidal effect [21–25]. This mechanism has not been fully elucidated, but observations from recent studies shed light on the interactions involved [26–29]. It is believed that silver ions interact with three main components of the bacterial cell to produce the bactericidal effect: the peptidoglycan cell wall [30] and the plasma membrane [31]; bacterial (cytoplasmic) DNA [23,24]; and bacterial proteins [23,30], especially enzymes involved in vital cellular processes such as the electron transport chain (Figure 2).

A comparative study of NS, silver nitrate and silver chloride revealed that NPs have higher antibacterial potency than free silver ions [11]. This suggests that NS has intrinsic antibacterial properties that do not depend on the elution of Ag^+. NS extensively interacts with bacteria cell walls and it has been proposed that it causes lysis [22,30,32]. There is substantial evidence that NS produce reactive oxygen species (ROS) [26], which might underlie and explain both the antibacterial activity of NS and its potential toxicity to humans [27].

The antibacterial mechanism of NS has been probed by proteomics using 2D electrophoresis in conjunction with mass spectroscopy of protein samples from NS-treated *Escherichia coli* cells [33]. Stringent analysis revealed that outer-membrane protein precursors (OmpA, OmpC, OmpF) are all upregulated after NS treatment [33] and reflect a

Table 1. Comparison of methods used to synthesize NS

Synthesis method	Size (nm)	Advantages	Disadvantages	Comments	Refs.
Soluble starch used as both a reducing and a stabilizing agent during synthesis by reduction of $AgNO_3$ to NS.	10–34	'Green' method. Silver NPs stabilized with starch were stable for >90 days.	Lack of control over NP size distribution.	Very simple, reproducible method for production of stable NS. Suitable for medical applications because of starch biocompatibility.	[70]
Enterobacteria supernatant containing bacterial enzymes and compounds that reduce $AgNO_3$ to NPs.	28–122	Environmentally friendly, using naturally occurring bacteria instead of chemicals to reduce Ag^+.	Lack of control over NP size, resulting in wide size distribution.	Not viable for medical applications owing to potential contamination with pathogens.	[18]
$AgNO_3$ is added dropwise to chitosan dissolved in acetic acid; Ag^+ in solution is then reduced to NS by γ radiation and stabilized by chitosan.	4–5	Very small NS with narrow size distribution. Chitosan used as a capping and stabilizing agent to prevent NP agglomeration.	Chitosan might alter the NS properties.	γ radiation ensures sterile NS synthesis, which is useful for medical applications. Silver NPs may be more toxic than larger NP; further *in vitro* and *in vivo* studies required.	[71]
Photoreduction of aqueous $[Ag(NH_3)_2]^+$ using poly(*N*-vinyl-2-pyrrolidone) (PVP) in the presence of UV light. $[Ag(NH_3)_2]^+$ aqueous solution synthesized by reacting ammonia and silver oxide at a 4:1 mol ratio.	4–6	Controlled synthesis of very small, spherical NS with a narrow size distribution. NS was monodisperse in solution, with no agglomeration, owing to the stabilizing effect of PVP.	Difficult to increase size. PVP may affect the biological properties of NS.	Silver NPs that are 4–6 nm might be too small for medicinal uses because there is evidence that smaller NPs are more cytotoxic than larger (>55 nm) NPs [27].	[72]
Electrical current is briefly applied between two silver wires in deionized water, causing surface Ag atoms to evaporate and condense back into aqueous NS.	5–35	Very simple, no need for any chemicals. Narrow NS size distribution. Colloidal NS produced and Ag^+ liberated, which has been shown to kill *Staphylococcus aureus*.	Without stabilizing agents, NP agglomeration could be a problem; long-term stability has not been investigated.	Potentially the best method for NS synthesis for medical applications because no chemicals are used (no toxic residues or impurities).	[73]
Biosynthesis of silver NPs using *Penicillium brevicompactum* to reduce $AgNO_3$ to NPs.	23–105	Environmentally friendly by using biomass. Silver NPs do not agglomerate.	Lack of control over NP size distribution. Long process (>72 h) for complete Ag^+ reduction.	Novel but not viable for medical applications owing to potential contamination with pathogens.	[19]
UV photoreduction of aqueous $AgNO_3$ in the presence of sodium dodecyl sulfate (SDS) and ethanol.	23–67	Product exhibited superior antibacterial activity against *P. aeruginosa* compared to sodium borohydride-reduced SDS-capped silver NPs and citrate-capped silver NPs.	Superior antibacterial activity possibly a result of facile insertion into the lipid bilayer, facilitating NS entry into bacterial cells; if NS can easily enter all cells, mammalian cytotoxicity might be an issue.	Further evaluation of selectivity of bactericidal effect required if mammalian cytotoxicity is an issue. Antibacterial effect is promising for medical applications.	[74]
Peptide-coated silver NP synthesis: $AgNO_3$ stirred with peptide solution in buffer at different pH values for 12 h in the dark; sodium ascorbate added to reduce Ag^+ in NPs.	19	Use of peptides as effective capping agents could decrease NS toxicity.	Peptide-coated silver NPs aggregate in response to changes in pH, which could be potentially dangerous for medical applications (e.g. the acidic pH of hypoxic or inflamed tissues might cause NS agglomeration at sites of pathology and could occlude blood vessels).	Aggregation seriously limits use in medical applications. Peptide sequences could be recognized as antigenic and trigger an unwanted immune response.	[75]

compensatory mechanism to counter damage to the cell wall that both silver ions and NS can inflict [22,28]. Disruption of the bacterial cell wall and membrane causes collapse of the proton motive force and inhibits ATP synthesis [33].

NS exhibits antibacterial effects against a large number of bacterial species (Table 2). There is much conflict in the literature regarding which action of NS has the major antibacterial effect, but we believe it is a combined effect in which each mechanism (interaction with the cell wall or plasma membrane, bacterial DNA and bacterial proteins) contributes to provide broad spectrum antibacterial activity. Furthermore, bacterial resistance to elemental silver is extremely rare [29], emphasizing the presence of multiple bactericidal mechanisms acting in synergy.

Medical NS applications

Research into the medical applications of NS has been extremely active, with a variety of commercially available products being used clinically (Table 3). As knowledge of NS is disseminated throughout medicine, more innovative applications are being proposed and evaluated.

Implantable devices are a major risk factor in the contraction of hospital-acquired infections during a medical intervention. There are two types of implantable invasive devices: devices that are fully implanted inside the patient, and devices placed partially within the body that are exposed to the external environment. Fully implantable devices, such as heart valves, can be contaminated during implantation and require prophylactic antibiotic treatment for the first few days post-operation to prevent

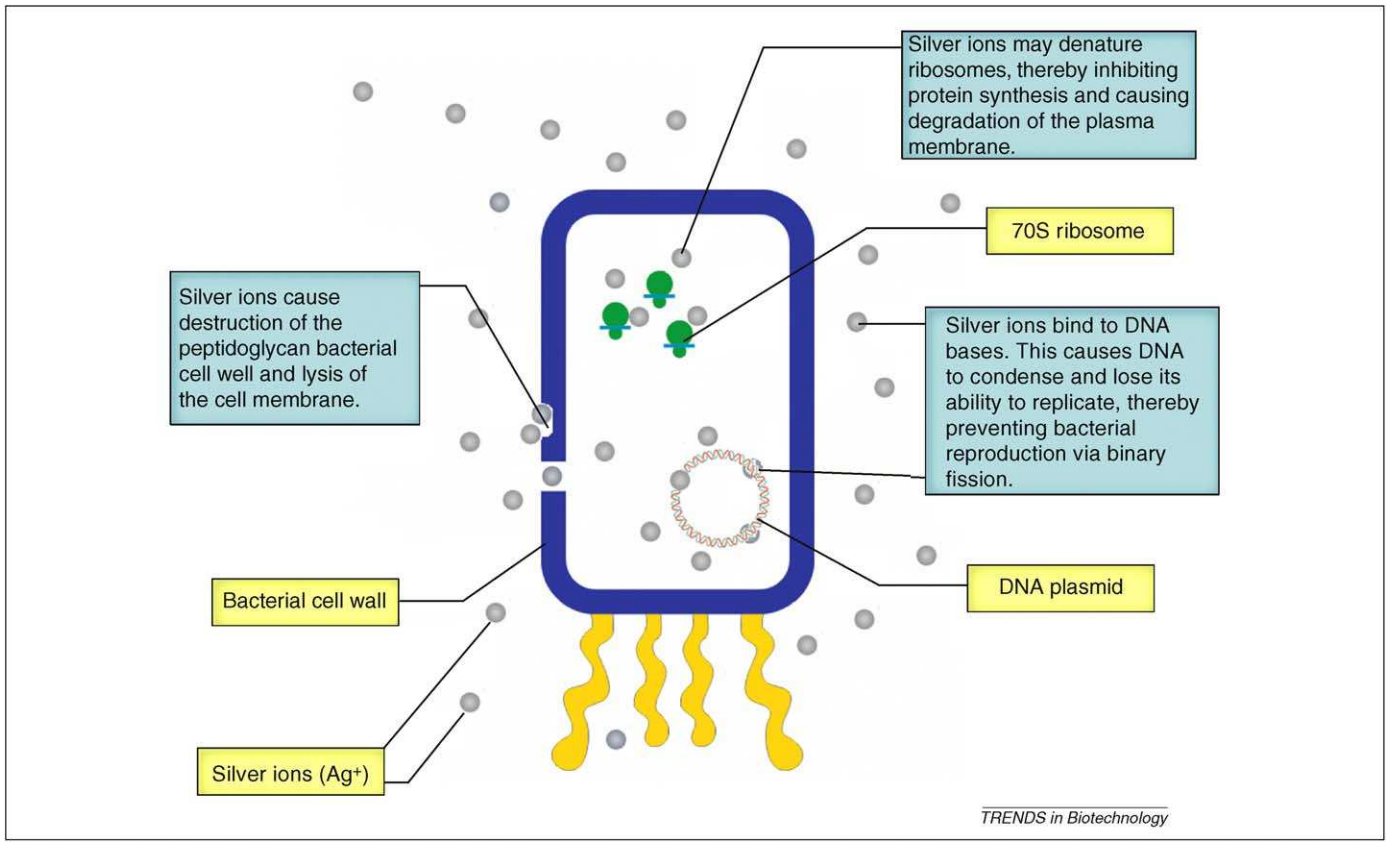

Figure 2. Mechanisms of the antibacterial activity of silver ions. It is widely accepted that the major antibacterial effect of NS is mediated by its partial oxidation and release of silver ions [22,25]. Silver ions interact with: the peptidoglycan cell wall [30] and the plasma membrane [31], causing cell lysis; bacterial (cytoplasmic) DNA [23,24], preventing DNA replication; and bacterial proteins [23,30], disrupting protein synthesis. Multifaceted antibacterial activity is the key to low bacterial resistance rates observed for silver and NS. NS also can directly damage and penetrate the cell wall and plasma membrane [22].

infection [34]. By contrast, devices such as urinary and venous catheters are prone to bacterial colonization owing to continuous exposure to the external environment. The increased risk of infection limits the use of such catheters in clinical practice.

The ideal properties of an antibacterial coating include prolonged activity, high levels of bactericidal and bacteriostatic activity, the ability to act against a wide spectrum of bacteria, biocompatibility, and low *in vivo* toxicity. In addition, the coating should be inexpensive, reproducible and disposable to minimize environmental damage. For use in cardiovascular applications (e.g. stents and venous catheters), an antibacterial coating must exhibit appropriate hemocompatibility to prevent thrombosis [35]. Here, we discuss various medical applications of NS, ranging from cardiovascular implants to wound dressings to catheters.

Cardiovascular implants

In 1998, spurred by promising *in vitro* and animal studies, a prosthetic silicone heart valve coated with elemental silver was designed (Silzone) to reduce the incidence of endocarditis following valve replacement and was subsequently used in a clinical trial (AVERT trial, n=4400) [36]. The rationale behind the use of silver was to prevent bacterial colonization on the silicone valve, thus reducing inflammation of the heart. Extensive toxicity testing of the silver heart valve showed promising biocompatibility. However, 4 years into the trial, Silzone heart valves were withdrawn because of elevated rates of paravalvular leakage in patients in the trial. Investigation revealed that inhibition of normal fibroblast function and hypersensitivity [37] led to failure of the trial. As a consequence, the use of silver in cardiovascular device coatings has been

Table 2. Compilation of recent studies on the antibacterial activity of NS

Microorganism tested	Findings	Refs.
E. coli, Vibrio cholera, P. aeruginosa, Salmonella typhi	Bactericidal effect of NS is size-dependent, with smaller NPs being more potent antibacterial agents. NP morphology was also important, with octahedral and decahedral particles having more highly reactive facets.	[22,76]
Enterococcus faecalis, S. aureus, E. coli, P. aeruginosa, S. epidermidis, Enterococcus faecium, Klebsiella pneumoniae	Different reducing saccharides were used to form NS of different sizes; smaller size NS exhibited more antibacterial activity against a range of different bacteria. Minimum inhibitory concentrations were 1.69–54.00 μg/ml.	[14]
E. coli, S. typhi, S. aureus	Antibacterial effect of NS was dose-dependent; NS more potent against Gram-negative (e.g. *E. coli* and *S. typhi*) than Gram-positive bacteria (e.g. *S. aureus*).	[23]
E. coli (4 strains), *Bacillus subtilis* (3 strains), *S. aureus* (3 strains)	Some strains of *E. coli* more resistant to bactericidal effects of NS than strains of *S. aureus*, contradicting earlier reports that Gram-negative are more sensitive to silver than Gram-positive bacteria; the authors attributed this to the fact that previous studies tested one strain of each bacterium rather than multiple strains.	[77]

Table 3. Commercially available medical products containing NS

Product	Company	Description	Clinical uses
Acticoat™	Smith & Nephew	Nanocrystalline silver wound dressing	Dressing for a range of wounds including burns and ulcers; prevents bacterial infection and improves wound healing.
Silverline®	Spiegelberg	Polyurethane ventricular catheter impregnated with NS	Neurosurgical drain of CSF for hydrocephalus. Also can be adapted for use as shunts. Antibacterial silver NP coating prevents catheter-associated infections.
SilvaSorb®	Medline Industries and AcryMed	Antibacterial products: hand gels, wound dressings, cavity filler	Wound dressings and cavity filler prevent bacterial infection. Hand gels used to disinfect skin in clinical and personal hygiene purposes.
ON-Q SilverSoaker™	I-Flow Corporation	Silver-NP-coated catheter for drug delivery	Delivery of medication (e.g. local anesthetics or analgesics) per-, peri- or post-operatively for pain management or for antibiotic treatment.

discontinued. At present, NS is being touted as a viable alternative in providing a safe, non-toxic antibacterial coating for medical devices.

A new nanocomposite material based on diamond-like carbon with 4-nm-NS embedded in the matrix has been synthesized and investigated for hemocompatibility properties when used as a surface coating for cardiovascular medical devices (e.g. heart valves and stents) [38]. Platelet adhesion studies have demonstrated decreased platelet attachment to the surface of the nanocomposite and those that did adhere were randomly distributed, indicating that the material has antithrombogenic properties. The authors posited that the nanocomposite material also possessed antibacterial properties, but no data were provided to corroborate this claim [38].

We have developed a polymer for cardiovascular applications that is composed of polyhedral oligomeric silsesquioxane (POSS) NPs and poly(carbonateurea)urethane (PCU) (POSS-PCU) [39]. Currently, antibacterial, mechanical and hemodynamic properties of NS-impregnated POSS-PCU are being investigated *in vitro*.

Central venous catheters

A number of different approaches have been used to investigate NS-impregnated polymers as antibacterial materials to retard biofilm growth on catheters [40]. Most commonly, polyurethanes, already established as plastic catheters, have been modified with NS. Plastic catheter tubes can be readily coated with a layer of NS to form effective antibacterial catheters [41]. *In vitro* testing revealed effective inhibition of biofilm growth and a prolonged effect for at least 72 h. A 10-day *in vivo* study in mice confirmed that the NS catheter was non-toxic [41].

Currently, a phase IV clinical trial is under way at Università Cattolica del Sacro Cuore in Italy (http://clinicaltrials.gov/ct2/show/NCT00337714) in which the efficacy of NS-impregnated catheters is being compared to control non-NS catheters in the prevention of infection at jugular and subclavian sites. It is hoped that the trial will bolster the reputation of NS-impregnated venous catheters for clinical use. The next step is then long-term clinical studies to assess the benefits and risks of *in vivo* human use.

Neurosurgical catheters

Catheters are used in neurosurgery to drain excess cerebrospinal fluid (CSF) [42], which can cause cerebral hypertension and brain damage. Neurosurgical catheters can be fully implanted and used as shunts to divert CSF permanently, or can be temporarily used as external drainage devices [42]. Both of these applications are prone to bacterial infection, which can rapidly spread to the brain and the surrounding meninges. Causative organisms include *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* [43].

NS is rapidly becoming established in everyday neurosurgical usage because the superior antibacterial properties and lack of observed toxicity can reduce the incidence of bacterial infection and complications during surgery. For example, NS-impregnated neurosurgical catheters have been fabricated to reduce catheter-associated infections. *In vitro* studies revealed prolonged silver ion release that lasted for at least 6 days, in addition to greatly decreased growth of *S. aureus* [44].

In a pilot clinical study of patients with acute occlusive hydrocephalus (in which blockage of the ventricular system causes ventricle dilation and increased intracranial pressure), NS-impregnated external ventricular drain catheters were used to determine whether NS is beneficial in preventing catheter-associated ventriculitis (CAV) [45]. CAV is inflammation of the ventricles of the brain, as confirmed by positive CSF cultures. Nineteen patients received the NS catheter and were assessed retrospectively against a control group that received normal catheters. Of the control group (n=20), five were positive for CAV. In the group with NS catheters, there were no cases of CAV and all CSF cultures were negative. The study showed that NS is potentially beneficial in the prevention of CAV and is promising for *in vivo* use in humans, with no reports of toxicity [45]. A large-scale randomized clinical trial is warranted to further validate this pilot study.

Bone cement

NS has been used as an antibacterial additive to poly(methyl methacrylate) (PMMA) bone cement [46]. Bone cement is used for the secure attachment of joint prostheses in, for example, hip and knee replacement surgery. Infection rates for total joint replacement are high at 1.0–4.0% [47]. The use of antibiotic-loaded bone cements has greatly reduced infection rates to between 0.4% and 1.8%, but reliance on antibiotics is undesirable because bacterial resistance can quickly develop [47]. NS-PMMA bone cement has been suggested as a means to decrease the incidence of resistance through its multifaceted mechanism of action, and has

further shown impressive *in vitro* antibacterial activity and low cytotoxicity [46]. Potent antibacterial action against methicillin-resistant *S. epidermidis* and *S. aureus* and retardation of biofilm growth have been observed [46]. NS bone cement has not demonstrated cytotoxicity in mouse fibroblasts or human osteoblasts, suggesting good biocompatibility [46].

Wound dressings

Nanocrystalline silver wound dressings have been commercially available for over a decade (e.g. Acticoat^{TM}) and are in current clinical use for the treatment of various wounds, including burns [8,48,49], toxic epidermal necrolysis [50], Steven–Johnson syndrome [51], chronic ulcers [5] and pemphigus [51]. Typical dressings consist of two layers of polyethylene mesh forming a sandwich around a layer of polyester gauze. Typical nanocrystalline coatings are 900 nm thick with a crystallite size of 10–15 nm [4], and are applied to the polyethylene layer.

Randomized clinical trials (RCTs) have evaluated the superior wound healing properties of nanocrystalline silver dressings over existing silver sulfadiazine and gauze dressing regimens in the treatment of burns. One RCT tested the efficacy of nanocrystalline silver versus a control group receiving conventional silver sulfadiazine on 166 different burn wounds in 98 patients [8]. Nanocrystalline silver dressings significantly decreased wound healing time by an average of 3.35 days and increased bacterial clearance from infected wounds. No adverse effects were observed for the dressing [8]. Another RCT examined NS dressings in treating second degree burns [48]; 191 patients were divided into groups treated with NS dressings, 1% silver sulfadiazine cream or plain Vaseline gauze. The results unequivocally showed the superiority of NS dressings in reducing the healing time for superficial burn wounds. However, there was no difference in healing of deep burn wounds when compared with 1% silver sulfadiazine [48], suggesting that NS accelerates re-epithelization, but not other phases of wound healing associated with new tissue formation, such as angiogenesis and proliferation.

As this field of research advances, new NS-containing dressings are being fabricated with the aim of further increasing the antibacterial efficacy and promotion of wound healing. A recent chitosan-nanocrystalline silver dressing showed superior healing rates (~89%) compared to silver sulfadiazine dressings (~68%) and chitosan film (~74%) [52]. In addition, the chitosan-nanocrystalline silver dressing deposited far less silver than conventional silver sulfadiazine [52], thus demonstrating that the use of NS may be safer in reducing the incidence of argyria (skin discoloration) and argyremia (elevated silver concentration in blood). As evidenced by RCTs and systematic reviews [53], the use of nanocrystalline NS is being embraced by medicine to provide effective treatment for a range of wounds [5] and for improved treatment of burns.

Anti-inflammatory NS properties

Basic scientific research has been carried out to elucidate the mechanism underpinning the anti-inflammatory activity of NS observed clinically. Anti-inflammatory properties of nanocrystalline silver were assessed by topically applying 1,2-dinitrochlorobenzene to induce contact dermatitis in swine [4]. Nanocrystalline silver outperformed saline- and silver nitrate-soaked wound dressings for treatment of the resulting contact dermatitis. Histopathology revealed near-normal pig epidermis at 72 h after treatment with nanocrystalline silver. Immunohistochemical staining revealed higher levels of the pro-inflammatory cytokines TGF-β and TNF-α in the saline and silver nitrate groups. The negative control and nanocrystalline silver groups showed similar levels of staining, leading to the conclusion that nanocrystalline silver has anti-inflammatory activity. Anti-inflammatory NS activity might be mediated by reducing cytokine release [54], decreasing lymphocyte and mast cell infiltration [55] and inducing apoptosis in inflammatory cells [4,7].

Matrix metalloproteinases (MMPs) contribute to tissue injury and inflammatory processes and their overexpression is associated with chronic ulcers rather than acute wounds, which suggests that MMPs might contribute to the non-healing nature of chronic ulcers. Nanocrystalline silver dressings significantly reduced MMP-9 levels in a porcine model and improved wound healing, although no mechanism was proposed [7]. In a human clinical study (n=15), nanocrystalline silver dressings promoted healing of stalled chronic leg ulcers [5]. This might have been achieved by reducing not only the number of bacteria in the wound, but also the inflammatory response, as evidenced by a decrease in neutrophil infiltration during biopsy.

NS toxicity: a need for concern?

The use of NS is increasingly widespread in medicine [56] and in daily life [57,58]. Toxicological NS issues are still unanswered. Assessment of NS toxicity is a two-fold problem. First, the inherent toxicity of NPs must be considered, which arises from having the same dimensions as biological molecules (e.g. DNA and proteins, ~2 nm) and thus may directly interact to damage DNA, denature proteins and enzymes and produce free radicals [59]. This is further compounded by the toxicity of elemental silver [60] and biologically active silver ions [12].

Studies have shown that NS is cytotoxic to several different cell lines, including NIH3T3 mouse fibroblasts [59], THP-1 monocytes [12], BRL 3A rat liver cells [61] and C18-4 male mouse germline cells [62]. Recent evidence strongly suggests that NS is cytotoxic through its interaction with mitochondria [27,59] and induction of the apoptosis pathway [59] via the production of ROS, which leads to cell death. One study identified a relationship between NP size and inhibitory effects on mitochondria, with smaller NPs of 15 nm significantly more toxic than larger NPs of 55 nm [27]. Studies on inhalation and oral toxicity of NS in Sprague-Dawley rats have shown that low concentrations induce toxicity after only 28 days of exposure [63]. Thus, long-term exposure to silver NPs in aerosols or food packaging might pose toxicity problems in humans.

The teratogenicity of NS in humans is unknown because no cases or studies have been reported in the literature. However, it has been shown that NS can affect the early

development of zebrafish embryos *in vivo* [64,65]. Furthermore, the level and type of embryonic abnormality depended on the NS dose [64]. Thus, NS assessment in humans for potential teratogenic effects is imperative.

In conclusion, *in vitro* and animal studies have demonstrated that NS can exhibit a significant level of toxicity. *In vivo* studies have demonstrated that long-term exposure is associated with increased argyremia. Limited studies have been performed in humans [8,53], but the safe and widespread use of NS wound dressings in the management of burns has not mirrored the concerns of *in vitro* [65] and animal studies [66]. However, longer-term studies and monitoring of humans exposed to NS are imperative to evaluate any potential toxicity. Care must also be taken in the use of NS in everyday applications (e.g. socks, cooking ware) so that the burden of NS exposure does not exceed sub-toxic levels [56]. Furthermore, the environmental impact of NS and silver ions, which leach into the water system, must be considered to prevent ecological disaster.

Future perspectives

The use of NS is already established for some medical applications, including wound dressings and treatment, with many new potential applications currently being investigated at the preclinical stage. NS exhibits remarkable biological properties, such as anti-inflammatory and anti-viral activities, in addition to more renowned antibacterial properties. Nanocrystalline silver dressings are now the new gold standard in the conservative treatment of wounds and burns. Implantable medical devices, such as neurosurgical and venous catheters, have greatly benefited from the broad antibacterial activity of NS by reducing both patient infection and dependence on antibiotic use and the associated costs.

Recent research has revealed exciting new biological properties of NS that could be translated into new therapeutic and pharmacological treatments (Box 1). Nanocrystalline silver powder dissolved in water has been investigated for treatment of interstitial cystitis (inflammation of the bladder) in a mouse model [55]. Aqueous silver nanocrystals had an anti-inflammatory effect, with fewer mast cells present in biopsy tissues and lower levels of the pro-inflammatory mediators TNF-α and histamine. This result is significant because it describes a therapeutic *in vivo* application of NS.

The full potential of this technology has yet to be discovered. The mechanisms underlying the impressive biological properties of NS are still not understood and this is a priority for future research *in vivo*. There is room for improvement in stabilizing and prolonging the antibacterial effects of NS coatings for medical applications to prevent infection and inflammation. Finally, with the widespread adoption of NS, several concerns about toxicity remain and need to be addressed.

Box 1. Emerging antiviral properties of NS

Novel antiviral properties of NS have been discovered *in vitro*, highlighting a possible therapeutic dimension to complement to its antibacterial activity. Early electron microscopy studies with NS and HIV-1 showed a size-dependent physical interaction, with NS of 1 nm in diameter able to directly bind to the virus [78]. Peculiarly, NS was bound in a regular punctate manner, suggesting it might bind to particular proteins on the viral envelope [78].

Further studies have attempted to elucidate the mechanism of antiviral activity of NS using *in vitro* assays [79]. A cell-based fusion assay using $CD4^+$ cells and cells expressing the HIV-1 envelope protein gp120 showed that NS disrupts the key interaction between gp120 and CD4, which facilitates viral entry. NS seemed to bind specifically to gp120, thereby preventing gp120 from binding to CD4. ELISA revealed that recombinant gp120–CD4 interactions were also prevented by NS, thus validating the cell fusion studies [79]. It will be interesting to determine whether these preliminary *in vitro* studies can be successfully extrapolated to *in vivo* models of HIV infection, which could potentially yield new HIV therapeutics.

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