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Engineering nanosilver as an antibacterial, biosensor and bioimaging material

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Abstract

The capacity of nanosilver (Ag nanoparticles) to destroy infectious micro-organisms makes it one of the most powerful antimicrobial agents, an attractive feature against “super-bugs” resistant to antibiotics. Furthermore, its plasmonic properties facilitate its employment as a biosensor or bioimaging agent. Here, the interaction of nanosilver with biological systems including bacteria and mammalian cells is reviewed. The toxicity of nanosilver is discussed focusing on Ag⁺ ion release in liquid solutions. Biomedical applications of nanosilver are also presented capitalizing on its antimicrobial and plasmonic properties and summarizing its advantages, limitations and challenges. Though a lot needs to be learned about the toxicity of nanosilver, enough is known to safely use it in a spectrum of applications with minimal impact to the environment and human health.

Introduction

Nanosilver is considered the most commonly used engineered nanomaterial [1] for antibacterial textiles [2], polymer films for food packaging [3], paints and pigments [4], filters for water [5] or air [6] treatment etc. It has attractive biomedical applications taking advantage of its a) antimicrobial properties to prevent infections, and b) plasmonic and metallic properties as a diagnostic (e.g. biosensors, in vivo biomarkers) and therapeutic tool (e.g. photothermal tumor treatment).

Nanosilver can be made by wet- and, in particular, gas-phase routes that are readily scalable even at academic laboratories [7]. Different nanosilver morphologies can be obtained also, including pure, surface-modified, supported on ceramics, coated (SiO₂, polymers) and heterodimers. Nanosilver of uniform size is made by the reduction of silver nitrate in the presence of citrate [8] (Figure 1a). Immobilized nanosilver on nanostructured silica is made by flame aerosol technology [9] (Figure 1b) or by wet-chemistry [10] (Figure 1c). Nanosilver can be hermetically coated by 2-3 nm thin SiO₂ film and form heterodimer or Janus-like particles with Fe₂O₃ (Figure 1d) that can be magnetically manipulated [11]. Depending on preparation route, different structures can be created onto nanosilver affecting its interaction with a host solution. For example, wet-made silica-coated nanosilver tends to have a porous silica coating [12] while when made in the gas-phase, hermetic or CVD-like coatings are achieved [11].

The broad use of nanosilver, however, raises concerns regarding its fate and potential adverse effects on environment and human health. Actually, nanosilver is the first nanomaterial to attract attention by the U.S. Environmental Protection Agency as, not long ago, petitions had been filed to be treated as pesticide [13]. Such concerns were created

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when it was shown that some nanosilver may “escape” to waste water treatment plants after washing products containing it [14]*. This leaching of silver into aquatic environments may be toxic for environment-friendly micro-organisms igniting intensive research.

One of the main ways that silver can be released into the aquatic environments is in the form of ions [15]**. Even though silver metal is not soluble in water, when in the nanometer size range, Ag^+ ions are released (leached) from its surface [15] that might be oxidized and readily dissolving in water [16]*. Nanosilver particle formation from such released Ag^+ ions can occur under certain conditions as with soil sediments [17] transforming to less toxic silver sulfide nanoparticles in sewage sludge [18]. The presence of silver sulfide, however, can influence the silver uptake and bioaccumulation into food chains [19]. By systematically varying the nanosilver size, it has been shown that Ag^+ ions dominate the toxicity of nanosilver less than about 10 nm in diameter [9]** (Figure 2).

The oxidation state of nanosilver strongly influences its ion leaching and therefore, its toxicity, since oxidized nanosilver exhibited much stronger antibacterial activity against *E. coli* than metallic nanosilver [16]*. Furthermore, the Ag^+ ion release of about 5 and 60 nm silver particles, as well as of a macro-sized silver foil scales with the exposed Ag surface area [20] (Figures 3a,b). So the antibacterial activity of nanosilver against *E. coli* is better expressed by surface area, rather than mass or number concentrations [21] (Figures 3c,d). Other factors can influence also Ag^+ ion leaching: pH, temperature, organic matter and surface coatings [22,23].

Here, the interaction of nanosilver with bacteria and mammalian cells is discussed focusing on Ag^+ ion release. The toxicity of nanosilver of different sizes and surface morphologies is compared, pointing out the need for a unified perspective when examining such studies. Finally, biomedical applications of nanosilver as antimicrobial agent, biosensor and bioimaging are discussed highlighting current opportunities, limitations and challenges.

Interactions of nanosilver with biological systems

Antimicrobial activity

This activity of nanosilver has been examined against prokaryotic organisms including bacteria, fungi or viruses [24,25]. *Escherichia coli* (*E. coli*) is the most commonly used culture with various nanosilver concentrations, morphologies and sizes as well as different *E. coli* concentrations and even life stages. This makes difficult to directly compare such studies, even for the same bacteria.

Nanosilver particles and released Ag^+ ions from their surface destroy sulfur and phosphorus containing compounds such as DNA and proteins [26]*. This harms the cell membrane as well as protein functions leading to cell death. For instance, higher nanosilver concentrations are required to inhibit the growth of Gram-positive bacteria than Gram-negative ones [27], as the former have thicker cellular wall (membrane) than the latter [28] that may inhibit Ag nanoparticle or ion transport through it. Coating nanosilver with increasing amounts of silica (up to 10 wt%) reduced the Ag^+ ion leaching and practically blocked the nanosilver toxicity to *E. coli* [29]. Bacteria are relatively easy to culture and perhaps could be considered a screening tool for the toxicity of nanosilver before other more complex biological systems are examined.

Cytotoxicity against mammalian cells

Nanosilver exposure to humans may occur either accidentally by the use of its products or deliberately when its theranostic (therapy and diagnostics) properties are sought [30]. There are various indications of the health state of a cell. The oxidative stress that is correlated

with the reactive oxygen species (ROS) is perhaps the most well-known [31]. Such ROS can cause toxic effects through the production of free radicals that influence the redox potential of the cell, thus damaging proteins, lipids and DNA. Therefore, monitoring ROS and oxidative stress can determine proinflammatory responses and cytotoxicity [31]. Other ways to measure cell viability or cytotoxicity exploit specific functions within the cell. These functions occur only when the cell is healthy and, therefore, any absence of them can be considered an indication of toxicity. One example is mitochondrial enzymes that normally change a dye color, indicating cell viability [32]. Another is to monitor the induced DNA damage, the so-called genotoxicity. Then, the DNA is stained with a dye and its structure is monitored since damaged DNA has a more relaxed structure than undamaged DNA [33].

As with antibacterial activity, a variety of nanosilver morphologies and sizes has been employed. Typical cell lines include macrophages, liver and lung cells. Macrophages are the first cells that nanosilver will encounter upon its entrance in a body, as they will try to neutralize Ag by phagocytosis [34]. Lung cells are of interest, as inhalation is one of major pathways that nanosilver and other engineered nanomaterials may enter the human body [35]. Finally, liver cells are examined because nanosilver will be cleared from a human body through the liver [36]. For example, nanosilver was more cytotoxic to rat liver cells when compared to other nanomaterials (including iron oxide, titania, aluminum, molybdenum oxide) by generation of ROS and oxidative stress [36].

When nanosilver 15-20 nm in diameter is coated with a biocompatible layer of glycolipids, it still exhibits higher cytotoxicity than similarly sized and coated gold nanoparticles, as measured by the mitochondrial function and DNA damage [30]. Furthermore, nanosilver particles of about 15 nm in diameter coated with a 2 nm thick hydrocarbon layer induced larger oxidative stress than those of 30 and 55 nm in diameter [37]. Caution, however, is required when evaluating such results since coatings such as hydrocarbons may interfere with the toxicity of nanosilver inducing toxicity and oxidative stress [38]. As a matter of fact, hydrocarbon-coated nanosilver was more toxic than similarly sized polysaccharide-coated nanosilver [39]. Different surface coatings can also influence the Ag⁺ ion release kinetics and the final degree of dissolution. The Ag⁺ ion release of citrate-coated nanosilver of about 50 nm was lower than that of PVP-coated [40]. Furthermore, nanosilver thinly (2 nm) coated with silica has exhibited no toxicity on HeLa cells for up to 24 hours incubation at 37 °C [11].

Biomedical applications of nanosilver

Antimicrobial material

In several health treatments such as in intravenous catheters, endotracheal tubes, wound dressings, bone cements, oral cavity fillings or implant surgery, the spread of an infection may result in poor living quality or even the life of a patient. Therefore, it is common to treat patients with antibiotics to minimize this risk. Several bacteria, however, start exhibiting resistance to antibiotics [41] so materials for passive protection are sought that exhibit bactericidal properties such as nanosilver and/or photoactive titania.

Nanosilver (5-50 nm) embedded in PMMA bone cement exhibited high antibacterial activity when tested against antibiotic-resistant bacteria, with no cytotoxic signs against human cells for identical nanosilver concentrations [42]. Similarly, plastic catheters impregnated with nanosilver particles of about 10 nm in diameter exhibited strong antibacterial activity [43]. Nanosilver in dental adhesives was quite effective against streptococci [44] without affecting the adhesive mechanical properties, enabling its use in orthodontic treatments.

Biosensors

The plasmonic properties of nanosilver strongly depend on its size, shape and dielectric medium that surrounds it [45]. In fact, the latter dependency can be exploited in biosensing. Most biomolecules have a higher refractive index than buffer solutions [46]** so when attached on nanosilver, the local refractive index increases shifting the Ag extinction (absorption and scattering) spectrum. Figure 4 shows the corresponding schematic and discusses the procedure. Biosensors utilizing plasmonic nanostructures (local surface plasmon resonance-LSPR) are advantageous over commercial thin, plasmonic, continuous films (surface plasmon resonance-SPR) [47]. The LSPR biosensors exhibit less interference from the refractive index of the buffer solution and possess greater spatial resolution than the SPR ones [48].

Triangular nanosilver particles made by lithography were deposited on substrates to monitor interactions between biomolecules, such as biotin-streptavidin [46] and to monitor two biomolecules related to Alzheimer's disease [49]*. Nanosilver cubes [50] or rhombi [51] can also be employed in biosensing of protein interactions. Lately, nanosilver plasmonic biosensors are promising for cancer detection [52].

Even though silver is more efficient than gold in terms of plasmonic performance, gold is typically used for biosensing because silver oxidizes and easily forms plasmonically unattractive compounds like halides in biological solutions [53]. Furthermore, surface sulfidation may deteriorate the plasmonic performance of nanosilver even in ambient conditions [54]. This is why a protective coating is applied often on nanosilver facilitating also its dispersion in solutions [53]. Recently, the employment of silica-coated nanosilver as biosensors for the detection of bovine serum albumin (BSA) was demonstrated with excellent solution dispersion and limited antibacterial activity [29].

Bioimaging

Plasmonic particles, such as nanosilver, can be detected by numerous optical microscopy techniques and are advantageous over commonly used fluorescent organic dyes that decompose during imaging (photobleaching). In contrast, nanosilver is photostable allowing thus its use as biological probe to monitor continuously dynamic events for an extended period of time [55].

The plasmonic properties of such small metallic nanoparticles enable them also to be employed also as an in vivo therapeutic tool. Plasmonic particles are conjugated to biological targets [56]** such as cancer cells or tissues and are used to absorb light and convert it into thermal energy. This destroys the targets by thermal ablation, enabling such particles to be used in a non-invasive cancer treatment. Typically, gold nanoparticles are used as they are relatively less toxic than nanosilver. In fact, this potential toxicity of plasmonically superior nanosilver is its main limitation, since it may destroy the cell [39] motivating the development of hermetically-coated nanosilver [11].

There are two ways to employ nanosilver for bioimaging: either incubated with cells and monitor their physical interactions and uptake, or to functionalize the nanosilver surface with a biomolecule that binds specifically to sites on the cell membrane. The former is easier as the latter needs a specific biofunctionalization molecule. Nanosilver particles incubated with neuroblastoma cells were detected nicely under dark-field illumination but exhibited toxicity [39]. Nanosilver attached on iron oxide nanoparticles were incubated with macrophages for their easy detection by two-photon imaging after their cell uptake [39].

The attachment of nanosilver to specific receptors on the membrane of fibroblast cells [57], or to intracellular proteins has been achieved for monitoring their internalization through the

cell membrane of neuroblastoma cells [58]. The selective binding on HeLa and Raji cells of silica-coated, Janus-like silver-iron oxide nanoparticles (Figure 1d) that could be magnetically manipulated was achieved by hDC-SIGN antibody [11]. The presence of a thin (about 2 nm) silica coating blocked quite effectively the toxicity of nanosilver.

Concluding remarks

Nanosilver is one of the leading nanotechnology materials and products for its unique antibacterial and plasmonic properties. The advantages of nanosilver, however, have to be balanced with any potential adverse effects that may induce to the environment and human health. Today there is a reasonable good understanding of the nanosilver toxicity. Very small nanosilver particles ($d_p < 10$ nm) readily release ions from their oxidized surface that dominate their antibacterial performance. Larger particles release much less ions so their bactericidal activity is manifested upon contact of bacteria or cells with their surface that is quite likely oxidized over time. This understanding facilitates the development of biomedical materials with bactericidal properties to fight micro-organisms that exhibit resistance to antibiotics.

Plasmonic and thermal properties of nanosilver can be readily exploited in biosystems provided that hermetic and long-lasting coatings are applied on its surface that can facilitate also its liquid suspension. That way powerful biosensors and bioimaging agents can be developed to monitor biomolecules related with chronic and life-threatening illnesses.

Given that there is a good understanding of nanosilver synthesis with closely controlled size by scalable technologies there is a strong likelihood to see further examples of its use and markets in bioapplications. This understanding may help to design and make safer nanosilver products that possess the desired properties for target applications and at the same time overcome any adverse toxic effects. This would result in sustainable engineered nanomaterials in a rather “eco-friendly” way that offers their sought-out performance and minimize risks.

Acknowledgments

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Figure 1.

Electron microscopy images of different nanosilver morphologies: (a) pure (Reprinted with permission from [8] copyright 1999 American Chemical Society), (b,c) supported on SiO_2 (Reprinted with permission from [9] copyright 2010 American Chemical Society, and [10] copyright 2001 American Chemical Society), (d) SiO_2 -coated and heterodimer or “Janus-like” (Reprinted with permission from [11] copyright 2011 American Chemical Society).

Figure 2.

The toxicity of nanosilver as a function of particle size. For small (< 10 nm) nanosilver, a large fraction of Ag⁺ ions is released from their oxidized and highly convex surface (Kelvin effect) dominating the Ag toxicity. Silver oxide is readily dissolved in liquids in contrast to metallic Ag. For larger (> 10 nm) particles, a small fraction of Ag⁺ ions is released so the Ag toxicity is affected by both ions and direct contact with the nanosilver particle surface [9].

Figure 3.

(a) The Ag^+ ion release (Ag^{dis}) over the total Ag amount kinetics for different nanosilver sizes and a macro-sized silver foil. (b) The same data renormalized on the basis of nanosilver surface area (Reprinted with permission from [20] copyright 2010 American Chemical Society). (c,d) The extent of *E. coli* growth of all data at 210, 270 and 330 minutes as a function of the Ag (c) mass concentration C in suspension and (d) surface area concentration $C \cdot \text{AgSSA}$. The vast variability of *E. coli* growth at identical Ag mass (or molar) concentration in contrast to Ag surface area concentration points out the limitation of using Ag mass concentration in assessing the antibacterial activity of nanosilver in liquid suspensions (Reprinted with permission from [21] copyright 2011 Elsevier).

Figure 4.

Schematic of plasmonic biosensors. In step 1, nanosilver with its characteristic plasmon extinction spectrum (black line) is deposited on a glass substrate. In step 2, the surface of nanosilver is biofunctionalized with a biomolecule (Y). The higher refractive index of the biomolecule than the surrounding buffer solution forces a red-shift in the Ag spectrum (orange line). In step 3, a ligand analyte (●) is selectively bound on the functionalized nanosilver surface, further red-shifting the spectrum to higher wavelengths (blue line). This shift, $\Delta\lambda$, can be considered the biosensor response.