

Antimicrobial surface functionalization of plastic catheters by silver nanoparticles

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Objectives: To test the antimicrobial activity and evaluate the risk of systemic toxicity of novel catheters coated with silver nanoparticles.

Methods: Catheters were coated with silver using AgNO₃, a surfactant and *N,N,N',N'*-tetramethylethylenediamine as a reducing agent. Particle size was determined by electron microscopy. Silver release from the catheters was determined *in vitro* and *in vivo* using radioactive silver (^{110m}Ag⁺). Activity on microbial growth and biofilm formation was evaluated against pathogens most commonly involved in catheter-related infections, and the risk for systemic toxicity was estimated by measuring silver biodistribution in mice implanted subcutaneously with ^{110m}Ag⁺-coated catheters.

Results: The coating method yielded a thin (~100 nm) layer of nanoparticles of silver on the surface of the catheters. Variations in AgNO₃ concentration translated into proportional changes in silver coating (from 0.1 to 30 µg/cm²). Sustained release of silver was demonstrated over a period of 10 days. Coated catheters showed significant *in vitro* antimicrobial activity and prevented biofilm formation using *Escherichia coli*, *Enterococcus*, *Staphylococcus aureus*, coagulase-negative staphylococci, *Pseudomonas aeruginosa* and *Candida albicans*. Approximately 15% of the coated silver eluted from the catheters in 10 days *in vivo*, with predominant excretion in faeces (8%), accumulation at the implantation site (3%) and no organ accumulation (≤ 0.1%).

Conclusions: A method to coat plastic catheters with bioactive silver nanoparticles was developed. These catheters are non-toxic and are capable of targeted and sustained release of silver at the implantation site. Because of their demonstrated antimicrobial properties, they may be useful in reducing the risk of infectious complications in patients with indwelling catheters.

Keywords: nanotechnology, nosocomial infections, biofilms, biodistribution, mice

Introduction

More than 200 000 nosocomial bloodstream infections occur each year in the USA and most of them are related to the use of intravascular devices.^{1,2} Central venous catheters are a particularly high risk category of devices.^{3,4} According to recent estimates, the use of 1 in 20 of the 7 million central venous catheters inserted annually is associated with catheter-related bloodstream infection.^{2,5} Chronic indwelling urinary catheters

also increase the risk of infection, accounting for ~80% of all nosocomial urinary tract infections.⁶

The insertion of catheters under sterile conditions is the most effective measure to prevent catheter-associated infective complications.⁷ Despite concerns that they would lead to complacency regarding septic techniques, catheters with antimicrobial properties have nonetheless been proposed as a means to provide additional protection and further reduce the risk of infection.^{8,9} A number of such devices have been developed^{10–}

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¹² and tested with variable success in clinical studies.^{4,6} However, the ideal catheter, a catheter that combines low-cost coating technology, wide-spectrum and long-lasting antimicrobial properties, and safe utilization, has yet to be developed.

The objective of the studies presented in this report was to develop a new method to deposit silver nanoparticles at low temperature on plastic catheters, determine the antimicrobial activity of these catheters *in vitro* and examine their silver-releasing properties both *in vitro* and *in vivo*. The data suggest that plastic catheters coated with silver nanoparticles may be effective in reducing the infectious risk associated with chronic catheterization in humans.

Materials and methods

Materials and reagents

All chemicals were purchased from Sigma (St Louis, MO, USA). Standard PE-BAX polyamide 20 gauge catheters were used (0.53 mm internal diameter, 0.94 mm outer diameter; surface = 0.463 cm²/cm) for all experiments. Radioactive silver nitrate (^{110m}AgNO₃, spec. act. 22.2 Bq/μg) was obtained from the Oregon State University Radiation Center (Corvallis, OR, USA).

Silver coating of the catheters

Catheters were coated on both sides with silver using AcryMed SilvaGard technology (published US patent application 2007/0003603). In brief, an aqueous solution containing 5.6 g/L Tween 20, 4 mM sodium saccharine and 1–5 mM silver nitrate was first prepared and stirred for 10 min before the addition of *N,N,N',N'*-tetramethylethylenediamine (0.33%, v/v). The solution was then placed in a microwave oven, heated to 56°C and then poured over pre-cut, 2 cm long catheter segments placed in screw-cap tubes. The tubes were centrifuged to remove the air trapped inside the catheters and quickly placed in a thermostated oven set at 53°C. After 16 h, the coating solution was removed and the catheters were rinsed once with each of the following: aqueous solution of Tween 20 (1.25 mM), 2% HNO₃, H₂O and isopropanol. After the isopropanol wash, the catheters were placed on bench-top paper, dried and sterilized (autoclave at 121°C for 15 min). The same method was used to prepare catheters coated with radioactive silver, simply replacing AgNO₃ with ^{110m}AgNO₃. Radioactive catheters were not sterilized.

Silver concentration was determined in randomly selected catheters by absorption spectrophotometry ('cold' silver coating) or by gamma counting ('hot' silver coating). Radioactivity was measured using a Beckman 5500 gamma counter. All counts were corrected for background radioactivity, counting efficiency and decay using a half-life of 256 days.

Silver nanoparticle analysis

Silver coating was examined by electron microscopy. For particle size analysis, a drop of silver nanoparticles prepared as described above was deposited at the surface of a carbon-coated copper grid and analysed using a Zeiss DSM-960 equipped with a Link Analytical multi-window, X-ray detector with a no-window capability. For coating thickness analysis, thin sections of silver-coated polycarbonate matrix were prepared and viewed by non-disruptive electron transmission microscopy using a dual beam STRATA 400S electron microscope (FEI Company, Hillsboro, OR, USA).

Biofilm formation inhibition assay and bactericidal activity measurements

Silver-coated catheters (650 μg silver/g) were placed in tubes (8 × 1 cm strips/tube) containing growth medium (1 mL/tube; 1% bovine serum albumin, 0.1% neopeptone, 0.25% glucose) and 10³ test microorganisms (test tubes). Six different microorganisms (clinical strains) were tested: (i) *Escherichia coli*; (ii) *Enterococcus*; (iii) *Staphylococcus aureus*; (iv) *Pseudomonas aeruginosa*; (v) coagulase-negative staphylococci; and (vi) *Candida albicans*. Tubes containing non-coated catheters and tubes containing growth medium but no catheter and no microorganism (controls) were prepared in parallel. The tubes were then incubated for either 24, 48 or 72 h at 35°C. A count of free floating viable bacteria and the determination of viable sessile bacteria by means of a biofilm formation assay were performed at each time point. Biofilm formation was estimated using the tetrazolium salt XTT assay as previously described.^{13,14} Results were expressed in per cent inhibition of growth of free floating organisms or biofilm formation in each test tube relative to the average growth or biofilm formation in control tubes (tubes with non-treated catheters).

In vitro silver release procedure

Catheters were coated with radioactive silver and placed in 5 mL plastic tubes (33 segments of ~2 cm/tube; *n* = 5 tubes for each type of catheters). Each tube was filled with 2 mL of saline (enough to completely immerse the catheters), capped, assayed for radioactivity and placed in an oven set at 37°C (day 0). Every day for 10 days, the saline solution was collected, assayed for radioactivity and replaced with fresh solution after brief washing of the catheters. Silver release was expressed in μg/day.

In vivo silver release and biodistribution

In vivo studies were performed with C57Bl/6J male mice. On the day of surgery, the animals were weighed and anaesthetized with an isoflurane–oxygen mixture (4% induction, 2% maintenance). A subcutaneous pocket (2 cm × 1 cm) was created on the dorsum of each animal, ~1–2 cm from the nape of the neck. Catheters (14 segments, 2 cm in length each; total catheter length = 28 cm) coated with ^{110m}Ag were placed in the pocket. The pocket was sealed with one suture and a drop of tissue glue (3M Vetbond™). The animals were allowed to recover from anaesthesia and then placed into individual metabolic cages. They had free access to food and water and were housed with a 12 h dark–light cycle at 20°C. Urine and faeces were collected every day in the morning, between 8:00 and 9:00 am. Urine and faeces samples were transferred in counting tubes and assayed for radioactivity on the same day. On day 10, the animals were weighed, anaesthetized and sacrificed by cervical dislocation. Organ and tissue collection was then performed followed by radioactivity measurement of the collected samples. All procedures were approved by the institutional (OHSU) Ethics Committee.

Results

Silver coating of the catheters

The coating method produced catheters coated with a reproducible and predictable amount of silver. In these experiments, batches of 35 catheter segments (each ~2 cm in length) were

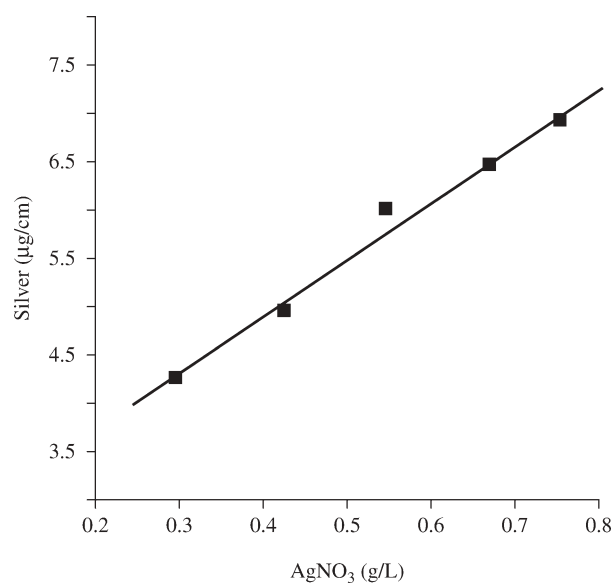


Figure 1. Silver coating of nylon catheters with silver nitrate. Coating was performed with varying concentrations of $^{110m}\text{AgNO}_3$ as described in the Materials and methods section. The y -axis represents silver deposited per centimetre length of catheters and the x -axis represents the concentration of silver nitrate in the coating solution. Each data point represents the mean (\pm SEM) of six independent measurements. The line represents the best (linear) fit of the data points. Error bars are smaller than the size of the points.

coated using initial $^{110m}\text{AgNO}_3$ concentrations ranging from 1.0 to 5.0 mM ($n = 6$ batches/given AgNO_3 concentration; five concentrations tested). As shown in Figure 1, the coating was proportional to the initial concentration of AgNO_3 and was highly reproducible from batch to batch (coefficient of variation was $0.7 \pm 0.1\%$, mean \pm SEM, $n = 5$). The relationship between temperature and coating was not fully characterized, but preliminary experiments suggest a quadratic behaviour of the relationship between 40 and 70°C , with higher temperatures resulting in higher silver concentrations deposited at the surface of the catheters (data not shown).

Silver deposition at the surface of the catheters was analysed by electron microscopy in parallel experiments performed with non-radioactive silver. In one experiment, a drop of a solution of silver nanoparticles prepared as described in the Materials and methods section was deposited at the surface of a carbon-coated copper grid and analysed by X-ray electron microscopy. Elemental composition of treated catheters showed peaks due to Ag in addition to peaks due to C, N and O, the elements making up the base polymer grid. As illustrated in Figure 2, distinct and round particles of silver were observed, with diameters ranging from ~ 3 to 18 nm (median = 10.7 nm; $n = 100$) (Figure 2a and b). Particle density was estimated at $10^4/\mu\text{m}^2$ on average. Particles showed typical polyhedral structures indicating twin boundaries^{15,16} (Figure 2c). Analysis of silver coating could not be obtained using silver-coated catheter sections as the base polymer softened during exposure to the electron beam. The thickness of the coating was thus examined using non-disruptive electron microscopy and thin (100–150 nm) sections of a polycarbonate matrix coated using conditions similar to those described for catheters. As illustrated in Figure 2(d), the coating

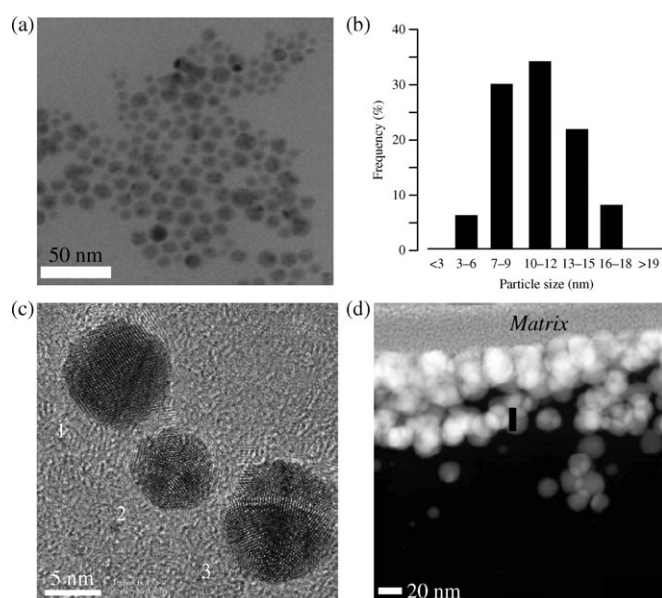


Figure 2. Silver nanoparticle analysis by X-ray electron microscopy. (a) A drop of silver nanoparticle was deposited on a carbon grid to obtain the image (scale bar = 50 nm). (b) The image was magnified and particle size distribution was calculated using the scale bar (50 nm) from 100 measurements. (c) Higher magnification reveals the characteristic polyhedral structure, most visible in particles 2 and 3 (scale bar = 5 nm). (d) Silver nanoparticle coating was further examined by non-disruptive electron transmission microscopy using a thin (100–150 nm) section of a polycarbonate support (*matrix*) coated under conditions similar to those described in the Materials and methods section (white horizontal scale bar = 20 nm; black vertical scale bar = 20 nm). The silver coating is 80–120 nm thick corresponding to ~ 4 –6 layers of silver nanoparticles.

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Bactericidal activity and biofilm formation

These experiments were performed using catheters coated with 600 μg of non-radioactive silver. The catheters demonstrated significant antimicrobial activity against all tested microorganisms. They inhibited both cell growth and biofilm formation for at least 72 h (Table 1). The effect on growth was complete growth inhibition of all microorganisms but *P. aeruginosa* (67% growth inhibition at $t = 72$ h). The inhibition of biofilm formation was almost complete for *E. coli*, *S. aureus* and *C. albicans*, and reached more than 50% for *Enterococcus*, coagulase-negative staphylococci and *P. aeruginosa* after 72 h.

Silver release from coated catheters (in vitro studies)

These experiments were performed with catheters coated with two concentrations of silver: 600 $\mu\text{g/g}$ of catheter, a concentration similar to that used in the antimicrobial activity experiments, and 1000 $\mu\text{g/g}$. Actual silver concentrations were 593 ± 2 and 1019 ± 3.7 $\mu\text{g/g}$. Five strands (cut in ~ 2 cm long segments) of each type of catheter were studied in this experiment. The average length and weight of these strands were 71.7 ± 0.7 cm (348 ± 4 mg) for the catheters coated with 1000 $\mu\text{g/g}$ silver, and 69.2 ± 1.1 cm (336 ± 5 mg) for those

Table 1. Silver-coated catheter antimicrobial activity

Pathogen	24 h	48 h	72 h
<i>E. coli</i>			
biofilm	88 ± 3.3	95 ± 1	83 ± 3
growth	100	100	100
<i>Enterococcus</i>			
biofilm	16 ± 7	32 ± 5	66 ± 4
growth	100	100	100
<i>S. aureus</i>			
biofilm	93 ± 2	95 ± 1	78 ± 4
growth	100	100	100
Coagulase-negative staphylococci			
biofilm	39 ± 17	86 ± 11	50 ± 16
growth	100	100	100
<i>P. aeruginosa</i>			
biofilm	59 ± 5	91 ± 1	66 ± 9
growth	100	100	67 ± 19
<i>C. albicans</i>			
biofilm	84 ± 2	98 ± 0	87 ± 7
growth	100	100	100

A count of free floating viable bacteria and the determination of viable sessile bacteria by means of a biofilm formation assay were performed at each time point. The results are expressed as percentage inhibition of growth or biofilm formation versus untreated catheters. Data are presented as mean per cent inhibition ± SEM with $n = 12$ for biofilm data and $n = 6$ for growth data. SEM are not shown when all data points show >99% inhibition.

coated with 600 µg/g (mean ± SEM; $n = 5$; $P =$ not significant). On average (10 day average), the amount of silver released daily from catheters was 45.1 ± 1.1 ng/cm (catheters coated with 1000 µg/g silver) and 24.1 ± 2.4 ng/cm (catheters coated with 600 µg/g silver).

Daily release rates were relatively constant in both groups although they were higher on the first days than on the last days (data not shown). To better characterize silver release kinetics, cumulative release data were examined (Figure 3). In 10 days, the catheters coated with the low concentration of silver released 0.38 ± 0.03 µg/cm of silver (14% of coated silver) and the catheters coated with the higher concentration released 0.45 ± 1.1 µg/cm (9% of coated silver). The data show a biphasic silver release over time, with a downward inflexion of release rates on day 4, less marked for the catheters coated with the highest concentration of silver. The total amount of silver released from these catheters in 10 days was ~20% higher than the amount of silver released from the catheters coated with 600 µg/g silver.

Biodistribution studies

On average, each animal was implanted with the equivalent of 28 cm of ^{110m}Ag -coated catheters, representing 221.7 ± 0.8 µg of silver and 6515 ± 24 Bq (mean ± SEM; $n = 7$). The mice showed no sign of toxicity and looked healthy during the 10 days of the experiment. In particular, there was no sign of inflammation or infection at the site of catheter implantation. Further, the weight of each organ collected (heart, brain, liver, lungs, spleen and kidneys) was similar to organ weight

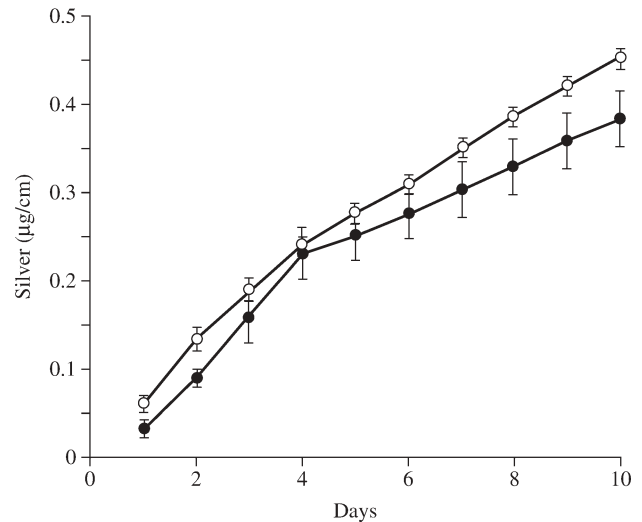


Figure 3. *In vitro* silver release kinetics from ^{110m}Ag -coated catheters. Two groups of catheters were studied: one coated with 593 ± 2 µg of silver/g of catheter (filled symbols) and another with 1019 ± 3.7 µg of silver/g of catheter (open symbols). Each data point represents the cumulative daily average (mean ± SEM; $n = 5$) of silver released on the day indicated in µg/cm of catheter length.

measurements performed in healthy adult animals of the same strain and sex (data not shown). On average, however, the body weight of the animals decreased by ~8% from 36.9 ± 2.2 g before surgery to 34.0 ± 0.7 g on day 10.

Urine and faeces excretion. On average, silver urine excretion was very low (0.02 µg/day, i.e. <0.01% of the silver present on the catheters on day 0; Figure 4a). The total excretion of silver in urine over the 10 day experimental period was 0.22 ± 0.04 µg, equivalent to 0.1% of the silver implanted at day 0. In contrast, silver excretion in faeces was significant and varied with time (Figure 4a). It started relatively high at 3.36 ± 0.44 µg on day 1 (~1.5% of implanted silver), peaked on day 2 (4.50 ± 0.40 µg; ~2.1% of implanted silver) and then declined in the following 8 days reaching a plateau at ~0.6–1.0 µg/day (~0.4%) on day 6. The cumulative excretion of silver in faeces in 10 days was 18.33 ± 0.99 µg, equivalent to $8.3 \pm 0.4\%$ of the initial silver load (Figure 4b).

Organ and tissue accumulation. Silver radioactivity was determined at day 10 in several organs (heart, brain, lungs, liver, kidney and spleen), other tissue samples (skin at the implantation site, underlying muscle with ribs, control skin area, duodenum, caecum, femur, thigh and blood) and in the catheters. The data are summarized in Table 2. Most of the silver remained associated with the catheters (~84% of implanted silver). A significant but small amount of silver was detected at the implantation site (skin with panniculus carnosus and scar tissue; ~3%) and in the muscle + rib cage sample underlying the implantation site (0.2%). All other organs or tissue samples including the lungs, which under normal breathing condition, are in contact of the rib cage had <0.1% of the implanted silver (caecum, liver, lungs, blood and heart) or no detectable levels of silver (control skin sample, duodenum, spleen, kidneys, brain, femur and thigh; data not shown). Silver recovery was high (~96% on average).

Silver nanotechnology and antimicrobial catheters

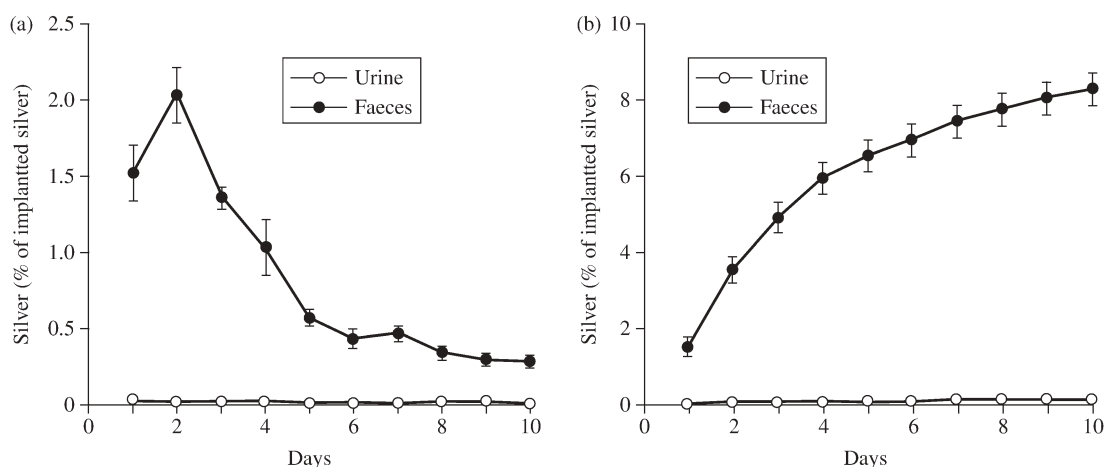


Figure 4. Silver urine (open circles) and faeces (filled circles) excretion in mice implanted with ^{110m}Ag -coated catheters. (a) Daily excretion. (b) Cumulative daily excretion. Data are expressed as percentages of implanted silver (mean \pm SEM, $n = 7$).

Table 2. Biodistribution of silver (μg per sample and % of implanted silver) on day 10 (mean \pm SEM; $n = 7$)

	Silver (μg)	Percentage of implanted silver
Catheters (initial)	221.7 ± 0.8	—
Catheters on day 10	186.2 ± 2.6	83.9
Implantation site (skin, panniculus carnosus, scar tissue)	6.8 ± 0.4	3.1
Rib cage + muscle underlying the implantation site	0.4 ± 0.1	0.2
All other tissues and organs: $\leq 0.1\%$ of implanted silver or no detectable silver		
Urine (cumulative 10 days)	0.22 ± 0.04	0.1
Faeces (cumulative 10 days)	18.33 ± 0.99	8.3
Recovery		95.6

Urine and faeces data are from Figure 4.

The 4% unaccounted for are likely to be found at the implantation site on the borders of the insertion pocket associated with either serous liquid or scar tissue.

Discussion

This report describes a method to prepare catheters coated with silver nanoparticles and presents evidence for the catheters' antimicrobial properties and safety of use in animals. The method uses silver nitrate and a mix of low-toxicity coating inducers. The coating process is slowly reversible, yielding sustained release of silver for at least 10 days. The released silver is active against microorganisms most commonly found responsible for nosocomial infections and predominantly accumulates at the site of insertion, thus suggesting that catheters coated with this method could provide enhanced local protection against infections with no risk of systemic toxicity.

Silver has long been known for its broad antimicrobial properties. These properties are believed to result from the disruption of the energy metabolism and electrolyte transport systems, which occurs when silver ions bind to bacterial sulphhydryl- or histidyl-containing proteins.¹⁷ The use of silver to reduce the risk of catheter-related infection was proposed 20 years ago by Maki *et al.*,¹⁸ who tested the efficacy of a biodegradable collagen matrix impregnated with bactericidal silver. Since then, a number of other methods have been developed to create catheters capable of delivering silver.¹⁹ These methods include electron beam assisted deposition,^{20,21} distribution of submicron particles of metallic silver in the polyurethane matrix of the catheter,¹⁷ placement of silver wires at the cutaneous extremity of the catheters and release of silver by iontophoresis,^{22,23} and catheter impregnation with silver nanoparticles using supercritical carbon dioxide.²⁴ These methods are all relatively complex and expensive.

To come up with a simpler and cheaper coating method, silver nanoparticle production technologies were reviewed. None of them seemed to provide a rate of nanoparticle formation and deposition that was fast enough to be compatible with the logistic of efficient industrial production. Further, many of them utilize either starting silver salt concentrations too low (10 mM or less) to yield potentially antimicrobial concentrations at the surface of the catheters or agents that are toxic or at least not biocompatible, thus precluding the use of silver nanoparticles made by these methods because of toxicity concerns.^{25,26} The method described in this report is simple, uses non-toxic chemicals and yields reproducible coating of plastic catheters with typical nanoparticles of silver as confirmed by electron microscopy. The amount of silver deposited is proportional to the concentration of silver nitrate used in the coating solution (Figure 1), at least within the range tested and thus can be reasonably predicted. Importantly, coating takes place on both the luminal and the external surface of the catheters, thus providing a double protection against microorganism penetration at the implantation site. Further, the coating method does not affect the size or the diameter of the catheters (Figure 2d) and is resistant to handling. Thus, this method preserves the original qualities of manufactured catheters and will likely not affect their handling during clinical use.

The size distribution of the nanoparticles is relatively broad (3–18 nm) but actually quite tight considering the simplicity of the production process. Whether or not the distribution impacts the microbicidal activity of the catheters is not known at this point. One could speculate that for a given amount of coated silver, smaller particles will yield a greater contact surface with the liquid environment and will be more active than larger particles. Alternatively, larger particles may provide a slower but more prolonged release of silver, a benefit for catheters destined for longer-term implantation. The issue of size–activity relationship is thus important to consider and will be the focus of future studies.

The overwhelming majority of nosocomial infections associated with catheter use are caused by coagulase-negative staphylococci such as *Staphylococcus epidermidis*.^{1,4,7} Infections caused by *S. aureus*, *Enterococcus* and *E. coli* are also frequent and are often associated with increased resistance to antibiotics. A smaller percentage of catheter-related infections are caused by other bacteria such as *P. aeruginosa* and *Klebsiella pneumoniae*, and by yeast, mainly *Candida* spp. Our data show that catheters coated with silver nanoparticles are very effective in preventing the growth of and biofilm formation by most of these pathogens *in vitro*. Whether they will be equally effective *in vivo* can only be speculated at this time and will require further testing both in the animal model and with controlled randomized clinical trials.

The data also show that not all microorganisms tested are fully sensitive to silver. One could thus question the potential advantage of catheters coated with silver nanoparticles since as long as viable organisms remain, they will regrow as soon as they are exposed to nutrient-containing infusion. It must be pointed out that the silver nanoparticle-coated catheters described in this report are not meant to treat infections, but rather to limit microorganism implantation, to slow their subsequent growth and to retard biofilm formation. The catheters were tested against an initial high bacterial load (1000 microorganisms/mL). According to Mermel *et al.*,¹ the presence of 100 cfu/mL in blood taken from central venous catheters (500–1000 cfu/mL in blood from peripheral intravenous catheters) is considered positive for infection and would prompt antibiotic treatment. In this context, silver-coated catheters should not be considered as antimicrobial devices but as one more weapon in the fight against infection.

The molecular mechanism underlying the antimicrobial activity of our silver-coated catheters was not explored. However, it is likely that growth inhibition was caused by the silver ions (Ag^+) released from the matrix of the catheter. Our *in vitro* experiments indeed indicate that these catheters release ‘soluble’ silver when placed in contact with physiological saline solution ($\sim 3 \mu\text{g/day}$ over 10 days; Figure 3). This soluble silver may in turn have inhibited the pathogens’ respiratory enzymes and electron transport components as proposed by others.^{17,27} Interestingly, the release is not constant over time. It proceeded in two phases: a relatively fast phase during the first 4 or 5 days and a slower phase during the next 5 days. The two-phase release may be explained by the presence of two different pools or layers of silver on the catheters, each having different solubility or surface density properties. Alternatively, the biphasic release could reflect silver accessibility, the silver present on the outer surface of the catheter being more accessible to exchange with the solution and thus releasing faster than the silver coating

the lumen of the catheters. More extensive microscopic analysis of the catheters after coating and at different times during elution may be useful in the future to test these hypotheses.

Bacterial resistance to silver has been a concern with the significant increase in the usage of silver-containing products to manage infections.^{28,29} Several studies suggest that it is secondary to a plasmid-related increase in silver binding to periplasmic membrane proteins and in silver efflux through two plasmid-encoded pumps.^{27,30} The potential benefits of widespread usage of silver-nanoparticle coated catheters may thus be in part offset by the emergence of new, silver-resistant bacterial strains. Such possibility exists with any new antimicrobial agent but is perhaps less to fear with silver than with other agents. As pointed out by Chopra²⁹, target-based mutation to silver resistance is unlikely because of the multiplicity of intracellular targets of silver ions and may explain in part the limited number of reports of silver resistance in bacteria published so far.

Catheters coated with silver nanoparticles could be used to perfuse various types of solutions. Some of these solutions may be poorly compatible with the silver coating and either damage it or reduce its antimicrobial activity. Laboratory experiments have shown that the coating is stable under pH conditions ranging from 2 to 12, hence the use of 2% HNO_3 as a rinsing agent in the final steps of the coating process. Thus, the pH of commonly used injectable solutions is unlikely to damage the coating of the catheters. Cysteine-, SH- or sulphide-containing compounds as may be found in albumin or amino acid solutions may bind silver ions and enhance their elution from the coating of the catheters. Chloride ions and phosphate ions, as found in many injectable solutions may also react with silver ions but decrease their release from the coating by forming an insoluble layer of AgCl or Ag_3PO_4 at the surface of the nanoparticles. Compatibility issues cannot be fully predicted at this time and will need to be examined on a case by case basis. It must be pointed out though that if the dissociation constants of AgCl and Ag_3PO_4 are low (1.6×10^{-10} and 1.6×10^{-5} M at 25°C , respectively), they are nonetheless high enough to allow some of the silver to be released as Ag^+ . Further, as shown by the data presented in Table 1, significant antimicrobial activity of the silver-coated catheters was observed in a medium containing both a protein digest (peptone) and sodium chloride. Thus, chemical interactions between silver-coated catheters and perfusion solutions may occur and change the kinetics of the release of silver ions from the catheters but are unlikely to significantly reduce overall Ag^+ availability for bactericidal activity.

The compatibility between injectable solutions and the silver-coated catheters may not be so much of a concern. Parenteral solutions are sterile and catheter-related infections are primarily caused by microorganisms invading the site of insertion of the catheter and the tissues in contact with the outside of the catheter. Of potentially greater concern is the possibility of systemic toxicity from the silver eluted from the catheter. Although silver is a low toxicity metal,^{31–33} this issue was addressed by evaluating the biodistribution of silver released from silver-coated catheters in the mouse.

The animals lost on average 8% of their body weight throughout the 10 day experimental period. Such a decrease is likely secondary to the change in housing conditions (from shoe box before surgery to individual metabolic cage after surgery) but could be attributable to silver toxicity. The data however

show that in 10 days the catheters released ~16% of their coating. The majority of the released silver is excreted in the faeces (~50%) and a significant amount remains associated with the tissues in direct contact with the catheters (~20%). No significant accumulation of silver was observed in the major organs examined. These data are in keeping with previous reports in rodents and other species.^{34,35} The amount of silver released from a 28 cm long catheter strand over the 10 day period is equal to ~35 µg. If all this silver accumulated in tissues, it would amount to a dose of ~1 mg/kg mouse body weight and 0.5 µg/kg human body weight (for an adult of 70 kg). With a LD₅₀ in the g/kg body weight range (in the mouse), such exposure to silver rules out the possibility that the animal weight loss was caused by the silver released by the catheters and suggests that the catheters coated with the silver nanoparticle technology will not cause any systemic toxicity in humans. The biodistribution data further indicate that there is a 4000-fold silver gradient over a distance of ~3–10 mm (from the catheter to the lungs and heart). This suggests a very limited diffusion of released silver through tissues and a very limited risk for subcutaneous catheters coated with silver to release toxic concentrations of silver to underlying tissues and organs in patients.

The excretion of silver in faeces, the major route of silver elimination *in vivo*, follows a biphasic pattern, with a rapid excretion rate for the first 3–4 days followed by a slower excretion rate from day 4 to day 10 (Figure 4). This mirrors the silver elution patterns observed *in vitro*, suggesting that silver release from implanted catheters follows the same kinetics *in vivo* and *in vitro*. Thus, the *in vitro* elution test might be an efficient and economic alternative to animal testing when it comes to predict the biological properties of new catheter formulations or other medical devices treated with our method.

In conclusion, a method to coat plastic catheters with bioactive silver nanoparticles was developed. These catheters are non-toxic devices capable of targeted and sustained release of bactericidal silver at the implantation site and may prove useful in preventing infectious complications in patients with indwelling catheters.

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Transparency declarations

Dr N. B.-S.: none to declare. Dr J.-B. R. was the recipient of a NIH subcontract from AcryMed, Inc. (AcryMed, Inc./Dr B. G. primary recipient). Drs B. G., B. K and D. R. own stocks or shares in a company that could be financially affected by the conclusions of this article. Dr B. G. is Chief Technical Officer and Chairman of AcryMed, Inc.

References

1. Mermel LA, Farr BM, Sheretz RJ *et al.* Guidelines for the management of intravascular catheter-related infections. *Clin Infect Dis* 2001; **32**: 1249–72.
2. Rosenthal K. Guarding against vascular site infection: arm yourself with the latest knowledge on equipment and technique to protect patients from catheter-related bloodstream infections. *Nurs Manage* 2006; **37**: 54–66.
3. Randolph AG, Brun-Buisson C, Goldmann D. Identification of central venous catheter-related infections in infants and children. *Pediatr Crit Care Med* 2005; **6** Suppl 3: 19–24.
4. Kline A. Pediatric catheter-related bloodstream infections. Latest strategies to decrease risk. *AACN Clin Issues* 2005; **16**: 185–98.
5. CDC NNIS System. National nosocomial infections surveillance (NNIS) system report, data summary from January 1992 through June 2004, issued October 2004. *Am J Infect Control* 2004; **32**: 470–85.
6. Johnson JR, Kuskowski MA, Wilt TJ. Systematic review: antimicrobial urinary catheters to prevent catheter-associated urinary tract infection in hospitalized patients. *Ann Intern Med* 2006; **144**: 116–26.
7. O'Grady NP, Alexander M, Dellinger EP *et al.* Guidelines for the prevention of intravascular catheter-related infections. *Pediatrics* 2002; **110**: 1–24.
8. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science* 1999; **284**: 1318–22.
9. Hanna H, Raad I. New approaches for prevention of intravascular catheter-related infections. *Infect Med* 2001; **18**: 38–48.
10. Walder B, Pittet D, Tramer MR. Prevention of bloodstream infections with central venous catheters treated with anti-infective agents depends on catheter type and insertion time: evidence from a meta-analysis. *Infect Control Hosp Epidemiol* 2002; **23**: 748–56.
11. Hanna HA, Raad II, Hackett B *et al.* Antibiotic-impregnated catheters associated with significant decrease in nosocomial and multidrug-resistant bacteremias in critically ill patients. *Chest* 2003; **124**: 1030–8.
12. Chaiban G, Hanna H, Dvorak T *et al.* A rapid method of impregnating endotracheal tubes and urinary catheters with gendine: a novel antiseptic agent. *J Antimicrob Chemother* 2005; **55**: 51–6.
13. Hawser SP, Norris H, Jessup CJ *et al.* Comparison of a 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2-H-tetrazolium hydroxide (XTT) colorimetric method with the standardized National Committee for Clinical Laboratory Standards method of testing clinical yeast isolates for susceptibility to antifungal agents. *J Clin Microbiol* 1998; **36**: 1450–2.
14. Tunney MM, Ramage G, Field TR *et al.* Rapid colorimetric assay for antimicrobial susceptibility testing of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2004; **48**: 1879–81.
15. Zheng J-G. Silver nanoprisms and nanotetrahedra investigated by transmission electron microscopy. *Microsc Microanal* 2003; **9**: 392–3.
16. Yang Z, Chang H-T. Anisotropic syntheses of boat-shaped core-shell Au-Ag nanocrystals and nanowires. *Nanotechnology* 2006; **17**: 2304–10.
17. Guggenbichler JP, Boswald M, Lugauer S *et al.* A new technology of microdispersed silver in polyurethane induces antimicrobial activity in central venous catheters. *Infection* 1999; **22**: S16–23.
18. Maki DG, Cobb L, Garman JK *et al.* An attachable silver impregnated cuff for prevention of infection with central venous catheters: a prospective, randomized multicenter trial. *Am J Med* 1988; **85**: 307–14.
19. Sticler DJ. Biomaterials to prevent nosocomial infections: is silver the gold standard? *Curr Opin Infect Dis* 2000; **13**: 389–93.
20. Sioshansi P. New processes for surface treatment of catheters. *Artif Organs* 1994; **18**: 266–71.

21. Trerotola SO, Johnson MS, Shah H *et al.* Tunneled hemodialysis catheters: use of a catheter for prevention of infection—a randomized study. *Radiology* 1998; **207**: 491–6.
22. Raad I, Hachem R, Zermano A *et al.* *In vitro* antimicrobial efficacy of silver iontophoretic catheter. *Biomaterials* 1996; **17**: 1055–9.
23. Raad I, Hachem R, Zermano A *et al.* Silver iontophoretic catheter: a prototype of a long-term antiinfective vascular access device. *J Infect Dis* 1996; **173**: 495–8.
24. Furno F, Morley KS, Wong B *et al.* Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection? *J Antimicrob Chemother* 2004; **54**: 1019–24.
25. Nickel U, Castell AZ, Poppl K *et al.* A silver colloid produced by reduction with hydrazine as support for highly sensitive surface-enhanced Raman spectroscopy. *Langmuir* 2000; **16**: 9087–91.
26. Green M, Allsop N, Wakefield G *et al.* Trialkylphosphine oxide/amine stabilized silver nanocrystals—the importance of steric factors and Lewis basicity in capping agents. *J Mater Chem* 2002; **12**: 2671–4.
27. Li X-Z, Nikaido H, Williams K. Silver-resistant mutants of *Escherichia coli* display active efflux of Ag and are deficient in porins. *J Bacteriol* 1997; **179**: 6127–32.
28. Percival SL, Bowler PG, Russell D. Bacterial resistance to silver in wound care. *J Hosp Infect* 2005; **60**: 1–7.
29. Chopra I. The increasing use of silver-based products as antimicrobial agents: a useful development or a cause of concern? *J Antimicrob Chemother* 2007; **59**: 587–90.
30. Silver S. Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. *FEMS Microbiol Rev* 2003; **27**: 341–53.
31. Armitage SA, White MA, Wilson HK. The determination of silver in whole blood and its application to biological monitoring of occupationally exposed groups. *Am Occup Hyg* 1996; **40**: 331–8.
32. Saber H, Anner RM, Anner BM. Cysteine protects Na⁺/K⁺-ATPase and isolated human lymphocytes from silver toxicity. *Biochem Biophys Res Commun* 1992; **189**: 1444–9.
33. Wright JB, Lam K, Buret AG *et al.* Early healing events in a porcine model of contaminated wounds: effects of nanocrystalline silver on matrix metalloproteinases, cell apoptosis, and healing. *Wound Rep Reg* 2002; **10**: 141–51.
34. Furchner JE, Richmond CR, Drake GA. Retention of silver-110m in the mouse, rat, monkey and dog. *Health Phys* 1987; **15**: 505–14.
35. Gammill JC, Wheeler B, Carothers EL *et al.* Distribution of radioactive silver colloids in tissues of rodents following injection by various routes. *Proc Soc Exp Bio Med* 1950; **74**: 691–5.