In vitro antimicrobial activity of silver-processed catheters for neurosurgery

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Objectives: To investigate the in vitro antibacterial activity of silver-processed catheters for use in neurosurgery using clinically predictive tests.

Methods: The antimicrobial activity of a commercially available silver-processed external ventricular drain catheter was evaluated against Staphylococcus epidermidis, methicillin-resistant Staphylococcus aureus (MRSA), Escherichia coli and Propionibacterium acnes. Non-impregnated catheters were used as controls. Two assays were performed: (i) testing the ability of the catheter to kill 100% of the attached bacteria (tK100); and (ii) in vitro challenge to determine the ability to prevent colonization under flow conditions. High and low inocula (10⁴ and 10⁷ cfu/mL) were used. Silver-processed and control catheters were examined by scanning electron microscopy and focused ion beam scanning electron microscopy; electron back-scatter and energy-dispersive X-ray analyses were used to investigate the distribution of silver within the processed catheter.

Results: The silver-processed catheters were not able to kill any of the bacteria tested in the tK100 assay at high inoculum. At low inoculum S. epidermidis was eradicated and some activity was seen against E. coli but without complete eradication. MRSA was also not eradicated even at low inoculum. The in vitro challenge test showed no prevention of colonization for any of the strains. Silver particles were shown to be >500 nm in size.

Conclusions: The commercial silver-impregnated catheter was not able to eradicate MRSA or E. coli and while it showed activity against S. epidermidis in one assay it was unable to prevent colonization in vitro under in-flow conditions. This is consistent with clinical studies on silver-processed catheters.

Keywords: silver impregnation, antimicrobial catheter, external ventricular drainage, hydrocephalus shunt

Introduction

Raised intracranial pressure due to head trauma, tumour, haemorrhage, meningitis or congenital or idiopathic obstruction of the CSF pathways can be relieved by placement of a temporary catheter in the cerebral ventricles, which drains excess CSF into a collecting system (external ventricular drainage (EVD)). In cases of hydrocephalus, where accumulation of CSF results in progressive CNS pathology, a permanent indwelling shunt is used. This consists of a catheter placed in the cerebral ventricle, draining CSF via a valve to the peritoneal cavity where it is reabsorbed. In shunts and EVD, infection is a serious complication. In shunts, the period of risk is virtually confined to the operation to insert the device or to revise it later, while in EVD the period of risk extends until the device is removed. Another difference between the two procedures is the infecting bacteria. In both cases staphylococci, usually coagulase-negative (mainly Staphylococcus epidermidis), predominate but in EVD the proportion of Staphylococcus aureus, including methicillin-resistant S. aureus (MRSA), and Gram-negative bacteria such as Escherichia coli is greater.1–4 Propionibacterium acnes is not usually found in EVD but is a problem in shunts.5,6 Many patients with EVD are cared for on a neurocritical care ward, and here the multiresistant Gram-negative bacterium Acinetobacter baumannii is a particular problem.7 All of these are capable of attaching to the catheters and developing biofilms, then going on to cause infection of the CNS. Prophylactic antibiotics are of no benefit in EVD and have been reported to increase the risk of infection with Gram-negative bacteria.8,9 This has led to development of antimicrobial catheters for both shunts and EVD.10–12

In view of its broad antimicrobial spectrum, silver has been an attractive proposition for EVD, and more recently for shunts.13 The use of silver in some form to confer antimicrobial activity on biomaterials, textiles or environmental materials is now...
widespread. The inclusion of silver in catheters for urinary tract, central venous access and neurosurgical use is increasing but the clinical results are inconsistent and often unconvincing. A meta-analysis has shown that silver alloy-coated catheters are superior to silver oxide-coated ones for short-term use. However, here again, results are inconsistent, and a trial of short-term catheter use in an intensive care setting using alloy urinary catheters showed no significant benefit. Another study reported a significant fall in infection when silver alloy urinary catheters were used, but historical controls were used. Silver can be applied to the catheter surface either as silver compounds or by ion beam deposition. Again, studies on ion beam-coated silver catheters for dialysis have shown no benefit. These processes coat only the outer surface, and as with other coatings give activity of only short duration. In order to overcome these difficulties, methods of impregnation of catheter material with silver nanoparticles have been developed. Materials containing nanoparticulate silver promise to give greater ion availability and therefore greater antimicrobial activity than coatings of silver salts or metallic silver.

This has led to commercialization of one nanosilver catheter for use in EVD (Silverline®; Spiegelberg GmbH & Co, Hamburg, Germany). Other antimicrobial catheters for EVD use have spectra of activity that are limited to Gram-positive bacteria, whereas silver, if effective, would be expected to have activity against all Gram-positive and Gram-negative bacteria and Candida spp. Silverline catheters have been used clinically in small numbers of patients with retrospective controls and more recently in seven patients with hydrocephalus shunt infections, though the case definitions and detailed results were not given. Pre-clinical evaluation of antimicrobial catheters for efficacy is often unsatisfactory; Silverline catheters have been tested by immersion in a broth culture and sampling by a roll plate method. We have previously described rigorous pre-clinical evaluation tests and have therefore used these to determine the antimicrobial activity of the commercial silver catheters. Both silicone and polyurethane silver-processed catheters are available, but only the silicone version was used here, as we have found no difference in their performance (data not shown).

**Methods**

**Test catheters**

Silverline silicone catheters were purchased from Forth Medical Ltd.

**Test bacteria**

Clinical isolates of *S. epidermidis* (F1228), MRSA (F1853), *E. coli* (F2365) and *P. acnes* (F1726) were resuscitated from frozen and maintained on sheep blood agar (Oxoid, Basingstoke, UK). *P. acnes* was manipulated, maintained and tested in anaerobic conditions.

**Time to kill attached bacteria (tk100)**

This assay determines the time taken to kill (notionally) 100% of bacteria attached to the silver-processed catheter. The limits of sensitivity of detection of viable bacteria by this method, as found by experiment, were: MRSA F1853, 3 × 10^3 cfu/ml; *S. epidermidis* F1228, 3 × 10^3 cfu/ml; *E. coli* F2365, 5 × 10^3 cfu/ml; and *P. acnes* 3 × 10^3 cfu/ml. It was carried out with and without a protein conditioning film and with and without prior soaking of the catheters. Catheter segments 1 cm in length were cut aseptically with a sterile scalpel, and then bisected longitudinally. The protein conditioning film was applied by immersing segments in 1:300 human plasma (National Blood Authority, Sheffield, UK) in PBS for 1 h, then rinsing in sterile PBS. For bacterial attachment, segments were immersed in a suspension of early log phase bacteria. MRSA and *E. coli* were used at a concentration of 1 × 10^7 cfu/ml for 1 h in one experiment. All subsequent tk100 assays were carried out at a concentration of 1 × 10^6 cfu/ml (except *P. acnes* where the catheter segments were immersed anaerobically in a suspension of 1 × 10^5 cfu/ml), then rinsed in sterile PBS. A separate set of experiments was carried out as above with the low inoculum but the segments were soaked in PBS for 5 days at 37°C with daily change of saline before plasma immersion and bacterial attachment.

After bacterial attachment, the segments were incubated separately in 0.1% tryptose soya broth (TSB; Oxoid) or, for *P. acnes*, anaerobe basal broth (ABB; Oxoid) at 37°C with gentle agitation. At intervals, three segments were removed, rinsed in sterile PBS and sonicated. Viable bacteria in the sonicate were enumerated by colony counting after 48 h of incubation on sheep blood agar.

**In vitro challenge**

This assay determines the ability of the catheters to withstand repeated bacterial challenge while being constantly perfused at 37°C. Catheters were aseptically introduced into a modular apparatus that bathed the outer surface of each catheter in sterile PBS while the lumen was perfused with TSB at approximately the normal CSF production rate of 24 ml/h. Bacterial challenge (1 × 10^6 cfu/ml) was administered into the proximal catheter lumen at intervals 14 days apart, and perfusion was then continued for a further 14 days before the next challenge. Between challenges, effluent from each catheter was collected, 200 μl of the effluent was aseptically introduced into a modular apparatus that bathed the outer surface of each catheter in sterile PBS while the lumen was perfused at 37°C with gentle agitation. At intervals, three segments were removed, rinsed in sterile PBS and sonicated. Viable bacteria in the sonicate were enumerated by colony counting after 48 h of incubation on sheep blood agar.

**Investigation of nanoparticulate silver impregnation**

Silverline catheters were prepared for transmission electron microscopy by focused ion beam milling using gallium ions in a FEI Quanta200 3D dual beam FIB/SEM (FEI Co., Hillsboro, OR, USA). Characterization and analysis of the silver–polymer nanocomposites was then performed using electron back-scatter SEM and energy dispersive X-ray (EDX) analysis.

**Results**

**Time to kill attached bacteria (tk100)**

MRSA was the first isolate to be tested. There was no overall difference between results with and without a plasma protein conditioning film (Figure 1), the differences in readings at *T* = 24 and *T* = 48 not being statistically significant (*P* = 0.42,
Consequently, all further tk100 experiments were carried out with a conditioning film. Figure 2 shows the effect of 5 day soaking on tk100 for MRSA using the low inoculum. Before soaking, there was some antimicrobial effect as shown by the lower counts immediately following challenge \((T=0)\) compared with control, and a further fall at \(T=24\), but this early activity was lost on soaking in PBS for 5 days, and bacteria were not killed at 96 h. The results of the tk100 assay for \(S. \text{epidermidis} \) with low inoculum are shown in Figure 3. Attached bacteria were eradicated by 72 h and soaking for 5 days made no significant difference. Using a high inoculum, attached bacteria were not eradicated with or without soaking (results not shown). Before soaking and with low inoculum, detectable \(E. \text{coli} \) numbers were reduced to almost zero by 48 h but by 96 h (Figure 4) they had returned to the 24 h level. After soaking a very similar picture was seen, but the numbers at \(T=0\) were considerably higher than those before soaking. There appeared to be little antimicrobial activity against \(P. \text{acnes} \) (Figure 5), and again the bacteria were not eradicated. The effect of using a high inoculum of MRSA and \(E. \text{coli} \) is shown in Figures 6 and 7. The numbers of MRSA were reduced (but not significantly, \(P=0.057\)) at 24 h, but increased again at 48 h, showing no difference from controls by 96 h. There was no detectable activity against \(E. \text{coli} \) using a high inoculum.

**Figure 1.** tk100 results for MRSA (low inoculum) with and without a plasma protein conditioning film (CF/NCF). Each point is the mean ± SD of three values. While the number of viable attached bacteria at \(T=0\) was greater without a conditioning film, the overall results were similar, with no eradication of MRSA. The differences for Silverline catheters (CF versus NCF) at \(T=24\) and \(T=48\) were not significant \((P=0.42, P=0.31)\). Open circles, plain catheter NCF; open squares, Silverline catheter NCF; filled circles, plain catheter CF; filled squares, Silverline catheter CF.

**Figure 2.** tk100 results for MRSA with conditioning film (low inoculum) before (a) and after (b) soaking for 5 days. Each point is the mean ± SD of three values. There was a slight loss of initial activity after soaking, but there was no significant difference between the results. MRSA was not eradicated in either case. Filled circles, plain catheter; open circles, Silverline catheter.

**Figure 3.** tk100 results for \(S. \text{epidermidis} \) before (a) and after (b) soaking for 5 days. Each point is the mean ± SD of three values. There was no significant difference between the results at \(T=24\) \((P<0.07)\) or at \(T=48\) \((P=0.4)\). The bacteria were eradicated by 72 h. Filled circles, plain catheter; open circles, Silverline catheter.
In vitro challenge

The results are shown in Figures 8–11. The challenge bacteria were not eradicated during perfusion over several days. Though multiplication of MRSA was slowed at low inoculum (Figure 8), colonization was not eradicated. No activity was demonstrated under flow conditions against *S. epidermidis* or *E. coli* at low inoculum (Figures 9 and 10). No activity was shown in these conditions against *P. acnes* (Figure 11). SEM showed no difference in the case of MRSA between Silverline and control (Figure 12). While there appeared to be fewer bacterial cells of *S. epidermidis*, *E. coli* and *P. acnes* in the Silverline catheters compared with the controls, they were shown to be producing exopolymer.
Investigation of nanoparticulate silver impregnation

Figure 13 shows back-scatter electron SEM images of Silverline catheter material. There was surprisingly little silver in the commercial catheter, and the silver particles were irregular and 500 nm in diameter (nanoparticles are defined as being <200 nm in diameter). The remaining particulate matter was barium sulphate. The nature of the particles was confirmed by EDX analysis.

Discussion

Silverline catheters are claimed to have activity against a wide range of bacteria, but the tests used to establish this usually do not evaluate catheter performance over time in flow conditions. Previous evaluation methods have used roll plates after flushing or non-microbiological methods. The methods used here have been developed to provide data that are more predictive of clinical performance. The tk100 assay is carried out using dilute (0.1%) TSB. The critical factor in the in vitro challenge assay is the flow rate and its effect on duration of antimicrobial activity. TSB was chosen in order to increase the chances of isolating surviving bacteria, thus making the test more sensitive than if CSF had been used. In any case, CSF in clinical cases varies widely in nutrient content, as demonstrated by EVD use after subarachnoid haemorrhage.
One problem with silver-processed biomaterials is that the availability of silver for ionization is often low, yet if the concentration of silver ions is increased to maximize antimicrobial action, cytotoxicity may result. From the point of view of neurotoxicity of silver by the systemic route, Lansdown classifies silver as a sequestered toxicant, meaning that the choroid plexus is able to protect the CNS by sequestering the silver from the bloodstream. However, long-term effects of direct introduction of silver ions into the parenchyma of the CNS by means of an inserted catheter, as in the Silverline catheter, have not been considered. Greil et al. tested aqueous extracts of the Silverline polyurethane catheter (containing 0.3% or 0.6% silver w/w) against various cell lines including phytohaemagglutinin-stimulated human lymphocytes and found no acute cytotoxic effect. They concluded that the Silverline catheter emitted silver ions only in non-cytotoxic concentrations, ‘if at all’.

Electron back-scatter SEM studies on Silverline showed few silver particles, with a diameter of ~500 nm. The low particle density and large particle size could account for the weak activity and short duration of antimicrobial activity of Silverline, as the special behaviour of nanoparticles depends on their very small size.
size, being defined as <200 nm. The results shown here indicate that Silverline does have antimicrobial activity but not enough to eradicate all attached bacteria with the exception of S. epidermidis in non-flow conditions. The tk100 assay is specifically designed to test the ability of an antimicrobial material to kill 100% of attached bacteria, as these are known to exhibit increased phenotypic non-susceptibility to antimicrobials, and are those encountered in clinical use. In the neurosurgical applications for which the catheters are intended, it is essential that all attached bacteria are killed in order to prevent progression to infection, and to avoid risk of resistance developing. Using in vitro test systems, the use of low bacterial inocula often leads to failure of positive controls, and for the sake of experimental success higher inocula than those found clinically are usually used. We were obliged to use this strategy in the case of P. acnes, which could not be tested using the low inoculum of 10⁴ cfu/mL. However, the other test bacteria were used at both high and low inocula, the latter made possible by titration of the nutrient medium. In fact, inoculum size made a difference only in the case of S. epidermidis, which passed the tk100 assay with a low, but not a high, inoculum. Zhao and Stevens have examined the killing activity of silver. Even though their work was carried out with silver nitrate against planktonic bacteria, the results were informative. They found that the ability of silver ions to kill all the bacteria was dependent on not only silver ion concentration but also inoculum size, and they identified an initial inhibitory concentration of low ion density where there was growth delay followed by recovery. This phenomenon might be seen in our Figures 1 and 4.

The tk100 assays were repeated after soaking the catheters in PBS to simulate depletion of silver during use but this made little difference to the overall results, as found by Becht et al. Similarly, the application of a plasma protein conditioning film, the use of which is obligatory in order to simulate in-use conditions, made no difference to the results, contrary to expectations. It is possible that a plasma protein conditioning film has the opposite effect, slightly enhancing the activity of silver ions.

The in vitro challenge has been validated as a clinically predictive test for antimicrobial catheters and tests the ability of the catheter to eradicate a microbial challenge in flow conditions. In this context, the same production technology used in the Erlanger catheter has failed to prevent catheter-related infection in another study. The silver-processed catheters evaluated here were unable to eradicate challenges of any of the test bacteria in flow conditions, even at low inoculum. In the case of successful antimicrobial catheters the test usually progresses to several challenges 14 days apart before failure but in this case failure occurred at first challenge. While Silverline catheters might be effective enough to eradicate very small numbers of contaminating bacteria, they do not appear to have the power to eradicate higher inocula. The results of the in vitro challenge show that they are unlikely to perform well in flow conditions where the flow rate is similar to that of CSF.

**References**


**Author contributions**

R. B. was responsible for the conception and design of the study, study coordination and drafting the manuscript. L. V., A. M., O. S. and W. A. were responsible for generating and analysing data. S. M. H. contributed expertise in inorganic and polymer chemistry. All authors critically reviewed the manuscript and gave final approval.

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**Transparency declarations**

There was no commercial influence or restriction on the design, execution or reporting of the project. Commercial catheters were purchased using normal procedures. R. B. receives financial support for research into antimicrobial biomaterials and related consultancy fees from Codman and Shurtleff Inc. and Medical Components Inc., and is named Inventor on a patent for antimicrobial biomaterials. No other authors have anything to declare.

**Figure 13.** Back-scattered electron SEM analysis. Silverline catheter material with silver particles highlighted with white rings. The silver particles were ~500 nm in diameter. The remaining light particles were barium sulphate (confirmed by EDX analysis).
Antimicrobial silver-processed catheters


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