Acknowledgment

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References


Silver Modification of Polyethylene Terephthalate Textiles for Antimicrobial Protection

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The safety and in vitro effectiveness of applying silver to polyethylene terephthalate fabric mechanical heart valve (MHV) sewing cuffs for the prevention of prosthetic valve endocarditis (PVE) were evaluated. PVE is an infrequent but grave complication of cardiac surgery associated with mortality rates potentially exceeding 50%. A poor response to antibiotic therapy is partly responsible for the high mortality rates. Silver is a well known antimicrobial agent with broad effectiveness. Preliminary in vitro microbial challenge studies of the coated fabric using the New York State 63 bacteriostatic test and Dow Corning Shafe Flask test showed a ≥97% reduction for most organisms tested. Sheep mitral valve replacement studies suggest...
comparable tissue ingrowth of uncoated and coated fabric with a more organized, thicker pannus formed on silver coated fabric. Low levels of silver were present in the serum at all time periods. These results indicate MHVs with silver coated cuffs may provide additional protection against PVE. ASAIO Journal 1997; 43:M475–M481.

Infective endocarditis involving prosthetic valves is an infrequent but grave complication following heart valve replacement surgery, associated with high morbidity and mortality rates despite aggressive antimicrobial therapy and surgical intervention. Mortality rates have been reported to be as high as 74%/no pt yr for early onset prosthetic valve endocarditis (PVE), which occurs within 2 months post implantation, and 43%/pt yr for late onset PVE.1,2 The higher mortality rate for early onset PVE is due to infection with more virulent organisms, a “non endothelialized” sewing cuff, and other aspects of incomplete healing in the early post operative period.2 The occurrence of PVE overall ranges from 1 to 4%/pt yr.1,2 Recurrent PVE is an even greater risk and has been reported to be as high as 15%/pt yr.3 Thrombotic complications concurrent with PVE have been reported to range from 13% for staphylococcus infections to 90% for fungal infections.3 The valve sewing cuff has been found to be the most frequent site of infection.1

An aortic valve allograft is the preferred prosthesis for surgically treating aortic valve endocarditis.1 Biologic tissue appears to be less prone to reinfection relative to synthetic prosthetic material and appears to limit the spread of infection.1,4 However, disadvantages of allografts include increased technical difficulty with implantation, limited availability, limited sizes, and structural failure due to calcification. In addition, infected native mitral valves often have to be replaced with a bioprosthetic or mechanical valve because a mitral valve allograft is not readily available.1

Silver has been used to reduce the incidence of infection since the 19th century when it became routine to apply silver nitrate solution to newborn eyes for the prevention of ophthalmia neonatorum (gonorrhea).5 More recently, silver nitrates and silver sulfadiazine cream are used for cutaneous infections, especially those associated with thermal wounds, because of their broad spectrum antimicrobial properties.5 With respect to medical devices, silver has shown promise in reducing infections caused by catheters and their subcutaneous cuffs in both intravenous and urologic applications,7,8 sutures,9 dental amalgams,10 vascular grafts,11 and orthopedic devices.12

This study was designed to determine the safety and in vitro efficacy of ion beam assisted deposition (IBAD) silver coated polyethylene terephthalate (PET) fabric for use in the sewing cuffs of the St. Jude Medical mechanical heart valve (MHV) SJM Masters Series prosthesis (SJM Masters Series valve) for the inhibition of PVE and as an alternative to the allograft heart valve, which is in limited supply.

Materials and Methods

Fabric Modification

PET fabric (Meadow double velour, uncrimped, scoured, and heat set (Meadow Medicals, Oakland, NJ) was coated with metallic silver using the patented IBAD SPI-ARGENT process developed by Spire Corporation (Bedford, MA). A nominal 3,000 Å thick coating has been engineered to substantially mediate silver release, yet provide an adherent, long-lasting antimicrobial surface. Film PET (Mylar; DuPont, Wilmington, DE) was also coated for surface analysis studies. Clinical grade, 25 mm SJM Masters Series valve prostheses with half coated and half uncoated PET polyester sewing cuffs were used for the mitral valve replacement model. With this cuff configuration, internal controls were established in each animal. All materials were sterilized using steam for 40 min at 121°C, 17 psi.

Microorganisms

Staphylococcus epidermidis ATCC 29868, Streptococcus pyogenes ATCC 8668, Candida albicans ATCC 10231, Pseudomonas aeruginosa ATCC 9027, and Staphylococcus aureus ATCC 6538 were used to test the in vitro efficacy of the silver coated fabric. All test organisms were prepared by inoculating nutrient broth with stock cultures and incubating for 24 hr at 20–25°C for C. albicans and 30–35°C for the other organisms. The microbial suspension was then refrigerated at 2–8°C until use to prevent further growth.

Surface Analysis

The surface chemistry and energy of uncoated and silver coated PET film were analyzed using X-ray photoelectron spectroscopy (XPS) and contact angle analysis. Contact angle analysis was performed with a Kruss–Hart contact angle goniometer using the method of advancing contact angles. The critical surface tension (γ_*) and dispersive and polar components of the surface free energy (γ_s and γ_p, respectively) were calculated by the methods of Zisman13 and Kaelble.14 XPS was performed using a Perkin Elmer model 5400 XPS spectrometer.

Table 1. Contact Angle Analysis of Silver Modified PET: Mean (SD)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Y_s (dyne/cm)</th>
<th>Y_p (dyne/cm)</th>
<th>Y_d (dyne/cm)</th>
<th>Water Contact (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET (n = 2)</td>
<td>35.6 (0.4)</td>
<td>10.0 (1.3)</td>
<td>28.1 (1.1)</td>
<td>70 (1)</td>
</tr>
<tr>
<td>PET/Ag (n = 2)</td>
<td>30.7 (0.4)</td>
<td>6.9 (0.7)</td>
<td>24.2 (0.9)</td>
<td>94 (0.8)</td>
</tr>
</tbody>
</table>

Table 2. Atomic Concentrations (%) of Silver Modified PET: Mean (SD)

<table>
<thead>
<tr>
<th>Sample</th>
<th>C</th>
<th>O</th>
<th>Ag</th>
<th>Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET (n = 2)</td>
<td>70.1</td>
<td>29.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PET/Ag (n = 2)</td>
<td>55.1 (6.2)</td>
<td>8.5 (2.7)</td>
<td>35.8 (2.5)</td>
<td>1.3*</td>
</tr>
</tbody>
</table>

* Detected in one sample only.
Table 3. Microbial Challenges: Mean % Reduction

<table>
<thead>
<tr>
<th>Organism</th>
<th>NYS63</th>
<th>Dow Shake</th>
<th>PET/Ag</th>
<th>PET/Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. epidermidis</td>
<td>NR</td>
<td>99.0 (1.9)</td>
<td>12.5 (17.7)</td>
<td>97.1 (3.3)</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>NR</td>
<td>99.7 (0.3)</td>
<td>NR</td>
<td>68.5 (43.8)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>NR</td>
<td>98.2 (1.2)</td>
<td>NR</td>
<td>96.7</td>
</tr>
<tr>
<td>C. albicans</td>
<td>29.2 (1.8)</td>
<td>99.5 (0.3)</td>
<td>NR</td>
<td>98.6 (1.7)</td>
</tr>
</tbody>
</table>

NR, no reduction.

(PHI, Eden Prairie, MN) with monochromatic Al Ka x-ray radiation. Average depth of penetration was 60 Å and the take-off angle was 65⁰. Depth profiling to 40 Å depth was also performed using a 4 KeV Ar⁺ ion beam at a sputter rate for silver of 32 Å/min.

In Vitro Efficacy

In vitro efficacy of the silver coated fabric was assessed using the New York State (NYS) 63 test for bacteriostatic activity, the Dow Corning Shake Flask test, and a static incubation test. Each assay was repeated on two separate days. The NYS63 test method for bacteriostatic activity consisted of incubating 2 × 10⁶ to 1 × 10⁶ CFU of S. aureus, S. pyogenes, C. albicans, or S. epidermidis in a humidified atmosphere with 1 in squares of uncoated and coated fabric at 37°C for 24 hr. The samples were diluted with 99 ml of Lethone Broth and vigorously shaken for 1 min. Serial dilutions were plated on nutrient agar, incubated again for 24–48 hr, and counted. The percent reduction of each organism was determined. Five replicates were done in each assay.

Dow Shake Flask test consisted of inoculating 70 ml of Kayser–Roth phosphate buffer with 5 ml of 1–2 × 10⁶ CFU/ml S. aureus, S. pyogenes, C. albicans, or S. epidermidis, placing 0.75 g of uncoated or coated fabric in the suspension, and shaking vigorously for 1 hr in a wrist action shaker (Burrell Scientific, Pittsburgh, PA) at 37°C. Aliquots of solutions are plated into petri dishes and incubated for at least 24 hr and then counted. A percent reduction was calculated. An initial count was obtained before the samples were added.

A final bioactivity assay consisted of incubating 1 × 1 cm² pieces of coated and uncoated fabric for 48 hr at 30–35°C in 10 ml of tryptic soy broth inoculated with 10–100 CFU of either S. aureus, P. aeruginosa, or S. epidermidis. Specimens were rinsed in saline, fixed in 2% buffered glutaraldehyde, serially dehydrated with ethanol, dried with hexamethyldisilazane, coated with a gold palladium film, and imaged using scanning electron microscopy (Hitachi 450; Hitachi, Tokyo, Japan) to assess the nature of organism interaction with the fabric.

Silver Leaching

The leach rate of silver from fabric sewing cuffs was tested in vitro in serum. Two 17 mm valves with uncoated cuffs and two 17 mm valves with coated cuffs were exposed to 495 ml fetal bovine serum (Hyclone Laboratories, Logan, UT) for 7 days at 37°C with rotation at 100 rpm (Environ Shaker; Lab-Line, Melrose Park, IL). Five milliliter samples of the serum were subjected to elemental analysis for silver pre-exposure and at 5 hr, 1, 2, 3, 4, and 7 days post exposure. Elemental analysis was performed using an inductively coupled plasma atomic emission spectrometer (Atom Scan 16; Thermo Jarrell Ash, Franklin, MA). Standard curves were prepared using 10,000 ppm NIST Silver in 14% nitric acid. The range was from 0 to 500 ppb. All samples were lyophilized and then hydrolyzed in 17.5% nitric acid at 115°C for 24 hr. The samples were diluted to 14% nitric acid before analysis. The quantitation limit was 25 ppb.

Cardiac Valve Replacement (CVR) Model

All animal care complied with the Principles of Laboratory Animal Care and The Guide for the Care and Use of Laboratory

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**Figure 1.** Scanning electron micrographs of S. epidermidis reaction to uncoated fabric (a) and silver coated fabric (b). Original magnification ×5,000. Magnification bar = 2.5 µm.
Animals (NIH Publication No. 82-23, 1985). All protocols were approved by the facility's animal care committee. SIM Masters Series valves with half silver coated and half uncoated PET cuffs were implanted in the mitral position of four Suffolk sheep, each weighing ~25 kg. The native mitral valve was either left intact or the anterior leaflet was removed. No anticoagulants were given postoperatively. Prophylactic antibiotics were given for 7–10 days postoperatively. The valves were sutured into the annulus using interrupted horizontal mattress sutures of 3-0 Tevdek (Deknatel) with or without Teflon felt reinforcing pledgets. Serum samples were prepared from whole blood drawn pre-implantation, 1, 2, and 3 weeks postoperatively, and pre-sacrifice and assayed for silver using elemental analysis as described above. The silver quantitation limit was 12.5 ppb. Values that fell below this limit were assigned the value 12.5 ppb. Four to five weeks after implantation, the valves were explanted after the animals were systemically anticoagulated with 300 units heparin/kg body wt and killed. Pre-operative, intraoperative, and postoperative techniques and husbandry methods are described in detail elsewhere. Explanted valves were fixed in McDowell-Trump fixative after rinsing with 0.1 M phosphate buffer and analyzed macroscopically and histologically for tissue reaction. Silver coated and uncoated sewing cuff histology samples were retrieved 45° from the two seams. The thickness of the tissue (pannus) covering the sewing cuffs was measured using a calibrated reticle.

**Results**

Surface energy and XPS measurements are shown in Tables 1 and 2. Modification of the PET film with IBAD silver resulted in a surface with increased hydrophobicity, decreased γ_w, and decreased polarity. XPS results show a surface that is predominantly C, Ag, and O. Analysis of the oxygen surface chemistry shows the oxygen is bound to C and not Ag. Depth profiling shows that both C and O drop off after no more than 16 Å is removed (data not shown).

The results of the NYS63 and Dow Shake Flask tests are shown in Table 3. The mean percent reduction of organisms on the silver coated fabric in the static NYS63 test ranged from 98.2% for *S. aureus* to 99.7% for *S. pyogenes* compared with little to no reduction with the uncoated fabric. The Dow Shake test showed a mean reduction that ranged from 68.5% for *S. pyogenes* to 98.6% for *C. albicans* for silver coated fabric compared with little to no reduction with the uncoated fabric. *S. pyogenes*’ apparent need to be in prolonged direct contact with silver to achieve inhibition may explain the difference in reduction in the Dow Shake test compared to the NYS63 test. The scanning electron microscopic analysis of uncoated and silver coated fabric incubated with microorganisms indicated that there were fewer organisms attached and less colonization of the bacteria on the silver coated fabric than the uncoated control for both *S. epidermidis* and *P. aeruginosa* (Figures 1 and 2). The silver coating on the fabric also inhibited the accumulation of *S. aureus*; most bacteria were attached as singlets or doublets (data not shown).

Cumulative and incremental *in vitro* silver leaching data are shown in Figure 3. The results show that ~2% of the silver is released from the cuff in serum within 7 days and the release has reached a plateau. The incremental release data show that the majority of the silver was released in the first 3 days. No silver was detected in uncoated controls, as anticipated. The maximum level of silver detected in the serum was 387 ppb (data not shown), which corresponds to 55 ppb in an adult human of normal blood volume (5 L). Ninety percent of silver

![Figure 2](image-url)  
*Figure 2.* Scanning electron micrographs of *P. aeruginosa* reaction to uncoated fabric (a) and silver coated fabric (b). Original magnification ×5,000. Magnification bar = 2.5 μm.

![Figure 3](image-url)  
*Figure 3.* Cumulative and incremental *in vitro* leaching of silver from mechanical heart valves with ion beam assisted deposition silver coated cuffs in serum. Error bars are SEMs. *Value went negative and was assigned 0.*
ingested or absorbed is excreted in the bile, which, in this case, would result in a residual 5.5 ppb serum silver level.

Representative gross macrographs and histology from the CVR study are shown in Figure 4. The gross reaction to the cuff was similar for both the uncoated and the coated halves (Figure 4a). Grossly, the pannus was >75% healed at the 4 to 5 week time period. Histopathologic analyses showed that the degrees of foreign body giant cell (FBGC) response and fibrous integration of the two halves were similar (Figure 4), with an average score of 1.25 (FBGC) and 1.6 (fibrous integration) on a scale of 0–4, 0 being none and 4 being marked. Also, there was a suggestion that pannus maturity was more advanced on the silver coated side than the uncoated side. The pannus in the control areas was characterized by randomly oriented, “activated fibroblasts” forming a non lamellar pattern (Figure 4b). Dystrophic calcification was found in control areas only (Figure 4c). The pannus in coated areas was characterized by regularly oriented “mature fibroblasts” forming a lamellar pattern (Figure 4d). The mean pannus thickness of the uncoated half was 400 ± 291 μm compared with 269 ± 235 μm for the silver coated half. Serum silver levels are shown in Figure 5. A slight peak is suggested at two weeks, after which it dropped to below quantitation by death (4–5 weeks).

Discussion

Silver salts and colloids have a broad spectrum and long history as antimicrobials. However, silver must be carefully administered to avoid silver toxicity syndrome manifested as argyria (blue skin pigmentation), leukopenia, and kidney, liver, and neurologic tissue toxicity. The lowest serum level reported for this syndrome is 300 ppb. Promising results have been published on the use of metallic silver and silver oxide coatings on various devices for the prevention of device associated infection. Silver in these forms ionizes slowly providing protection against silver toxicity while offering surface contact antimicrobial benefits. Our in vivo CVR and in vitro leach studies indicate silver ionization from PET fabric to be far below toxic levels. Surface chemical analysis of the coating confirms the presence of a metallic silver state, with a thin layer of organic contaminant on the outermost surface. The XPS profile, together with the more hydrophobic and nonpolar nature of the coated surface, suggest that the IBAD process may have facilitated a surface coating of PET fragments with its hydrophobic groups (benzyl or methyl) outermost.

Our results support the broad spectrum antimicrobial activ-
ility of IBAD silver that others have reported for silver in general. IBAD silver inhibited the colonization on contact of gram positive organisms, a gram negative organism, and a fungus, all of which are implicated in PVE. Silver is postulated to inhibit microorganisms by binding to microbial DNA and subsequently preventing replication and by binding to the sulphydryl groups of key metabolic enzymes resulting in denaturation and inactivation of those enzymes. Although silver ion is considered one of the most potent heavy metal inhibitors of enzymes and microorganisms, its toxicity to higher organisms and humans is small because of its slow absorption and ease of reaction with chloride ion, proteins, and sulphydryl groups.

Metallic silver has been reported to be relatively nontoxic and inert to mammalian tissues, which is confirmed in this study. Tissue ingrowth was not inhibited in this application, and there was a suggestion that pannus organization was more advanced on the silver coated fabric. A fully organized pannus would provide protection from cuff associated thrombosis and infection. The relative blood compatibility of silver is supported in this study and by other groups who have reported on its use in intravascular catheter and vascular graft applications. Bambauer et al. reported a decrease in platelet attachment and thrombus formation on silver coated silicone rubber catheters for dialysis applications. Silver has also been shown to adsorb high levels of albumin, which perhaps contributes to its relatively good blood compatibility.

The heart valve prosthesis sewing cuff evaluated is designed to aggressively promote tissue ingrowth with its rough, double velour texture. However, the rough morphology and the PET polymer itself are highly attractive to microorganisms involved in endocarditis as supported by the clinical finding that infection begins in the sewing cuffs of MHV prostheses. It has been suggested that most contamination leading to early onset PVE occurs intraoperatively. One group found that 52% of valve prostheses and 64% of the myocardia had positive cultures prior to closure of the wound in a study of 66 patients undergoing open heart surgery. Adhesion mediated infections have proven to be so resistant to both host defenses and medical management with antibiotics that PVE of MHV, in particular, usually results in the prosthesis having to be removed and replaced. Therefore, it is critical to prevent the infectious organism from adhering to or propagating on the device in the first place.

Some surgeons dip MHV in antibiotics to lessen the perioperative contamination. However, the drug is rapidly eluted from the prosthesis into the blood and surrounding tissue. Methods to stabilize the antibiotic on prostheses using surfactants also result in relatively fast elution. Other approaches to reducing microbial colonization of medical devices have included coating with drugs to reduce inflammation and surface modification with nonadhesive materials such as silicone, polystyrene, and hydrogels. Antibiotic and other drug therapies pose difficulties in dosing appropriately to maximize efficacy and minimize toxicity. Passive coatings aimed at preventing protein and cellular attachment have been shown to be inevitably ineffective.

This study showed that IBAD silver coating for a MHV sewing cuff was tightly adherent and low leaching inhibited colonization of the fabric by microorganisms implicated in PVE (and may therefore provide added protection from intraoperative contamination), and facilitated controlled tissue ingrowth and incorporation. These data support further investigation into the benefits of providing a MHV prosthesis with an antimicrobial silver modified sewing cuff as an additional option to the cardiac surgeon for the treatment of native valve endocarditis (NVE) or prosthetic valve endocarditis (PVE).

Acknowledgment

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References


