

## Silver-Nylon: a New Antimicrobial Agent

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The potential effectiveness of a silver nylon (SN) fabric as an antimicrobial agent was evaluated in a series of in vitro experiments. The results indicated that silver ions from the SN fabric penetrated 2 mm of agar and killed a challenge of  $6.9 \times 10^7$  *Pseudomonas aeruginosa*,  $4.1 \times 10^7$  *Staphylococcus aureus*, and  $1.4 \times 10^7$  *Candida albicans* organisms per cm<sup>2</sup> of SN fabric after 24 h of incubation at 37°C. To determine some of the microbicidal limits of the SN, the distance between the SN fabric and the microbial challenge was increased. Increasing the height of the agar column overlying the SN fabric diminished the inhibitory effect of SN on microbial growth. For each increase in agar height of 2 mm, up to a total height of 8 mm, the effectiveness of SN to inhibit microbial growth decreased by a factor of 10. This distance-related decrease in the microbicidal ability of SN could be overcome by placing the SN fabric in contact with the agar column for 24 to 72 h before microbial challenge. On the basis of these experiments, it appears that SN is an effective antimicrobial agent, although further work must be performed before it is applied clinically.

Silver in various forms has a long history in medicine as an antimicrobial agent (4). The choice of an appropriate delivery system has been a significant problem, and over the years a variety of creams, solutions, foils, and mixtures have been studied. Presently, the major clinical uses of silver are in the prophylaxis of gonococcal ophthalmological infections in newborns and in the treatment of burn patients.

Silver compounds produce their antimicrobial effects by the time-dependent release of silver ions, and their clinical efficacy is directly related to the constant presence of free silver ions in the local microbial environment (2). Substances that release silver ions rapidly, such as silver nitrate, require frequent applications to achieve clinically effective concentrations of silver ions in local wounds. In contrast, an agent such as silver sulfadiazine, which releases silver into burn wounds more slowly, is associated with a more constant level of local silver ions and thus requires drug application only twice a day. Although both these agents have clearly been beneficial in the prophylaxis of burn wound sepsis and in the treatment of soft-tissue infections, they have definite limitations. They are expensive, technically cumbersome and time consuming to apply, and, most importantly, they are frequently ineffective in preventing or treating wound infections in patients with extensive burn injuries (6). This latter result occurs even though the infecting organisms may be susceptible to the antimicrobial effect of silver ions in

vitro. Thus, it seems likely that silver therapy could be of significant clinical benefit if a convenient, safe, and economical means of achieving constant local microbicidal levels of silver ions could be found. Recently, silver-coated nylon fabrics have been developed which offer the possibility of solving some of the problems associated with the use of silver compounds.

The silver-nylon (SN) fabric we used (Swift Textile Metalizing Corp., Hartford, Conn.) consists of a nylon substrate coated with metallic silver. The SN fabric is available in a variety of weaves and meshes and was originally developed as a means of providing flexible electrical shielding. Because of the low cost of SN fabric (as compared with silver foils or wires), porosity, and flexibility, it seemed that it might be a useful substance for delivering sufficient levels of silver to eradicate local soft-tissue infections. We therefore performed in vitro experiments to examine the antimicrobial potential of SN.

### MATERIALS AND METHODS

The SN fabric used was heavy rip-stop material (style A-25989-5) weighing 2.5 ounces per square yard (ca. 87.5 g/m<sup>2</sup>) of which 0.5 ounces per square yard (ca. 17.5 g/m<sup>2</sup>) was metallic silver. The SN fabric was briefly rinsed in distilled water and, except where noted, was not subjected to further treatment before use. The principal test system consisted of plastic culture tubes from which the bottoms had been removed and replaced with SN fabric (Fig. 1). A column of tryptic soy agar was poured on the SN fabric, and the surface of the agar was seeded with 50  $\mu$ l of serial

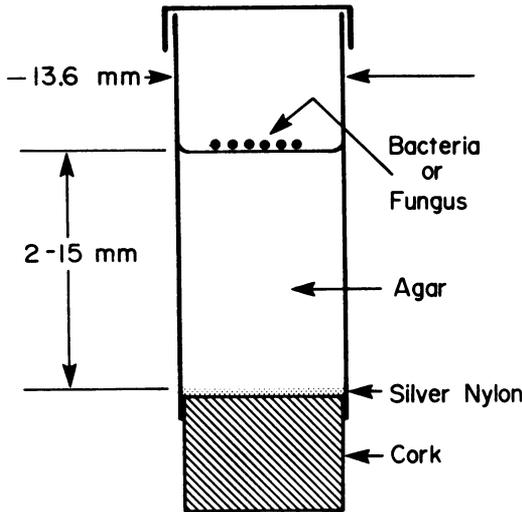


FIG. 1. Experimental apparatus used to determine the antimicrobial activity of SN.

dilutions of an 18-h broth culture of either *Staphylococcus aureus*, *Pseudomonas aeruginosa*, or *Candida albicans*. Both heavy rip-stop nylon without silver impregnation and aluminum foil were used as control materials in these experiments.

After the organism to be studied had been pipetted onto the agar surface, the units were incubated for 24 h at 37°C. The surface was then examined microscopically (×20) for the presence of visible bacterial colonies, a surface film, or opacity. For inhibition of growth to be recorded, the agar surface had to be both translucent and without visible colonies. The highest microbial inoculum that did not result in visible growth was recorded for each agar height. After visually inspecting for colonies or opacity, a swab of one-half of the surface area of the agar was taken and immediately subcultured to tryptic soy agar culture plates. The plates were incubated at 37°C for 24 h and read to determine whether the silver-ion concentration at that height was bactericidal.

To study the antimicrobial spectrum of SN, 1-cm squares of SN fabric were placed in culture dishes and overlaid with tryptic soy agar to a height of 2 mm. After the agar solidified, the plates were seeded with 50 μl of an overnight undiluted broth of each organism to be tested ( $5 \times 10^7$  to  $1 \times 10^9$  CFU). After 24 h of incubation at 37°C, the presence or absence of visible colonies on the agar surface directly above the SN fabric was recorded.

To determine whether SN was more effective when the silver ions were allowed to diffuse into the agar before microbial challenge, the following experiment was performed. Units containing SN fabric overlaid by various agar heights were stored at 37°C for up to 3 days. At 24, 48, and 72 h after the agar-SN assay tubes had been prepared, 50 μl of an undiluted overnight broth of the test organism was inoculated onto the agar surface. The presence or absence of microbial growth was determined 24 h later.

The microbial challenge was quantitated by serial pour-plate assays of each test organism.

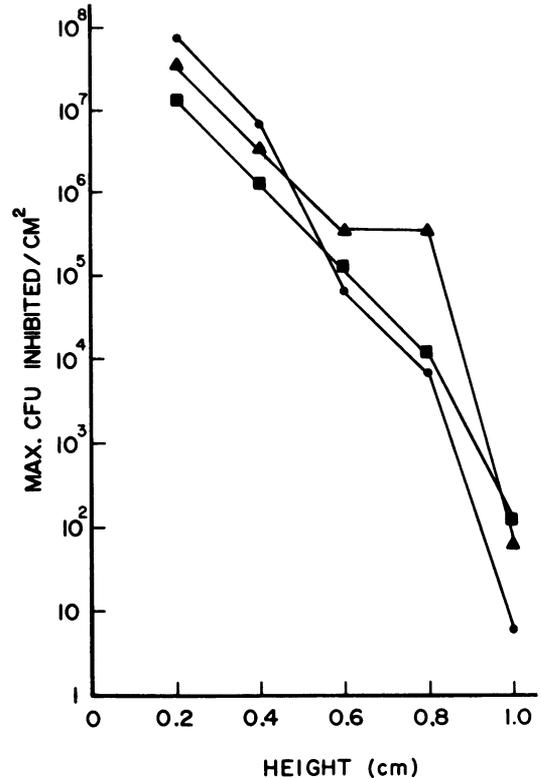


FIG. 2. Maximum number of CFU/cm<sup>2</sup> inhibited with increasing agar height between inocula of *P. aeruginosa* (●), *S. aureus* (▲), and *C. albicans* (■) and SN.

RESULTS

Serial inocula of *S. aureus*, *P. aeruginosa*, and *C. albicans* were placed onto the surfaces of SN-agar columns of various heights to determine the bacteriostatic and bactericidal penetration limits of SN. At an agar height of 2 mm, all the organisms present in 50 μl of an 18-h broth culture were killed after 24 h of incubation at 37°C. At this height, silver released from the SN fabric was capable of killing at least  $6.9 \times 10^7$  *P. aeruginosa*,  $4.1 \times 10^7$  *S. aureus*, and  $1.4 \times 10^7$  *C. albicans* organisms per cm<sup>2</sup> of SN fabric (Fig. 2 and 3). As the height of the agar column was increased, both the bacteriostatic and bactericidal ability of the SN decreased (Fig. 2 and 3). For each increase in agar height of 2 mm up to a total height of 8 mm, the effectiveness of the SN to inhibit microbial growth decreased by a factor of 10 (Fig. 2). A comparable trend for microbial killing was also seen as the height of the agar column was increased (Fig. 3). Neither aluminum foil nor nylon fabric without silver had any microbicidal action. Microbial growth occurred even at an agar height of 2 mm.

Experiments were performed to determine

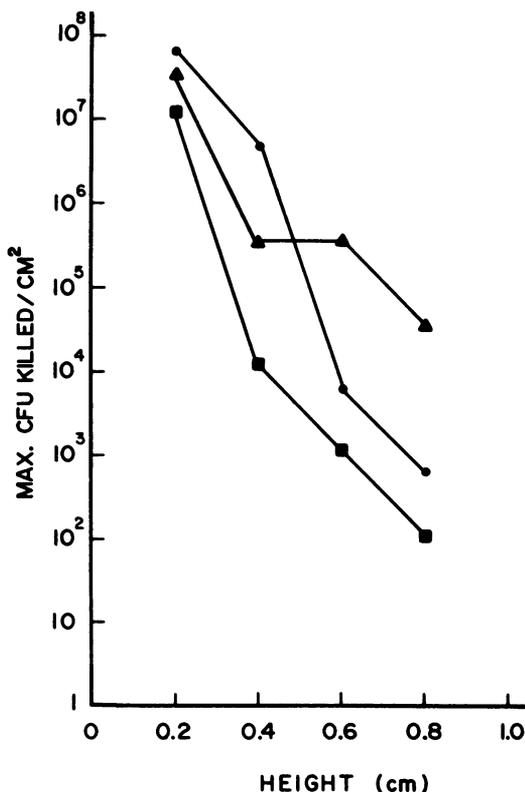


FIG. 3. Maximum number of CFU/cm<sup>2</sup> killed with increasing agar height between inocula of *P. aeruginosa* (●), *S. aureus* (▲), and *C. albicans* (■) and SN.

whether SN was more effective when the silver ions were allowed to diffuse into the agar before microbial challenge. The longer the SN fabric was in contact with the agar before inoculation with the test organism, the more effective was its antimicrobial action (Fig. 4).

To determine the spectrum of antimicrobial activity of SN, 50  $\mu$ l ( $5 \times 10^7$  to  $1 \times 10^9$  CFU) of an overnight broth of 22 different organisms was placed on the surface of an agar column 2 mm in height overlying a 1-cm<sup>2</sup> piece of SN fabric. No growth of the following 17 organisms was seen after 24 h of incubation: *Acinetobacter calcoaceticus*, *Shigella sonnei*, *Salmonella typhimurium*, *Salmonella typhi*, *Enterococcus* sp., *Serratia marcescens*, *Pseudomonas maltophilia*, *Listeria monocytogenes*, *Enterobacter cloacae*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, group A *Streptococcus*, group B *Streptococcus*, and *Pseudomonas cepacia*.

In contrast, the following five organisms did show some microbial growth at the outer border of the SN fabric: *Flavobacterium* sp., *Aeromo-*

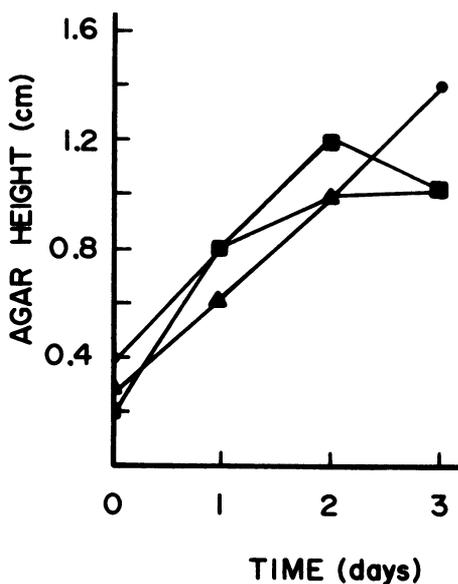


FIG. 4. Relationship between duration of prediffusion of silver ions and inhibitory effect with increasing agar height against *P. aeruginosa* (●), *S. aureus* (▲), and *C. albicans* (■). The microbial challenge used was  $1.4 \times 10^7$  to  $7 \times 10^7$  CFU.

*nas hydrophila*, *Vibrio cholerae*, *Micrococcus* sp., and *Proteus vulgaris*.

## DISCUSSION

The constant presence of free silver ions in the bacterial environment is a critical factor needed to ensure the effectiveness of silver as an antimicrobial agent in the treatment of established infections. Results of experiments in which microbial cultures containing from  $10^7$  to  $10^1$  CFU were used indicated that SN is an effective broad-spectrum antimicrobial agent with a spectrum of activity which is similar to those of other silver compounds, such as silver-sulfadiazine (1) and silver nitrate (3). We presume that the release of silver ions from the SN fabric was the basis of the antimicrobial action of SN. We base this hypothesis on the following facts and observations. It is recognized that distilled water that has come in contact with metallic silver becomes bacteriostatic even after the silver has been removed; this is a physical phenomenon known as oligodynamic action (3). A silver-ion concentration of 0.05  $\mu$ g/ml has been reported in silver-treated distilled water (3), and even higher levels of silver have been documented in broth media (8). Spadaro et al. (8) reported that a silver wire with a surface area of 24 mm<sup>2</sup> placed in a broth media for 4 h at 37°C resulted in a silver-ion concentration of 0.7  $\mu$ g/ml.

Although the bacteriostatic and bacteriocidal

sensitivity of organisms to silver vary widely, they are generally in the range of 10 to 20  $\mu\text{g/ml}$  (1). The surface area of the SN fabric used in this study was constant at 140  $\text{mm}^2$ . The antimicrobial effect of the SN was optimized for a given microbial challenge by either decreasing the distance between the inoculum and the surface of the SN fabric or increasing the length of time the SN fabric was in contact with the agar before microbial challenge (Fig. 2, 3, and 4). In both cases, the net effect was to increase the concentration of silver ions and thus to increase the antimicrobial action of SN.

To screen for the presence of organic or inorganic agents other than silver that may have accounted for the antimicrobial activity of SN, some SN fabric was treated for 24 h with either ethyl alcohol or distilled water before use. Neither treatment substantially affected the antimicrobial activity of SN. Although we consider it unlikely, it is still possible that some substance other than silver was liberated from the SN and was responsible for the observed antimicrobial effect of the SN.

One potential major advantage of SN over presently available silver compounds is its theoretical ability to release silver ions continuously over the entire time period that it is in contact with the aqueous wound environment. In general, agents that allow constant prolonged release of silver, such as silver sulfadiazine, are more effective clinically in both prevention and treatment of established infections than are agents that release silver rapidly, such as silver nitrate. Our results indicate that adequate levels of silver ions were released from the SN to inhibit growth of  $10^7$  organisms per  $\text{cm}^2$  of test material. Although the clinical applicability of SN cannot be determined from this study, SN has several features which make it clinically attractive. Silver is not associated with significant side effects, is not an allergen, and is only rarely associated with the induction of resistant strains of bacteria (5, 7). In addition, the silver ions delivered by the SN are not accompanied by a carrier molecule or anion which may be associated with systemic side effects, such as occurs with both silver nitrate and silver sulfadiazine.

An additional advantage of SN which is not shared by silver compounds is the ability it may provide to electrically augment silver ion release. Because SN is electrically conductive, it can be operated as the anode in a direct-current electrical circuit. Faraday's Law predicts that, under ideal conditions, 4  $\mu\text{g}$  of silver will be liberated per h per  $\mu\text{A}$  of current flow. This level of current flow is well below the level of cutaneous sensation. Even though in vivo electrical conditions are not ideal and multiple electrode processes will occur, microbicidal concentrations of silver ions could theoretically be generated in a few hours and maintained almost indefinitely via the passage of a weak electrical current. Electrically generated silver ions from the surface of SN, using currents in the range of 1 to 2  $\mu\text{A/cm}^2$ , have been reported to be effective in treating established orthopaedic infections (R. O. Becker and J. A. Spadaro, *J. Bone Joint Surg.* 60A:871, 1978). At this time, the clinical effectiveness of electrically augmented silver ion release from SN is being evaluated in a controlled clinical trial with osteomyelitis patients at our institution.

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