

Silver Nylon Cloth: In vitro and in vivo Evaluation of Antimicrobial Activity

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The topical agents used in burn care today, although beneficial, do have limitations, the most important of which is the failure to prevent or treat burn wound infections in patients with extensive injuries. Therefore, we evaluated the antimicrobial activity of two silver nylon fabrics (SN and HRS) on the growth of *S. aureus*, *P. aeruginosa*, and *C. albicans*. The theoretical advantages of these nylon fabrics over presently available silver compounds is their ability to continuously release silver ions into the burn wound as long as the material is in contact with the wound. Additionally, the release of silver from these fabrics can be augmented electrolytically. The results of these experiments indicated that both materials were microbicidal in vitro for all three test organisms. Furthermore, the antimicrobial activity could be significantly augmented by passing a weak DC current through the material, which increased the rate of release of silver ions from the fabrics. In vivo, HRS was as effective as silver sulfadiazine in preventing colonization of the burn wound. The use of silver nylon fabrics thus appears to be a promising way of delivering large quantities of silver ions into the burn wound without the potential side effects of a carrier molecule or cream.

The heavy metals, especially silver, were frequently used to treat infections before the development of systemic antimicrobial agents (5). Recently the development of topical antimicrobial agents containing silver, such as silver nitrate and silver sulfadiazine (4), have revolutionized the clinical practice of burn care. Yet these silver compounds have definite limitations: they are expensive, cumbersome, and require considerable nursing time to apply. More important, these agents are not effective in the treatment or prevention of burn wound sepsis in patients with extensive thermal injury (7). This occurs even though the infecting organisms may be susceptible to the antimicrobial effect of silver ions in vitro. For these reasons, the search for a more effective topical agent has continued.

The nature of the bond between the silver moiety and the carrier molecule appears to be critical for the silver compound to be an effective antimicrobial agent, since silver compounds produce their antimicrobial effect by the time-dependent release of silver ions from the carrier molecule. That is, the antimicrobial effect of a silver compound is due to the constant presence of free silver ions in the local wound environment (5). For example,

substances that release silver ions rapidly, such as silver nitrate, require frequent applications to achieve clinically effective local concentrations of silver in the burn wound. Compounds such as silver sulfadiazine, which releases silver into the burn wounds more slowly, are associated with a more constant level of silver ions locally and thus require less frequent applications (2). Based on these considerations, we believe that if the rate and number of silver ions delivered into the wound could be increased, then better local control of microorganisms in the burn wound would be achieved. We have begun testing a series of silver-coated nylon fabrics as antimicrobial agents. In preliminary work, we demonstrated that this cloth is an effective antimicrobial agent for a wide variety of bacteria and fungi (3). The purpose of the present experiments was to further characterize the antimicrobial properties of the silver nylon fabric in vitro and in vivo, as well as to determine if the effectiveness of this material could be increased by using an electrical current to augment the rate of release of silver ions. The results of these experiments indicate that silver nylon is a promising method of delivering large quantities of silver ions into the burn wound without the side effects of a carrier molecule or cream.

MATERIALS AND METHODS

Two silver nylon fabrics were tested. The first was a woven heavy rip-stop (HRS) material (Swift Textile Metalizing Corp., Hartford, CT), weighing 84.6 gm/M² of which 20% (16.9 gm/

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m²) was metallic silver. The second material was a knitted fabric (SN) which weighed 111.7 gm/m² of which 26% (29.1 gm/m²) was metallic silver. The silver nylon fabrics were briefly rinsed in distilled water and except when noted were not subject to further treatment before use.

The in vitro antimicrobial effect of each material (19 cm²) was measured in a liquid medium (50 ml tryptic soy broth at 37°C) which had been inoculated with an 18-hour broth culture of *Staphylococcus aureus* (1 x 10⁶ CFU), *Pseudomonas aeruginosa* (1 x 10⁶ CFU), or *Candida albicans* (3 x 10⁴ CFU). Samples were tested after 7 and 23 hours of incubation. All experiments performed in duplicate. Silver wire (SW) (212 cm of 10-mil) was used in all experiments as the positive control. The number of viable organisms in each sample was quantitated by serial pour-plate assays and recorded as colony forming units (CFU). Simultaneously, aliquots of each sample were removed for the measurement of silver concentration by atomic absorption spectroscopy. With this system, the rate of passive dissociation of silver ions (oligodynamic action) of each material could be measured.

To determine whether the antimicrobial effectiveness of these materials could be increased by augmenting silver ion release electrolytically, the test system was modified as follows. Two 400-ml beakers were connected by a salt bridge and had a DC electrical current passed across the beakers. The test material (SW, HRS, SN) was placed in the anodic (positive) beaker which contained 50 ml of tryptic soy broth. Silver wire immersed in 50 ml of tryptic soy broth was used as the return electrode in the cathodic (negative) beaker. Serial measurements of silver ion concentration and microbial levels were made at 0 and 1 volt, which produced 0 and 22 microamperes of current, respectively. As a negative control, similar measurements were made in the absence of silver materials in the anodic beaker. The current was monitored continuously during this 23-hour period and all experiments were performed in duplicate.

To determine whether the silver ions liberated from the silver nylon fabric could penetrate a semisolid medium, 1-cm squares of HRS fabric were placed in 50-mm plastic petri culture dishes and overlaid with tryptic soy agar to a height of 2 mm. Once the agar had solidified, the plates were seeded with 50 l of undiluted broth of each test organism (5 x 10⁷ to 1 x 10⁹ CFU). After 24 hr of incubation at 37°C, the presence or absence of visible colonies on the agar surface directly above the HRS fabric was recorded. After visually inspecting the agar surface, a swab of one half the surface area above the HRS was taken and immediately subcultured to a second tryptic soy agar culture plate. These plates were incubated at 37°C for 24 hr and read to determine if the silver ion concentration was bactericidal or bacteriostatic.

In vivo experiments were carried out to determine if silver fabric could reduce the level of bacterial colonization of the burn wound. Female Holtzman rats (180 to 220 gm) were subjected to a 20% dorsal scald burn using the method of Walker et al. (9). The animals were divided into a control group, a silver sulfadiazine (AGSD) group, and a test (HRS) group. The control group did not receive any topical agents; the AGSD group had AGSD applied to the eschar daily; and the HRS group had the silver fabric placed directly on the burn wound and changed daily. The number of organisms present in the eschar was measured by quantitating the number of bacteria (CFU) present in three 4-mm punch biopsies. In preliminary experiments, we determined that on the third postburn day, the control animals' eschars had become heavily colonized with bacteria, without any evidence of spontaneous sloughing of the eschar. Thus, we chose day 3 postburn as the test day to evaluate the silver fabric.

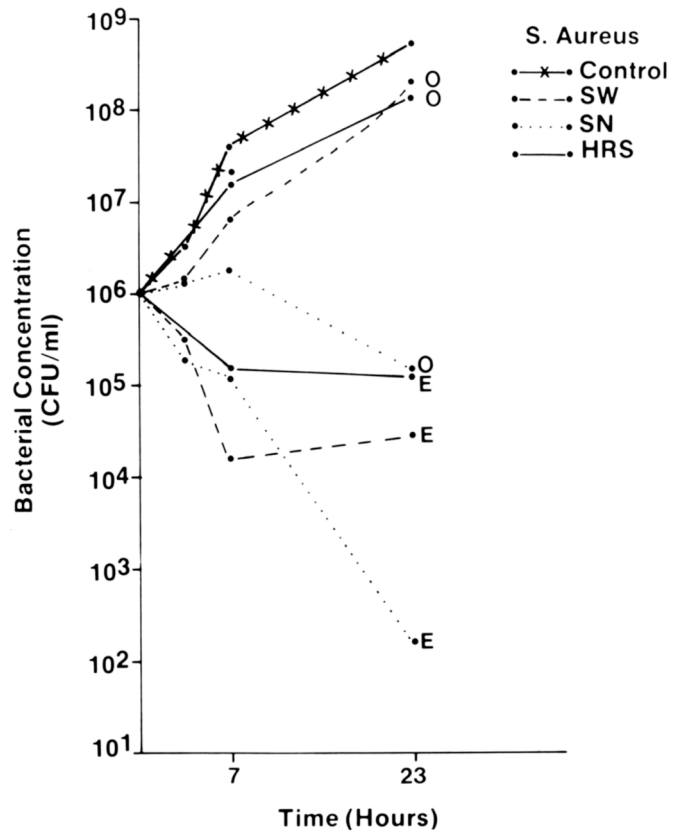


FIG. 1. Antimicrobial effect of SW, SN, and HRS on the growth of *S. aureus* (1 x 10⁶ CFU/ml) in tryptic soy broth after 7 and 23 hours of incubation at 37°C. All materials were tested under oligodynamic (O) and electrolytic (E) conditions.

RESULTS

The effects of the two silver nylon cloths (SN, HRS) and silver wire on the growth of *S. aureus*, *P. aeruginosa*, and *C. albicans* at 37°C under oligodynamic and electrolytic conditions are shown in Figures 1-3. The microbicidal effect of all three materials was enhanced by the administration of a 1-volt DC electrical current. The increased antimicrobial effect of the electrolytically stimulated silver composites appeared to be due to the increased liberation of silver ions from the composites into the media by the electrical current (Table I). Further experiments demonstrated that enough silver was liberated oligodynamically to penetrate a 2-mm agar column and kill an inoculum of *S. aureus*, *P. aeruginosa*, or *C. albicans* (1 x 10⁷ to 1 x 10⁹ CFU) (Fig. 4).

A series of in vivo experiments were performed to test the prophylactic antimicrobial effect of HRS in rats receiving a 20% total body surface area dorsal scald burn. The HRS was as effective as silver sulfadiazine in preventing colonization of the burn wound (Table II), and both HRS and silver sulfadiazine decreased the concentration of bacteria in the burn wound eschar below that of the untreated control animals (p <0.01).

DISCUSSION

Silver in various forms has a long history of use as an antimicrobial agent, however, because of the difficulties

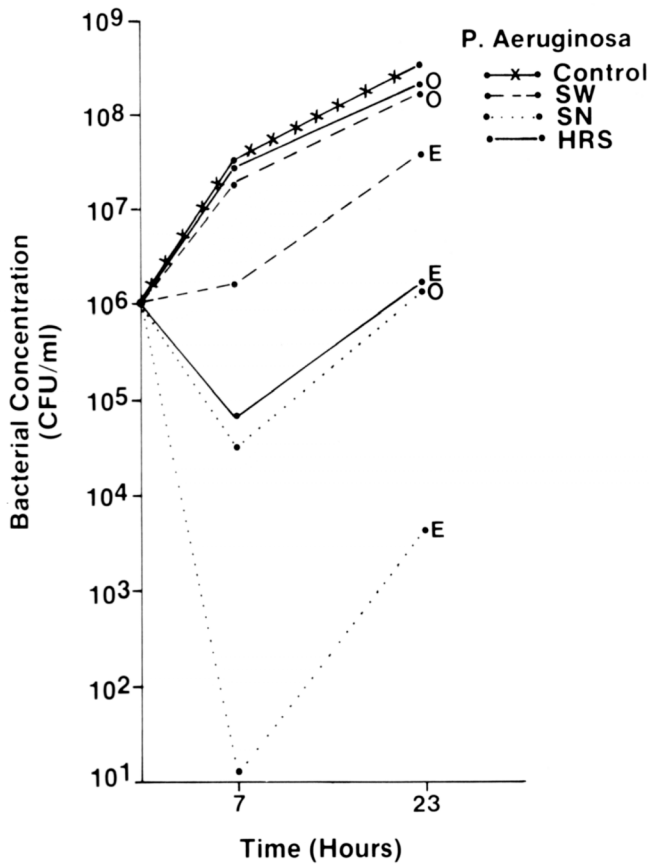


FIG. 2. Antimicrobial effect of SW, SN, and HRS on the growth of *P. aeruginosa* (1×10^6 CFU/ml) in tryptic soy broth after 7 and 23 hours of incubation at 37°C. All materials were tested under oligodynamic (O) and electrolytic (E) conditions.

of achieving consistent and sustained clinically effective local silver ion concentrations, silver has not been widely utilized. However, by improving the delivery system for liberating silver into the wound, such as by combining silver with sulfadiazine, a clinically effective agent has resulted (2). Yet, neither silver sulfadiazine nor the other topical agents have fully controlled or prevented burn wound sepsis. Silver sulfadiazine and silver nitrate frequently fail to prevent burn wound infection from occurring even though microbial resistance to silver is rare (7, 8). Although the bacteriostatic and bactericidal sensitivity of an organism to silver can vary from species to species, these concentrations generally are in the rate of 10 to 20 $\mu\text{g/ml}$ (2). The results of our in vitro experiments document that both silver nylon fabrics, SN and HRS, will spontaneously release silver in an aqueous environment by oligodynamic action. The passage of a weak DC electrical current through these materials increased the antimicrobial action of each material by increasing the rate of silver release. Theoretically, according to Faraday's Law, up to 4 micrograms of silver could be liberated per hour per μa of current flow. This level of current flow is well below the level of cutaneous sensation. Thus within a few hours, microbicidal levels of silver could be liberated into the wound, and these levels could be main-

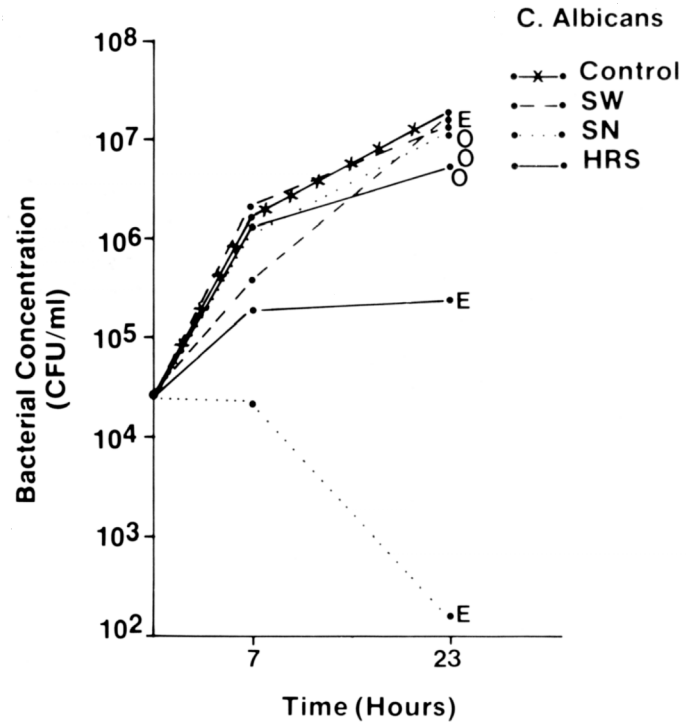


FIG. 3. Antimicrobial effect of SW, SN, and HRS on the growth of *C. albicans* (3×10^4 CFU/ml) in tryptic soy broth after 7 and 23 hours of incubation at 37°C. All materials were tested under oligodynamic (O) and electrolytic (E) conditions.

TABLE I
Silver concentration in $\mu\text{g/ml}$

	Oligodynamic			Electrolytic* at 1 Volt		
	SW	SN	HRS	SW	SN	HRS
4 hr	0	4.4	1.4	4.4	8.3	7.9
7 hr	0	5.8	1.8	7.1	12.1	7.3
23 hr	0	11.5	2.6	21.3	21.3	14.0

*Averages of triplicate determinations

tained almost indefinitely by the continuous passage of an electrical current. This concept of electrolytically releasing silver ions to treat an infection has been used effectively to treat established orthopedic infection (1).

However, just because a material liberated sufficient levels of silver in vitro to theoretically inhibit microbial growth does not mean that it will be effective. Thus, we tested these materials in vitro in both liquid and semi-solid media. In a liquid medium, the liberated silver ions must diffuse throughout the liquid to be effective, while in the semisolid medium, the silver ions must penetrate the medium to inhibit the growth of microorganisms. The silver nylon materials tested were effective antimicrobial agents in both of these test systems. In vitro activity, however, does not guarantee in vivo activity. Thus, we performed a series of in vivo experiments, the first of which was to test the effect of one of these fabrics (HRS) on the prevention of spontaneous colonization of the burn wound. The results of these experiments indicated that HRS appeared to be as effective as silver

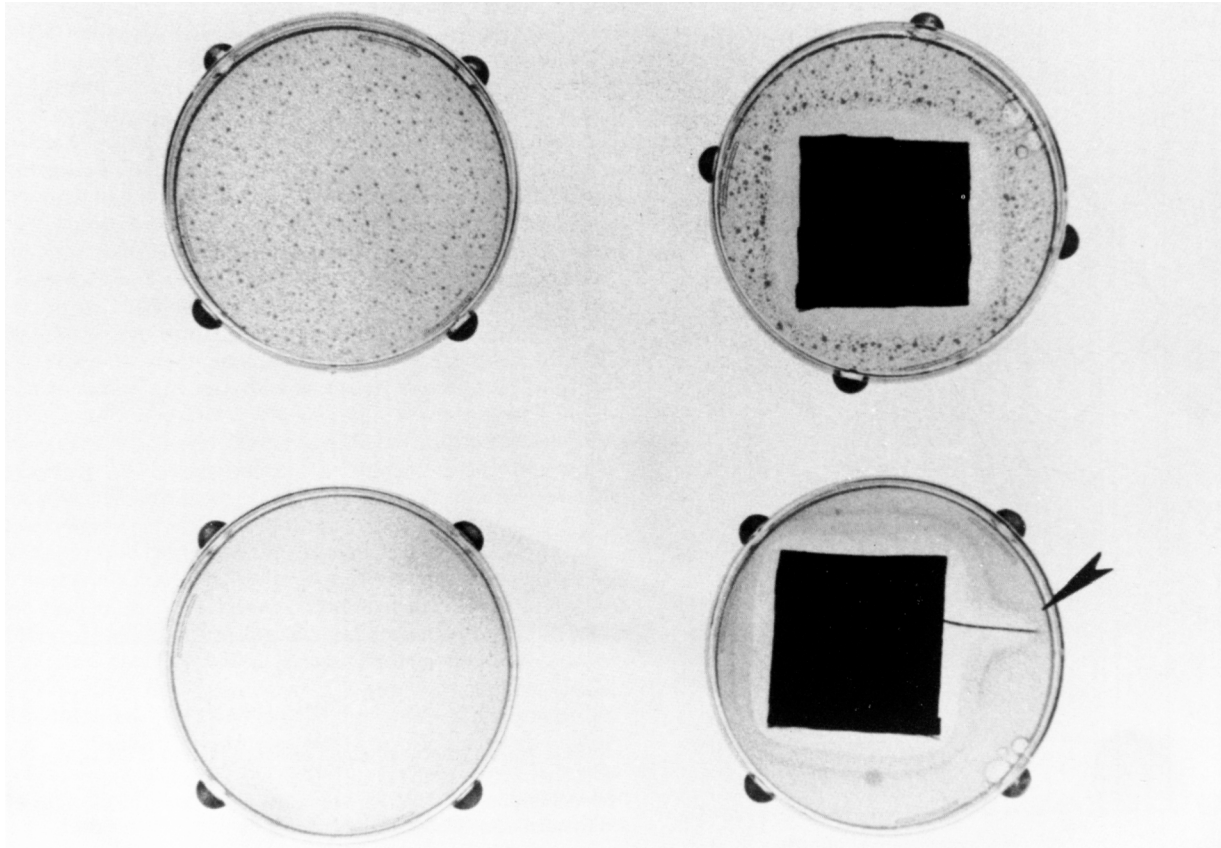


FIG. 4. Antimicrobial effect of HRS on the growth of *S. aureus* (1×10^7 and 1×10^9 CFU/ml) after 24 hours of incubation at 37°C .

TABLE II
Bacterial colonization of eschar

	<i>n</i>	CFU/gm Eschar	
Control	32	$6.8 \pm 8.8 \times 10^5$ *	
Silvadene	23	$8.3 \pm 9.4 \times 10^2$	NS
HRS	14	$1.2 \pm 1.5 \times 10^3$	

* $p < 0.001$ versus Silvadene and $p < 0.01$ versus HRS.

sulfadiazine in preventing burn wound colonization in a rat model. Both HRS and silver sulfadiazine significantly reduced the level of bacteria in the burn eschar compared with control untreated animals ($p < 0.01$).

Silver in the form of a nylon fabric has many features which make it clinically attractive. First, silver is not associated with significant side effects, is rarely associated with the induction of resistant strains of bacteria (6, 7), and is not an allergen. Additionally, since silver ions released from a silver fabric would not be accompanied by a carrier molecule or anion, there would not be any associated potential side effects due to the carrier molecule, such as occurs with both silver nitrate and silver sulfadiazine. We believe a major potential advantage of silver nylon fabrics over currently available silver compounds is the ability of these materials to continuously release silver ions into the wound as long as the material is in contact with the wound. This phenomenon

of continuous and sustained release of silver is clinically very important, since agents that function by the constant, prolonged release of silver, such as sulfadiazine, are more clinically effective in both preventing and treating infections than are agents that release silver rapidly, such as silver nitrate.

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