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Linda Jean Laatsch-Lybeck
Marquette University

Poh Chye Ong
Marquette University

Luther C. Kloth
Marquette University, luther.kloth@marquette.edu

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In Vitro Effects of Two Silver Electrodes on Select Wound Pathogens

Linda J Laatsch, MA, MT(ASCP)SM [1]
Poh Chye Ong, MS [2]
Luther C Kloth, MS, PT [3]

ABSTRACT: The use of electrical current to promote wound healing is well documented. However, little is understood about the effects of micro-amperage direct current (μADC) on growth of wound pathogens. The purpose of this project was to investigate the antibacterial effects of a silver wire/silver nylon electrode carrying μADC in vitro on several Gram positive and Gram negative bacteria. The current was delivered via silver wire and silver nylon electrodes at an amplitude of 100 μA for a 30-minute duration in an in vitro system. Results demonstrated that only silver wire carrying current inhibited bacterial growth around the anode. In contrast, the silver nylon electrode with or without current exhibited antibacterial activity around both the anode and cathode. The results of this study provide convincing evidence that the silver ion (AG^+) is responsible for suppressing bacterial growth. Both silver electrodes were bactericidal with all Gram negative bacteria tested and bacteriostatis with most Gram positive bacteria tested, suggesting that the cell wall composition may be a determining factor in the effectiveness of the AG^+.

INTRODUCTION

There are numerous studies that report antibacterial activity occurring in the presence of silver cations (AG^+) deposited in vivo or in vitro by low levels of direct current.[1-15] Deitch and colleagues[1-3] have shown that *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Candida albicans* can be killed in vitro by silver ions electrolytically liberated (activated) by a silver-nylon fabric electrode. Chu and associates[4] reported that silver nylon when used in vivo on a rat model as a cathode does not show any antibacterial activity. They reported that therapeutic effects were observed only when weak direct currents between 0.4 and 40 μA were delivered through silver nylon electrodes. Colmano et al[5] produced bactericidal effects in vivo on *Staphylococcus aureus* in the femurs of rabbits using 9.0 μA of direct current for 1 hour. Another report by Thibodeau et al[6] also showed inhibition and killing of oral bacteria by anodic silver ions activated with 5.0 μA of direct current applied for 20 minutes. Alvarez and colleagues[7] demonstrated that the healing of partial-thickness wounds inflicted on healthy pigs is enhanced by application of direct current (50 to 300 μA) via a silver-coated electrode.

A number of studies have reported that anodic Ag^+ at a direct current amplitude ranging from 0.4 to 40 μA has inhibitory and fungicidal properties.[8-14] These studies also demonstrated that increasing voltage and current or time had little effect on inhibitory zone size in agar cultures. One limitation of this antibacterial application reported by Spadaro[15] is that the maximum extent of the inhibitory zones with electrically activated silver is generally small (3 to 8 mm in radius). Collectively, these reports suggest that the use of μADC delivered with silver electrodes may be clinically beneficial in killing bacteria in the treatment of infected or contaminated wounds.

Our previous study also demonstrated that bacterial growth inhibition is caused by Ag^+[16] repelled into the culture medium at the anode. We found that silver anode wire electrodes had a bactericidal (killing) effect on the tested Gram negative bacterium *(Pseudomonas aeruginosa)* and a bacteriostatic (inhibitory) effect on the Gram positive bacterium *(Staphylococcus aureus)* tested. Bacteria are designated as either Gram positive or negative depending upon their cell wall composition. The Gram positive cell wall is thick and rigid, with approximately 90% of its weight composed of a unique polysaccharide, peptidoglycan.[17] Conversely, the Gram negative cell wall is flexible and thin, containing primarily lipopolysaccharide and lipoprotein, with only 10% peptidoglycan. In a review of the literature, we found no reports on the possible influence of bacterial cell wall composition on the effectiveness of silver electrodes carrying μADC in vitro.

Additionally, we found no reports comparing silver wire electrodes with other types of silver electrodes, such as silver nylon. Therefore, the purpose of this project was to investigate how various Gram positive and Gram negative bacteria that typically infect chronic human wounds are affected by Ag^+ that is electrically released by either silver wire or silver nylon electrodes carrying μADC in vitro.

METHODS

The μADC device used in this study was the TS 2100 Therapeutic Stimulator.* This device, when used in the DC mode, delivers constant current ranging from 100 to 5,000 μA. Sterile, pure silver wire† electrodes were positioned as shown in Figure 1. The positive electrode was configured into a rectangle having dimensions of 1.5 cm x 2.5 cm, and the negative electrode was simply a straight wire, 1.5 cm in length. For testing silver nylon electrodes (fabric style A-2589-5§), the anode was shaped into a 3 cm x 2 cm rectangle, and the cathode was a thin strip of fabric, 1.8 cm in length and 0.6 cm wide (see Figure 2). The silver nylon electrode also had an external tail for attachment to the TS 2100 electrical stimulator. For both electrode types, the anode dimensions were larger than the cathode dimensions to more evenly distribute current density at the anode.

The Gram positive and Gram negative bacteria chosen for this experiment are listed in Table 1. The Gram positive cocci *(Staphylococcus and Streptococcus)* were chosen because of their possible

[1] Medical Laboratory Technology Program, Marquette University, Milwaukee, WI 53233.
[2] Mr Ong was a student in the Biomedical Engineering Department at Marquette University, Milwaukee, Wis, when this study was completed in partial fulfillment of the requirement for his master's degree.
[3] Program in Physical Therapy, Marquette University, Walter Schroeder Health Sciences and Education Complex, Milwaukee, WI 53233. Address all correspondence to Mr Kloth.

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* Myo Kinetic Systems Inc, N84W13562 Leon Rd, Menomonee Falls, WI 53051.
† Cooner Wire, 9265 Owensmouth, Chatsworth, CA 91311.
§ Swift Textile Metalizing Corporation, Hartford, CT.

FIGURE 1. Schematic of experimental set-up for silver wire electrodes.

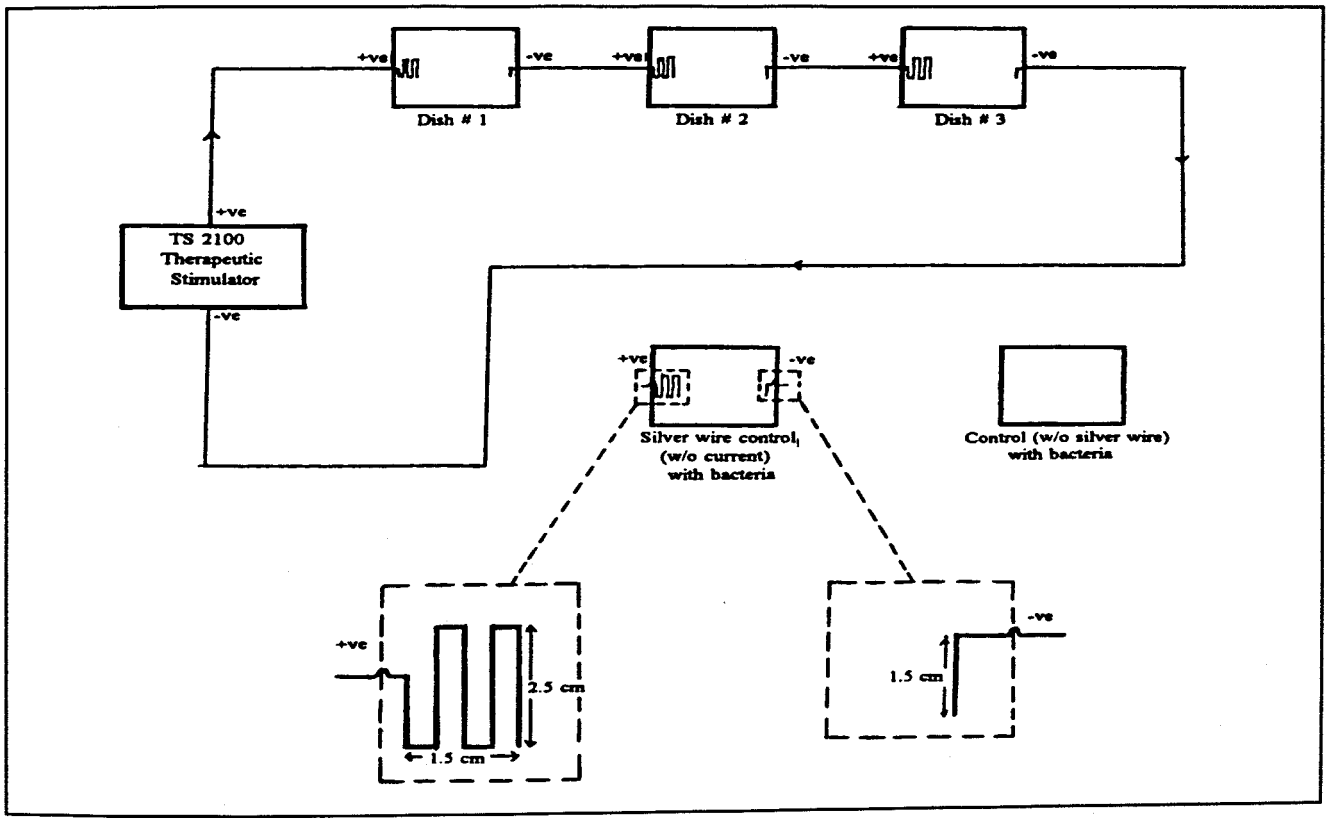
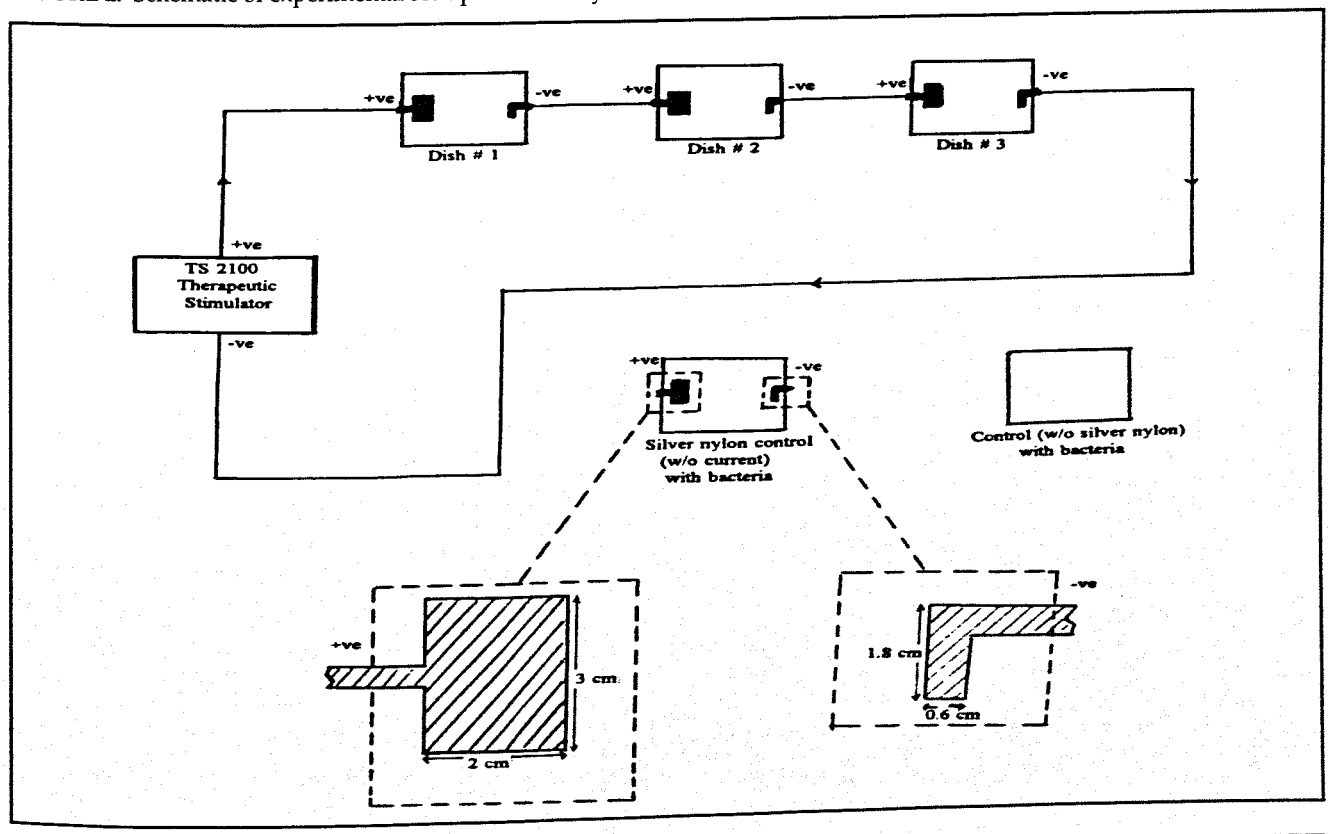


FIGURE 2. Schematic of experimental set-up for silver nylon electrodes.



association with chronic, infected wounds. The *Corynebacterium*, although not involved in infected wounds, enabled testing of a Gram positive bacterium that was a bacillus rather than a coccus. Similarly, the testing of *Neisseria sicca*, a nonpathogenic Gram negative coccus, provided a comparison with the pathogenic Gram negative bacilli. *Candida albicans*, a fungus and opportunistic pathogen, was also tested for comparison to the bacteria. It has a rigid, thick cell wall composed of 80% to 90% polysaccharides, predominantly chitin.[18]

All microorganisms were grown for 18 to 24 hours at 37°C in tryptic soy broth.* Each suspension was diluted to a final concentration of 1×10^7 colony-forming units per mL. Molten tryptic soy agar of sufficient quantity to cover the electrodes was inoculated with the organism, poured into a sterile petri dish, and allowed to harden.

For test runs, silver wire or silver nylon electrodes in the dishes were connected in series by alligator-clip leads to the TS 2100 Therapeutic Stimulator. Each microorganism was tested in three separate culture dishes. Current of 100 μA was applied to the test organisms for 30 minutes duration by passing current through the culture medium. We chose to use 100 μA because our previous study showed no significant difference in bacterial growth inhibition when using currents ranging from 26 to 800 μA.[16] Additionally, 100 μA is the lowest constant current available on the TS 2100 stimulator device.

After incubation at 37°C for 18 to 24 hours, each culture was observed for clear zones of growth inhibition surrounding the anode and cathode. The inhibition (clear) zone surrounding the silver wire/silver nylon electrode was noted and photographs were taken of observed inhibition zones (Figures 3-5). Subcultures from the zones of inhibition were checked for sterility by transferring the medium with a sterile scalpel into tubes of sterile tryptic soy broth and incubating them at 37°C for 18 to 24 hours. Visible turbidity was used as an indication of growth.

Control plates (seeded plates with and without silver wire/silver nylon electrodes) that were not exposed to electrical current were incubated at 37°C for 18 to 24 hours. This was done to determine whether the electrodes themselves would have an antibacterial effect.

* BBL Microbiology Systems, PO Box 243, Cockeysville, MD 21030.

TABLE 1. Microorganisms tested with silver wire and silver nylon electrodes.

Microorganism	Source	Gram Stain
Staphylococcus aureus	ATCC* 25923	Gram positive cocci
Staphylococcus aureus, β-lactamase positive	Clinical Isolate**	Gram positive cocci
Staphylococcus aureus, Methicillin resistant	Clinical Isolate	Gram positive cocci
Coagulase Negative Staphylococcus	Clinical Isolate	Gram positive cocci
β-Streptococcus Group A	Clinical Isolate	Gram positive cocci
Corynebacterium species (diphtheroids)	Clinical Isolate	Gram positive bacilli
Escherichia coli	ATCC 25922	Gram negative bacilli
Enterobacter aerogenes	ATCC 13048	Gram negative bacilli
Proteus vulgaris	ATCC 13315	Gram negative bacilli
Pseudomonas aeruginosa	ATCC 27853	Gram negative bacilli
Acinetobacter calcoaceticus	ATCC 19606	Gram negative bacilli
Neisseria sicca	Clinical Isolate	Gram negative cocci
Candida albicans (fungus)	Clinical Isolate	Yeast cell

* ATCC: American Type Culture Collection.

** Clinical Isolate: Microorganism isolated from human specimen.

RESULTS

Table 2 shows that the anodic silver wire electrode carrying μADC produced growth inhibition zones, whereas wire without current (control) had no effect on growth. In contrast, the silver nylon electrode with or without μADC produced growth inhibition around both the anode and cathode for all microorganisms except *P vulgaris*, which was not inhibited at the cathode. It was interesting to note that for all microorganisms tested a smaller zone of inhibition was found at the cathode in the silver nylon test group (exposed to current) compared with the silver nylon control group (not exposed to current).

Table 3 shows that silver wire carrying μADC was found to be bacteriostatic with most Gram positive bacteria (except *β–Steptococcus Group A*), bactericidal with all Gram negative bacteria tested, and bacteriostatic with the fungus *C albicans*. The silver nylon electrode with or without μADC produced the same results except that it had a bactericidal effect on *Corynebacterium*, a Gram positive bacillus.

DISCUSSION

Our results showing that silver wire had an antimicrobial effect at the anode only when μADC was passed through it agree with the findings of our previous study.[16] This effect has been associated with the presence of silver ions activated or repelled away from the silver wire into the culture medium by anodal μADC.[1-15] We did not quantify the inhibition zone size because in a previous pilot study we found these areas to be proportional to the area of the electrode used. The limiting factor on zone size appears to be the distance traveled by silver ions through the medium, a conclusion substantiated by the findings of Berger et al[11] and Marino et al.[15] For human applications, the treatment electrode (anode) dimensions should be large enough to provide sufficient inhibition of wound pathogens without causing tissue damage.

When silver nylon was used as the electrode, the zones of inhibition were consistently located at both the anode and cathode. The presence of an inhibition zone at the cathode does not agree with Chu et al,[4] who reported that a silver nylon dressing does not show any therapeutic effect when used as a cathode in an in vivo system. The presence of an inhibition zone at both the anode and cathode may be accounted for by the pre-existence of Ag^+ as silver nylon is simply a nylon fabric precoated with metallic silver.

TABLE 2. Summary of inhibition zones produced by silver wire and silver nylon electrodes.

Microorganism	Inhibition Zone Silver Wire		Inhibition Zone Silver Wire (control)		Inhibition Zone Silver Nylon		Inhibition Zone Silver Nylon (control)	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Gram positive bacteria								
Staphylococcus aureus	++++	-	-	-	++++	+++	++++	++++
Staphylococcus aureus, β-lactamase positive	++++	-	-	-	++++	+++	++++	++++
Staphylococcus aureus, Methicillin resistant	++++	-	-	-	++++	+++	++++	++++
Coagulase Negative Staphylococcus	++++	-	-	-	++++	+++	++++	++++
β-Streptococcus Group A	++++	-	-	-	++++	+++	++++	++++
Corynebacterium species (diphtheroids)	++++	-	-	-	++++	+++	++++	++++
Gram negative bacteria								
Escherichia coli	++++	-	-	-	++++	+	++++	++++
Enterobacter aerogenes	++++	-	-	-	++++	+	++++	++++
Proteus vulgaris	++++	-	-	-	++++	-	++++	++++
Pseudomonas aeruginosa	++++	-	-	-	++++	+++	++++	++++
Acinetobacter calcoaceticus	++++	-	-	-	++++	+++	++++	++++
Neisseria sicca	++++	-	-	-	++++	+++	++++	+++
Yeast								
Candida albicans	++++	-	-	-	++++	+++	++++	++++

Pos: Positive terminal

Neg: Negative terminal

Control: Silver wire/nylon without current, with microorganism

-: No effect
+: Slight effect
+++: Moderate effect
++++: Strong effect

FIGURE 3: Silver wire electrode; with inhibition around the anode.

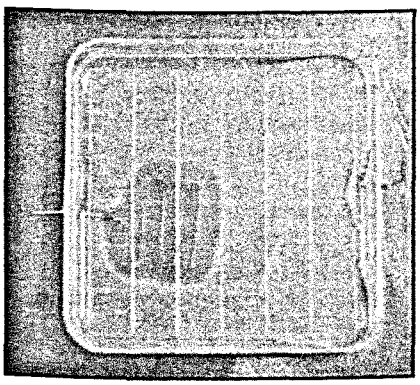


FIGURE 4: Silver nylon electrode; with inhibition around the anode and the cathode.

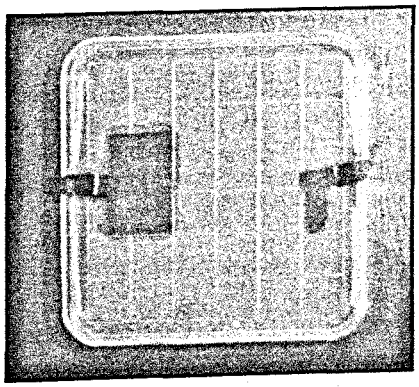
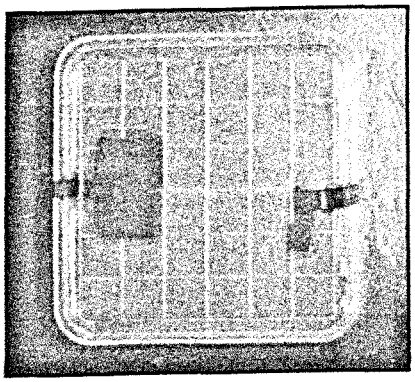


FIGURE 5: Silver nylon electrode (control); with inhibition around the anode and the cathode.



For silver nylon electrodes, the inhibition zone size was observed to be smaller at the cathode in the test group (with current) when compared with the control group (without current). This provides strong evidence for the presence of Ag^+ on the surface of the fabric. The reduction in zone size at the cathode may be explained in terms of polarity. Because like charges repel, Ag^+ on the fabric surface were repelled by the positive polarity electrode into the surrounding culture media around the anode, resulting in bacterial growth inhibition. At the cathode, Ag^+ were attracted by the negative polarity, making them less available to the surrounding bacterial culture, thereby reducing the inhibition zone size.

Whereas the size of the zone of inhibition was observed to be consistently large for all microorganisms on the silver nylon control plates (without current), this was not the case for all microorganisms on the test plates (with current). Some Gram negative bacilli *(E coli, E aerogenes, P vulgaris)* showed little or no inhibition at the cathode after exposure to μADC. The reason for this is unknown, and further testing is needed to study this phenomenon.

Silver wire carrying μADC was found to be bactericidal for all Gram negative bacteria tested and bacteriostatic for all Gram positive bacteria tested except for *β–Streptococcus Group A*. These results support our speculation that cell wall structure may determine the ability of Ag^+ to penetrate into the bacterial cell. Perhaps Ag^+ can penetrate the thin cell wall of Gram negative bacteria and bind to cellular components more easily than they can penetrate the thicker cell wall of Gram positive bacteria. The bactericidal effects on *β–Streptococcus Group A* present the only contradiction to this theory. Perhaps *β–Streptococcus Group A* was more easily killed by the Ag^+ because of the increased amount of protein that can be found in streptococcal cell walls compared with other Gram positive cell walls.[19] Or, they may have been more susceptible to killing because their growth may be poor in culture media that have not been enriched with blood or tissue fluids.[20]

The silver nylon electrode carrying μADC was found to be bactericidal with all Gram negative bacteria tested and bacteriostatic with most Gram positive bacteria except for *β–Streptococcus Group A* and *Corynebacterium* species. These findings echo those with silver wire electrodes except for the *Corynebacterium* species, which was bacteriostatic with silver wire. Some *Corynebacterium* species grow slowly on unenriched media,[21] which, along with the "high concentration" of Ag^+ in the silver nylon electrode, could account for the bactericidal result found.

TABLE 3. Summary of antibacterial effects produced by silver wire and silver nylon electrodes.

Microorganism	Antibacterial Effects Silver wire	Antibacterial Effects Silver Nylon
Gram positive bacteria		
Staphylococcus aureus	Bacteriostatic	Bacteriostatic
Staphylococcus aureus, β-lactamase positive	Bacteriostatic	Bacteriostatic
Staphylococcus aureus, Methicillin resistant	Bacteriostatic	Bacteriostatic
Coagulase Negative Staphylococcus	Bacteriostatic	Bacteriostatic
β-Streptococcus Group A	Bactericidal	Bactericidal
Corynebacterium species (diphtheroids)	Bacteriostatic	Bactericidal
Gram negative bacteria		
Escherichia coli	Bactericidal	Bactericidal
Enterobacter aerogenes	Bactericidal	Bactericidal
Proteus vulgaris	Bactericidal	Bactericidal
Pseudomonas aeruginosa	Bactericidal	Bactericidal
Acinetobacter calcoaceticus	Bactericidal	Bactericidal
Neisseria sicca	Bactericidal	Bactericidal
Yeast		
Candida albicans	Bacteriostatic	Bacteriostatic

Both silver wire and silver nylon electrodes carrying μADC had a bacteriostatic effect on *Candida albicans*. This yeast has a thick polysaccharide cell wall which supports our theory that the cell wall thickness/composition may affect the entry of Ag^+ into the microbial cell. Further testing needs to be performed on other fungi to verify this theory.

CONCLUSION

Based upon observations made in this investigation, we agree with other studies that only anodal silver wire carrying μADC repels Ag^+ into the medium culture, resulting in actual growth inhibition. On the other hand, silver nylon with or without μADC produced growth inhibition around both the anode and cathode. These observations provide evidence that the Ag^+ are responsible for the antibacterial effect. Clinically, these findings suggest that the use of the silver nylon fabric alone or silver wire with μADC may suppress bacterial growth in infected chronic wounds, thereby enhancing the wound healing process.

Furthermore, the type and structure of pathogens present in human wounds may influence the effect of the Ag^+. In our study, Ag^+ were generally bacteriostatic with Gram positive bacteria, and bactericidal with Gram negative bacteria. Additional research is needed to study the role of the microbial cell wall in limiting Ag^+ penetration and to verify whether these effects produced in vitro can be reproduced in vivo.

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