Laboratory Study

Antibacterial effects of electrically activated vertebral implants

Kutsal Devrim Secinti [a], Murat Ayten [a], Gokmen Kahilogullari [a,*], Gulsah Kaygusuz [b],
Hasan Caglar Ugur [a], Ayhan Attar [a]

[a] *Department of Neurosurgery, Ankara University School of Medicine, Ankara, Turkey*
[b] *Department of Pathology, Ankara University School of Medicine, Ankara, Turkey*

Abstract

Bio-implants in the human body act as passive surfaces that are prone to bacterial adhesion potentially leading to deep body infections. Pedicle screws made of uncoated or silver-coated titanium alloy were used both *in vitro* and *in vivo* to determine whether silver-coated materials have antimicrobial properties when they are anodized. Twenty-four New Zealand Albino rabbits were divided into four groups with six in each. In Group 1, the rabbits were exposed to 8 μA direct current (DC) via silver-coated screws. In Group 2, the rabbits were not exposed to any electrical current, but silver-coated screws were used. In Group 3, the rabbits were exposed to 8 μA DC using uncoated screws. In Group 4, the rabbits were not exposed to any electrical current, but uncoated screws were used. *Staphylococcus aureus* (106 cfu) was inoculated into the rabbits before any electrical current was applied. All the animals were killed, and the areas surrounding the screws were histologically and microbiologically examined. Silver-coated titanium screws prevented implant-associated deep bone infections when they were polarized anodically. The antibacterial effects of the same screws with the same bacterium were confirmed in *in vitro* experiments on agar plates. When the screws were anodized with the same electrical parameters *in vitro*, a marked inhibition zone was detected around the silver-coated screws but not around the uncoated screws. Our findings suggest that silver-coated titanium implants can be used to prevent implant-associated deep bone infections when they are polarized anodically.
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1. Introduction

Silver ions have been known to have antibacterial properties since the time of Avicenna. However, there is no evidence to date that silver has an essential function in plant or animal metabolism.[1]

Inhibition of bacterial growth in response to weak electrical currents has been reported.[2–7] If a pure (>99.9%) silver wire is connected to a weak direct current (0.1–10 μA) in a semi-solid culture medium, a bacteria-free inhibition zone appears near the anode after an incubation period, but not near the cathode. This antibacterial effect persists for at least 3 weeks after the electric current is cut off and sustains for at least 3 weeks.[8] This procedure is called silver anode treatment. In the relevant literature, electrically induced silver ions have been shown to have antifungal, antibacterial and antiviral properties, and have been found to be non-allergenic and nontoxic to mammalian cells.[6,9]

Inhibitory concentrations of electrically induced silver ions are approximately 100 times lower than they are for silver sulfadiazine.[6] This shows that electrical polarization confers extensive bactericidal specificity on silver. No other metal has as strong an antimicrobial effect as anodized silver. The specificity of silver suggests that electricity is not the key factor in silver anode antisepsis. The mechanism of anodic silver antisepsis may originate from either free silver ions scattering from the anode surface to the medium or from the electrical current. It is known that the antibacterial effect does not occur via silver iontophoresis.[6]

* Corresponding author. Present address: Ankara Universitesi Ibni Sina Hastanesi, Beyin ve Sinir Cerrahisi Anabilim Dalı, 06100 Sihhiye, Ankara, Turkey. Tel.: +90 312 3103333x2598; fax: +90 312 3094340.
E-mail address: gokmenkahil@hotmail.com (G. Kahilogullari).

In contrast, when pure silver metal is cathodized, it gains osteoblastic activity. Because of its specificity, silver has been used for the treatment of osteomyelitis and infected non-unions.[10,11] In this approach, silver electrodes are anodized for a few days using a small electrical current to obtain an antibacterial effect, and then the poles are reversed to establish fusion of the ununited fracture.[11,12] Thus, non-union can be successfully treated, with a low infection rate.

In neurosurgery, the proportion of spinal implants that become infected is not negligible. To the best of our knowledge, there is no information in the literature regarding whether electrical stimulation of silver-coated vertebral implants is effective in preventing implant-associated infections. Therefore, in this study we aimed to investigate the *in vivo* antibacterial effect of anodized silver-coated vertebral implants in order to establish a basis for future clinical applications. Additionally, we confirmed the antibacterial effect of silver-coated titanium alloy anodes *in vitro* using the same test bacterium.

2. Materials and methods

2.1. Screws

Ninety-six standard titanium (Ti) alloy bone screws (3 mm diameter, 20 mm length; M 36340; Trimed, Istanbul, Turkey) were obtained. The surface area of the part of each screw inserted into the animal was estimated to be 1.84 cm^2.

Forty-eight of the 96 screws were coated with silver by electroplating (LPW Ag, Spiegelglanz H137 T; LPW Chemie, Neuss, Germany). The thickness of the silver layer of the coated (Ag-Ti) screws was 5–8 μm. The change in surface area caused by silver coating was negligible. The durability of the coated screws was tested by inserting some of the screws into fresh-frozen cadaveric sheep vertebrae. The same screws were then tested in agar media *in vitro*, as described below, to confirm that the coating could withstand insertion.

2.2. Bacteria

Coagulase-positive *Staphylococcus aureus* was provided from the stock solutions of the Department of Infectious Diseases of the School of Medicine at Ankara University, Ankara, Turkey. The bacteria were kept in a 5-mL broth medium at 4 °C. Freshly cultured bacterial cells obtained from stock solutions were used during the experiment.

2.3. Animals

Twenty-four New Zealand rabbits (10 weeks old; weight range: 2000–2500 g; mean weight: 2250 g) were randomly divided into four groups of six rabbits (Table 1). The study was approved by the ethics committee of Ankara University in accordance with the Helsinki Declaration of Animal Rights.

Group 1 was exposed to 8 μA direct current (DC) via silver-coated screws. Group 2 was not exposed to any electrical current, but silver-coated screws were used. Group 3 was exposed to 8 μA DC via titanium alloy screws. Group 4 was not exposed to any electrical current, but titanium alloy screws were used.

The animals were anesthetized with ketamine hydrochloride (Pfizer, New York, NY, USA) and 2% xylazine hydrochloride (Bayer, Mannheim, Germany). The animals' lower backs were shaved and surgically scrubbed. Then, a 4-cm skin incision was made on the midline starting from the level of the iliac crest. After bilateral exposure of the iliac bones, two burr holes (diameter: 2.0 mm) were drilled into each iliac bone (FF055R, FF068R; Aesculap, Tuttlingen, Germany). The distance between the two holes on each bone was approximately 15 mm. Each hole was irrigated with 3 mL of sterile saline. Cotton balls in 0.1 mL of bacteria solution (10^6 colony forming units [CFU]) were inserted into each hole, and then removed. All the screws were then tightly inserted into the holes. Well-insulated (Teflon-coated) titanium alloy wires were soldered to the tip of each screw. After the wound was closed with surgical sutures, the skin was covered with sterile dressings moistened with 4 mL of benzalconiumhydrochloride (0.3% w/v). The same researcher operated upon all the animals in order to standardize screw placement.

Plain X-rays of all the animals were obtained immediately after the procedure to determine the positions of the screws (Fig. 1). The free ends of the wires were kept outside the wound; all the screws together represented one electrode (anode). The upper back skin of the animals was shaved, and 10 mm of a pure silver wire (0.5 mm diameter)

Table 1
Animal experimental groups, screw types inserted and electrical parameters

Animals	Screw			Actual charge (μA)	Exposure time (min)	Total charge delivered (C)	Charge density (C/cm^2)
	Ag-Ti	Ti	Total				
Group 1 ($n = 6$)	24	0	24	8	360	0.17	0.015
Group 2 ($n = 6$)	24	0	24	0	0	0	0
Group 3 ($n = 6$)	0	24	24	8	360	0.17	0.015
Group 4 ($n = 6$)	0	24	24	0	0	0	0
Total ($n = 24$)	48	48	48				

Only animals in Groups 1 and 2 were exposed to electrical current (2 h/day).
Ti, titanium; Ag-Ti, silver-coated titanium.

Fig. 1. Plain X-ray showing the post-operative locations of the screws.

was inserted under the skin. Then, the wire was sutured to the skin in order to provide a good electrical contact. This silver wire served as a cathode ($\sim$0.3 cm^2). At this stage, tissue impedance between the screws (anode) and silver wire (cathode) was measured using the two-terminal method, and the mean value was calculated. All the animals in Groups 1 and 3 were exposed to 8 µA DC (Table 1) produced by a battery-driven (1.55 V, 317-SRS16SW; Sony Energytec, Fukushima, Japan) DC source for 2 h/day for 3 days. The screws were anodized and the pure silver wires were cathodized. In order to adjust the current passing through the tissue, appropriate resistors were connected to the DC sources. The DC source, cables, and cathode were tightly bandaged on to the backs of the animals to avoid poor electrical contact when the animal moved.

At the end of the third day, the electrical current was switched off. All animals were killed on the 10th day by injection of thiopental sodium via the auricular vein. The screws were removed under sterile conditions.

2.4. Microbiological examination

The burr-hole cavity was irrigated with 10 mL sterile saline and samples of this saline (0.1 mL) were inoculated into freshly prepared Mueller-Hinton agar. All the microbiological samples were incubated at 37 °C for 2 days. Bacterial colonies were counted for each screw and recorded as cfu. The purity of each culture was evaluated using a coagulase test and Gram staining of randomly chosen colonies on the culture plates. Gram-positive, mass-forming and coagulase-positive cocci were evaluated as *S. aureus*. No further identification procedure was needed if no contamination was detected or suspected.

2.5. Histological examination

In order to examine each burr hole separately, each iliac bone was divided into two pieces, with each bone piece containing only one burr hole. Each bone specimen was

immediately fixed in 10% neutral buffered formalin and decalcified in 20% formic acid. Paraffin-embedded 5-µm serial tissue sections were stained with haematoxylin-eosin and examined under a light microscope (Courtesy; Zeiss, Oberkochen, Germany). Histological evaluation was performed by two double-blinded pathologists (Fig. 2a–d). The degree of inflammation was semiquantitatively scored as none, weak, moderate or severe, depending on the intensity of the neutrophil leukocytic infiltration and the presence and extent of necrosis, and devitalized bone tissue.

Bone marrow cellularity, bone tissue inflammation, multifocal exudate accumulation the presence of devitalized bone, necrotic changes in the bone marrow, new bone formation, and inflammatory cell counts were recorded for each screw as indicators of inflammation.

2.6. In vitro confirmation

Sterilized Ag-Ti and Ti electrodes were embedded into four Petri dishes (the first and second Petri dishes had a pair of silver-coated Ti alloy electrodes; the third and fourth had a pair of uncoated titanium alloy electrodes). After electrical connections were established, 5 mL of solution containing 1.3×10^6 cfu of *S. aureus* was poured into 50 mL of cooling agar medium. The temperature of the agar was 40 °C. Then, the agar was vortexed and poured into the Petri dishes, as previously described by Spadaro et al.[4] In the present experiment, the same bacteria and same materials (i.e. the electrodes) were used to confirm *in vitro* anodic silver antisepsis. The electrodes were polarized using the same electrical regimen (8 µA for 2 h/day for 3 days) as used for the in vivo experiment. Agar impedance was 16 kΩ ($\pm$1.8 kΩ); total charge was 1.2 C. The distance between the electrodes was 4 cm. Finally, the inhibition zone radius was measured, and the Petri dishes were photographed.

2.7. Statistical analysis

The data were analyzed statistically using the Kruskal-Wallis variant analysis test.[13] Comparisons of the parameters for each group were performed using the multiple comparison test. A p value of < 0.05 was considered statistically significant.

3. Results

3.1. In vivo experiment

The surgical procedure was well tolerated by all the animals. Tissue impedance between the inner surface of the iliac bone and the shaved skin was 75 kΩ (SD: $\pm$8.4 kΩ; median: 74.4 kΩ, $n = 24$). Current levels tended to decline with time in each session.

The mean bacterial count in Group 1 was 2.4 cfu, mean inflammatory cell count was 3.0, and the bone marrow cellularity was normal. The mean bacterial count in Group 2
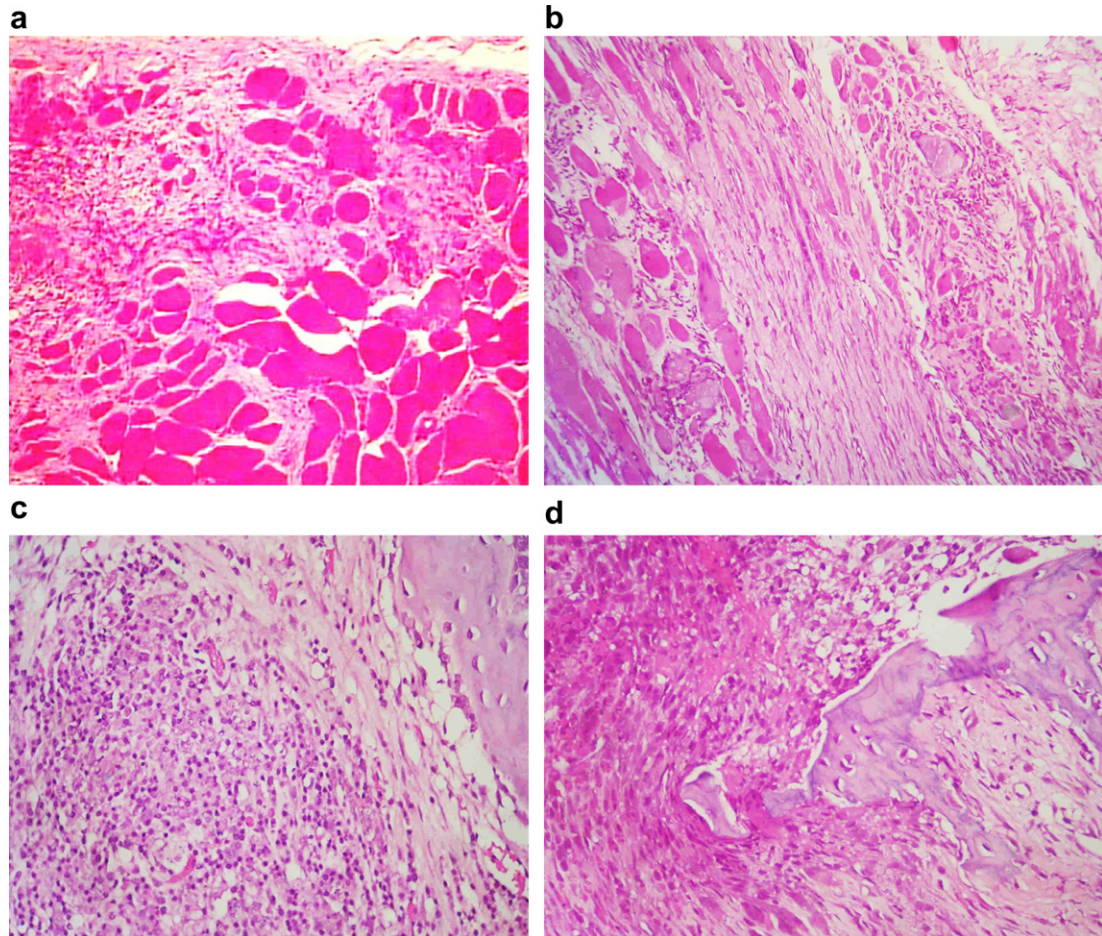
Fig. 2. Sections of tissue from around the electrodes. (a) Mild tissue inflammation around the silver coated anode (Group 1). (b) Mild inflammatory reaction and fibrosis in the soft tissue around the silver, sham group (Group 2). (c) Severe inflammation characterized by multifocal micro-abscesses around the titanium anode (Group 3). (d) Severe acute inflammation and devitalized bone tissue around the titanium screw, sham group (Group 4) (haematoxylin and eosin; $\times 400$).

was 76.0 cfu, the mean inflammatory cell count was 3.0, the bone marrow cellularity was normal, and there was minimal bone tissue inflammation. The mean bacterial count in Group 3 was 161.5 cfu, the mean inflammatory cell count was 10.29, significantly increased bone marrow cellularity and severe bone tissue inflammation were observed, and necrotic changes in the bone marrow were noticed for two screws. The mean bacterial count in Group 4 was 248.2 cfu, the mean inflammatory cell count was 13.0, there was significantly increased bone marrow cellularity, and severe bone tissue inflammation and exudate formation were observed.

There was no devitalized bone tissue or new bone formation in any of the groups. Necrotic change in the bone marrow was seen in only two screws in Group 3.

Using multiple comparison tests, the results of each group were compared with the results of the other groups for bacterial count and inflammatory cell count (those with a bacterial count over 300 were considered to have a count of 300).

There was no difference between Groups 1 and 2 with respect to mean inflammatory cell count ($p > 0.05$). Howe-ver, for all other paired comparisons, a significant difference was detected ($p < 0.001$). The bacterial count of Group 4 was the highest, and that of Group 1 was the lowest. There were significant differences between the mean bacterial counts of all groups ($p < 0.001$).

3.2. In vitro experiment

On Petri dishes, the Ag-Ti anodes (screws) but not the uncoated anodes had 23 mm of inhibition zones (Fig. 3). Microbiological samples obtained from the inhibition zones did not show bacterial growth. No bacterial growth was detected beneath Ti electrodes or on the electrode surface.

4. Discussion

The idea of inserting silver electrodes into living tissue is not new in orthopedics and dentistry. Becker et al.[12] trea-ted infected non-unions with silver anodes. Similarly, Aydın et al.[3] treated infected teeth with inserted silver anodes. In such cases, actual current passes through the
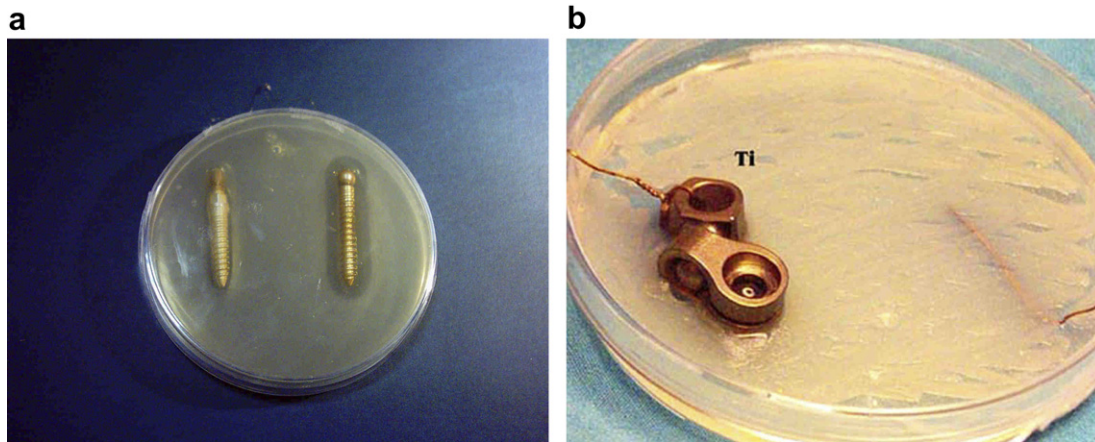
Fig. 3. *In vitro* inhibition experiments (a) Silver-coated titanium cathode (left) and anode (right); note the inhibition zone near the anode but not near the cathode. (b) Uncoated titanium anode; note the lack of any inhibition zone around the electrodes.

tissue and the total charge is based on tissue changes. In the literature, the threshold for living eucaryotic cells is given as 20 μA for actual current and 2 C/day for total charge.[2] In the present study, these electrical parameters were maintained to avoid irreversible tissue damage. However, in six cases the peri-implant tissue developed severe inflammation due to the electrical current used. However, the same electrical parameters caused a lesser degree of inflammation when silver anodes were used, which shows that silver ions emitted from the anode surface are more easily tolerated than are Ti ions. Comparison of the inflammatory cell counts of Groups 1 (Ag-Ti) and 3 (Ti) shows that, anodized silver causes less inflammation in living tissue. In the control groups (Group 2 *vs.* 4), Ag-Ti screws were associated with a lesser degree of inflammation; however, Ti screws were associated with a moderate degree of inflammation. Darouiche found no differences between silver-coated and uncoated metal and other implants, such as silver-impregnated silicon catheters or silver-coated external fixation pins.[14] However, in our study, there were significant differences.

Our microbiological results support the use of silver material. Silver is known to have antibacterial effects (oligodynamic effect). When anodized, silver ions emitted from the metal surface penetrate deeply into the bone tissue. This may be the reason for the lesser degree of bacterial growth in the tissue surrounding the silver-coated screws. In this study, we did not measure the silver ion concentration in deep bone tissue; however, data in the literature show that silver ions are capable of penetrating into bone structures as deep as 1 cm[12] or 1.38 mm in dental tissues.[3] The penetration depth of silver is correlated with the electrical current applied, while the antibacterial effect of a silver anode is mostly independent of electricity. In a liquid medium, even a 1 μA current was sufficient for a silver anode to inhibit bacteria.[8] If the electrical current had directly influenced the bacteria, we would have detected a lesser degree of bacterial growth when anodes were charged with higher electric current. However, there was significant difference in the microbicidal properties of the silver and titanium anodes.

Bacterial biofilm formation on the surface of implanted metal objects is a major clinical problem.[15,16] Bio-implants in the human body act as passive surfaces that are prone to bacterial adhesion and, thus, may cause implant-associated infections (peri-implantitis).[17] Antibiotic treatment of infected implants is not satisfactory because the biofilm architecture protects the adhering organisms. The minimal inhibitory concentration of antibiotic needed to inhibit free-floating bacteria has been reported to be approximately 50 or 500 times lower than that required for bacteria in a biofilm.[15] Infections associated with foreign materials are expensive to manage. For example, findings from the Dutch Trauma Trial indicated a mean cost of US$22 000 (in 1991) to treat a patient who developed a deep tissue infection after surgical fixation of a closed fracture.[18] Post-operative surgical site infections remain a major source of illness but a less frequent cause of death in the surgical patient.[19] These infections number approximately 500 000/year, among an estimated 27 million surgical procedures,[20] and account for approximately one-quarter of the estimated 2 million nosocomial infections in the US each year.[21] Major complications such as deep body infections continue to have a grave impact, increasing the duration of hospitalization by as much as 20-fold and the cost of hospitalization five-fold.[22]

Biofilms may cause antibiotic resistance by harbouring pathogenic micro-organisms.[23] Electricity has been used to remove biofilms from medical surfaces.[24] Pootringa et al.[25] have stated that it is possible to stimulate bacterial detachment from conducting indium tin oxide-coated glass by using a 10 μA/cm^{2} electrical current. Similarly, Borden et al.[26] have reported electrical current-induced detachment of *S. epidermidis* from surgical implants. A 100-μA DC yielded 78% detachment, whereas a 100-μA block current under the same experimental conditions yielded only 31% detachment. The same trend was found for 60 μA, with 37% detachment for a DC and 24% detachment for

a block current. These results suggest that DC is optimal for preventing infections. We believe that in our study, application of electrical current to the screws contributed to the removal of bacterial biofilms from their surface.

Aydin et al.[27] studied antibiotic sensitivity after exposure of bacteria to a silver anode. When the Ag-Ti screws were anodized, the antibiotics became more effective. This phenomenon may be useful in the prevention and management of implant-based spinal infections or may make traditional antibiotherapy possible.

The question remains as to how long the antibacterial effect of polarized silver is sustained in peri-implant tissue. Interestingly, once silver is anodized in culture medium (1 µA for 1 h), no bacteria can grow for at least 3 weeks despite frequent washing of the culture medium and cessation of the electrical current.[23] In our opinion, anodic silver ions may remain for longer than 3 weeks in the trabecular bony structures, and continue to exert a clinically significant effect over that time.

In conclusion, our results strongly support the use of electrically activated Ag-Ti implants for infected non-union, but with a lower electrical current. Given that silver cathodes have osteoblastic activity, inserted Ag-Ti screws should be first anodized, then cathodized throughout treatment. We suggest the use of silver-coated titanium to prevent implant-associated deep bone infections when implants are polarized anodically.

Acknowledgements

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