

Deposition Profile of Antibacterial Anodic Silver in Root Canal Systems of Teeth

Murat Aydin¹, İsmail Günay², Aykut Pelit², Mehmet Sami Serin¹

¹ Department of Microbiology, Faculty of Medicine, Çukurova University, Turkey

² Department of Biophysics, Faculty of Medicine, Çukurova University, Turkey

Abstract: Electrically activated silver was shown to have an antibacterial effect in vitro and in vivo. In this study the effect of placing a silver anode in the root canal systems of teeth was examined to establish a base for treatment of infected teeth. Pure silver wires were placed in the main canals of extracted human teeth (n=26) whose roots were partly submerged in a lactated Ringer's solution. Seventeen microamperes of anodic direct current were applied to one group of silver wires (n=15) for 4 days. Then the wires were removed and the roots of both group teeth were cut into six sections and demineralized. Silver concentrations of the root sections and their bathing solutions were measured with an atomic absorption spectrophotometer. In the anode group, the electrically activated silver concentrations (range, 1-30 µg/mL) exceeded the antibacterial levels (minimum inhibitory concentration 0.1 µg/mL Ag) in all sections, particularly in the middle and lower sites of the root. The amount of anodic silver that leaked out of the root was found as to be 0.4± 0.2 µg/mL in the fluid medium. This was 10 to 100-fold higher than that found in the nonactivated controls.

1997 John Wiley & Sons, Inc. *J Biomed Mater Res.* 38(1) 1997:49-54

Received 5 July 1996; Accepted 8 November 1996

Keywords: silver anode; root canal system disinfection; endodontics; teeth

INTRODUCTION

The bactericidal activity of a silver anode is mainly based on the emission of silver ions from the metal surface into tissue or medium when the silver metal is anodized by a low current^{1,2}. Complete bacterial inhibition is observed above the oxidation potential of silver (0.2 V).² The lack of increase in inhibition above that potential suggests a diffusion-limited process rather than iontophoresis. The presence of oxygen during electrical activation does not affect the antibacterial action and the antibacterial spectrum of the silver anode includes many anaerobic bacteria that are frequently present in the microbial flora of an infected tooth.³⁻⁶

The minimum inhibitory concentration (MIC) for anodic silver has been determined for a variety of bacteria. Generally the antibacterial effect appears

at an approximate level of 0.1 µg/mL⁷. However the MIC of nonpolar silver compounds was found to be higher^{2,5,7}. An extensive literature search found no indication of deleterious or irreversible effects on mammalian cells by the anodic silver unless the charge delivered exceeds 2 Coulombs per day. It is noteworthy in contrast that the silver cathode contributes to osteogenesis but is not microbicidal.^{8,9}

Usually the emitted silver produces proteinate and chloride complexes in the target tissue. Free silver ions and most silver compounds that have high solubility can easily penetrate any tissue, even when the tissue is avascular. Thus, the antibacterial effect of the anodic silver continues even when the electric current is stopped^{5,7}. For this reason antibacterial silver anode was employed in orthopa-

edics using silver electrodes to counter persistent deep-bone infections.¹⁰⁻¹²

Whether or not the silver anode can be used in root canal disinfection of an infected tooth has not been directly investigated. To establish a basis for the silver anode treatment of infected teeth, knowing the amount and concentration

profile of silver released into the root canal system is required. The goal of this study was to determine the amount of silver deposited into the root canal system and tooth tissue and to see if bactericidal levels can be achieved by this new method when a silver anode was placed in the root canal.

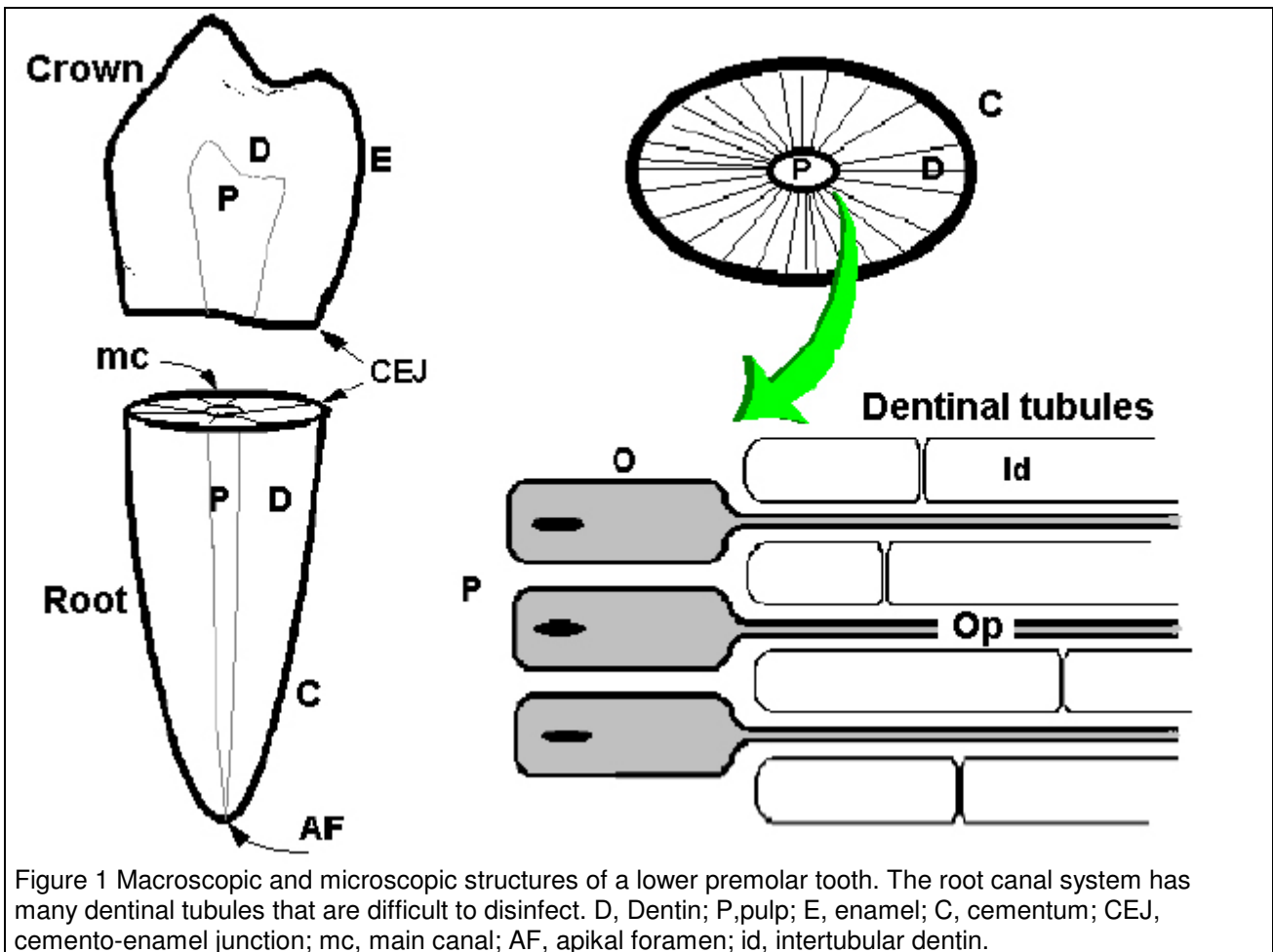


Figure 1 Macroscopic and microscopic structures of a lower premolar tooth. The root canal system has many dentinal tubules that are difficult to disinfect. D, Dentin; P, pulp; E, enamel; C, cementum; CEJ, cemento-enamel junction; mc, main canal; AF, apical foramen; id, intertubular dentin.

Background Information

A tooth contains dental pulp composed of connective tissue. The pulp is vascularized through the apical foramen (radius, 0.01-1mm) and is covered by the dentin that constitutes part of the tooth. The dentin contains 30,000 to 75,000 tubules/mm² with diameters of 0.74 to 3.24 μm. These are positioned radially to the pulp (Fig.1). The peritubular area is highly mineralized. The outer surface of root dentin is covered by a thin cementum layer (0.04-0.1 mm) and the crown dentin by enamel. In a normal tooth, a dentin tubular contents provide

nutrients for bacterial growth when the tooth is infected. Also, it is difficult to completely eliminate the bacterial deposits in the dentinal tubules, which may cause reinfections. Silver diffusion and deposition in the dentinal tubules via the root canal system may be a superior method for achieving sustained tooth disinfection.

METHODS AND MATERIALS

Thirty-two single rooted lower premolar teeth extracted because of infection or trauma were included in this study. The

teeth had not been exposed to any silver amalgam and had no abnormally shaped roots, either forked or hooked. The average age of the patients (16 males;12 females) from whom the teeth were extracted was 33 (median,31). All teeth were stored in a lactated Ringer's solution at room temperature to prevent decrease in their electrical resistance.¹³

All the root canals were cleaned and shaped by one operator in order to standardize the root canal preparation. Each root canal was enlarged to the size of a no 30 file. The canal was irrigated with 5 mL of sodium hypochlorite and 10 mL of saline but was dried in order to provide a good fluid contact between the electrode and inner surface of the main canal. Pure (%99) silver wire (0.5 x 100 mm) was inserted into the main canals of

the teeth. The upper part of the access cavities were tightly sealed with a carboxylate cement. The teeth were divided randomly into two equal groups.

The samples were clamped to test chambers that were filled with lactated Ringer's solution (20 ± 0.21 mL, pH 7.4) up to level of cemento enamel junction as seen in Fig 2. A non silver cathode (Ni) was introduced into the solution. The impedance of this system was measured to be 28 ± 4.12 kΩ with use of two terminal method.¹⁴ For each tooth in one group of teeth, direct current (DC) was applied from 1.5V battery through a serial rheostat and was maintained continuously at 17 μA with the silver wire as the anode. The teeth of the second group was not exposed to any electric current. Both groups of teeth were kept at 37 °C.

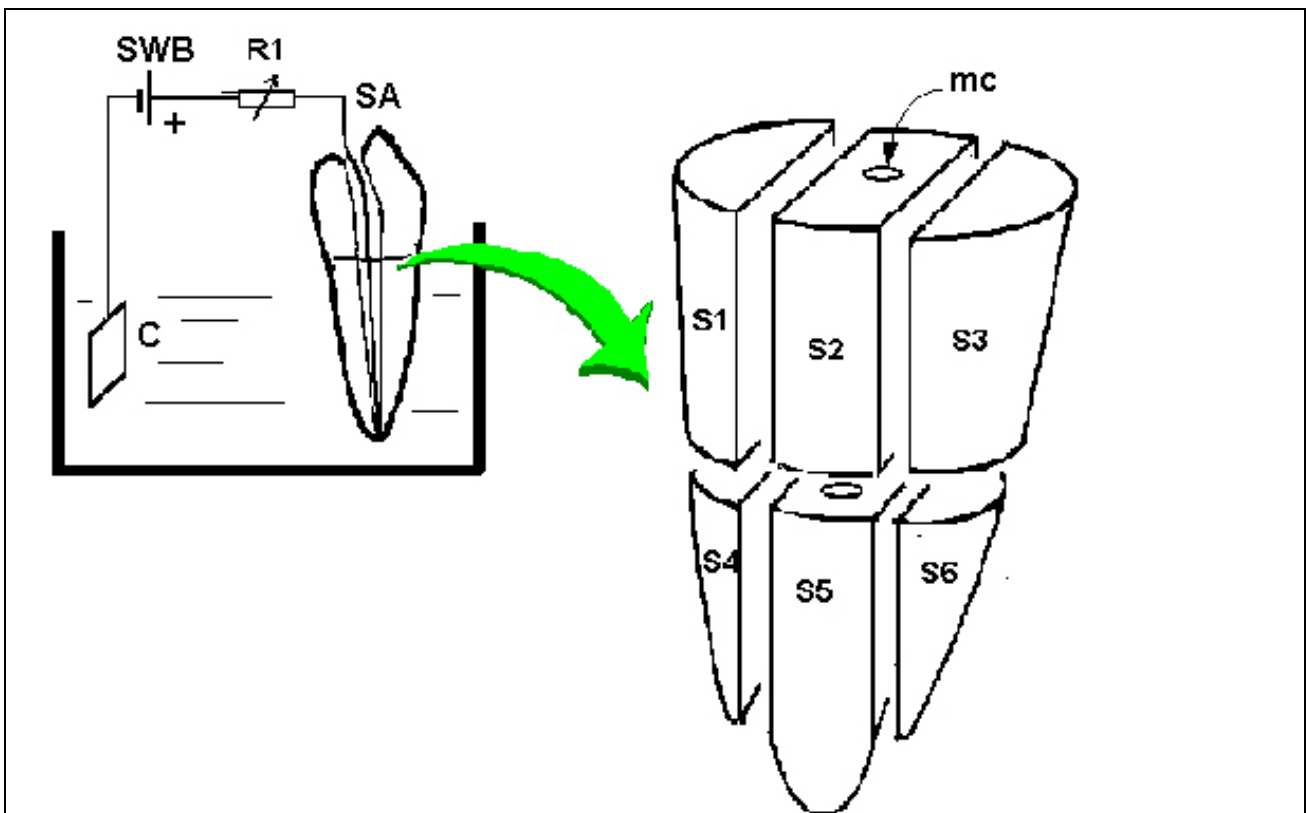


Figure 2 The teeth were attached to test chambers (5 x 5 x 5 cm, glass) after their root canal preparations and electrode placements. The first group of teeth were exposed to direct current for 4 days. Then roots of both groups were cut into six sections (S1-S6). SWB, Silver watch battery (1.55 V); R1, rheostat (100kΩ); C, cathode (22 x 14 x 0.5 mm, Ni); SA, Silver anode; mc, main canal.

After 4 successive days, the teeth, electrodes, cement residues, and wires were removed. Two milliliters of Ringer's solution was taken from the test chambers and labeled. This fluid bath contained the amount of silver that leaked out of the root canal through the apical foramen. Each one of the 32 teeth was cut at the cemento-enamel junction by a low speed cutter (Buehler, IL). The diamond disk blade was irrigated with 20 mL of water between each cutting to prevent to transfer of silver residues. All teeth crowns were excluded. The root samples were cut into six sections as shown in Figure 2. Any perforation of the main canal was avoided. Root samples were excluded from the experiment if the main canal was unintentionally exposed during the cutting. One root sample from both groups was cut horizontally near the apex and photographed (Fig.3). At the end, 15 of 16 roots remained in the first group and 11 roots in the second group. The six specimens per one root were labeled (S1-S6). They were dried overnight in a desiccator, measured, weighed, and recorded. They were demineralized in standard test tubes for 1 week with 0.5 mL of 25 %HCl at 22°C. Then 0.25 mL of 10.5% NaOCl was added to the test tubes and kept for 3 more days to completely dissolve the organic matrix. Then their final volumes were brought to 2 mL with deionized water. Their silver concentration was determined with an atomic absorption spectrophotometer² (Perkin Elmer 3100).

RESULTS

The length of the 26 roots examined in this experiment varied from 11.5 to 18 mm (median, 13 mm). Each of the effective anodes were the same length as the roots of the respective teeth. The thicknesses of the roots varied from 3.42 to 5.3 mm (median, 4.21 mm). The mean thickness of the central sections, S2 and S5, was 2.77 mm. The thickness of the lateral sections, S1, S3, S4, and S6, was found to vary from 0.8 to 1.1 mm (median, 0.94 mm). Their dry weights are summarized in Table I. The total current applied to one root was calculated to be 1.46 Coulomb/day, and the calculated current density at the anode surface was 0.83 $\mu\text{A}/\text{mm}$. The central sections (S2 and S5) contained the maximum silver in both groups and also maximum silver fluctuations were found in these sections with or without electricity. The magnitudes of the silver diffusing into specimens and their fluid baths are shown in Table I. Silver diffused into dentin tubules can be seen in Figure 3. The penetration depth of anodic silver was estimated to be 1.38 mm, equal to at least half of the thickness of the S2 section judging by measurable silver present in the S1 and S3 sections.

After the experiment there was a minimal pH shift in the liquid media (0.2). Six roots of the first group and their silver electrodes showed a gray discoloration, particularly in the apical region.

TABLE I. Average Concentration (mean \pm SD) of Silver Found in Root Canal System with or without Electricity.

Anode group (n=15)						
	S1	S2	S3	S4	S5	S6
Weight (g)	0.036 \pm 0.013	0.094 \pm 0.034	0.040 \pm 0.012	0.028 \pm 0.022	0.062 \pm 0.027	0.018 \pm 0.007
Ag ($\mu\text{g}/\text{ml}$)	1.149 \pm 0.0397	28.319 \pm 10.373	1.161 \pm 0.323	1.378 \pm 1.907	30.048 \pm 6.531	1.343 \pm 0.681
Ag ($\mu\text{g}/\text{g}$)	0.067 \pm 0.036	0.601 \pm 0.252	0.065 \pm 0.012	0.117 \pm 0.059	1.252 \pm 0.549	0.135 \pm 0.082
Fluid bath	0.412 \pm 0.19					
Control group (n=11)						
Weight (g)	0.034 \pm 0.012	0.179 \pm 0.287	0.037 \pm 0.015	0.018 \pm 0.01	0.058 \pm 0.023	0.021 \pm 0.017
Ag ($\mu\text{g}/\text{ml}$)	0.141 \pm 0.053	0.355 \pm 0.116	0.132 \pm 0.036	0.088 \pm 0.029	0.262 \pm 0.162	0.093 \pm 0.041
Ag ($\mu\text{g}/\text{g}$)	0.009 \pm 0.004	0.006 \pm 0.002	0.007 \pm 0.002	0.007 \pm 0.002	0.009 \pm 0.004	0.009 \pm 0.003
Fluid bath	0.001 \pm 0.0001					

Silver ion concentrations (mean \pm SD) were measured in the six sections of the root samples with electricity and without electricity. The fluid baths represent the silver quantity that was seeped out root into periapical tissues.

DISCUSSION

In the first group a significant amount of silver was found in the fluid bath. This can be explained by the fact that the current distribution was mainly directed toward the cathode through the apical foramen by a voltage gradient. In the fluid bath, the amount of silver was sufficient to provide for certain antibacterial activity when the lowest MIC of silver was accepted to be $0.1 \mu\text{g/mL}$ ⁷. Also, the silver concentrations were found to be enhanced in the peripheral sections of the anode group roots more than the same sections of the control group. These observations suggest that electrical polarization aided the silver diffusion along dentinal tubules. In each section of the active anode treated roots, the silver concentrations were sufficient for antibacterial activity despite the fact that the access of many dentinal tubules were partly obstructed by the smeared debris during the root canal preparation. In addition, the real quantity of the silver diffused into the dentin was much more than we measured because some silver was lost during the cutting of the roots.

In the second group, a major ratio of the amount of the silver released spontaneously from the nonpolarized silver wire appeared spectroscopically to be retained in the main canal. It is likely that the narrowness of the apical foramen limited silver transfusion into the fluid bath. The amount of released silver in the control case could be expected to eliminate some sensitive bacteria in the main canal space only, but not in all of the root canal system. Also, the silver that

leaked from the apical foramen in controls did not reach antibacterial concentrations. Histologically, the access of the dentin tubules in anode group roots were found to be completely obstructed by the silver deposits but were not in their controls as seen in Figure 3. This obstruction was already a major target of root canal treatment. The root canal filling materials that are routinely used in root canal treatment contain incidental antibacterial agents. Their antibacterial effects may be maintained for hours, days, and even weeks. For instance, wheares Diaket-A (ESPE, Germany) retains an antibacterial effect (except on *Pseudomonas* sp.) for months, Endomethasone (Septodont, Saint-Maur, France) loses its antibacterial effect in serum within days. Chloride compounds are briefly antibacterial and are inactivated within days. N2 Normal (Ghima S.p.A, Bologna, Italy) is antibacterial for only a few weeks. AH26 (De-trey, England) is inactivated within days¹⁵. Spadaro⁵ demonstrated that silver anodized for 1 h at $1 \mu\text{A/mm}$ is able to keep its antibacterial effect for at least 10 weeks in vitro, even when the culture fluid is changed 3 times/week. The low solubility of metallic silver and its precipitates can remain antibacterial for a longer period than the above endodontic materials because any fluid circulation is not present in dentin tissue when the root canal is prepared. Furthermore, it was shown for the silver material that chemical irritation to living tissues is minimal.^{5,16,17}

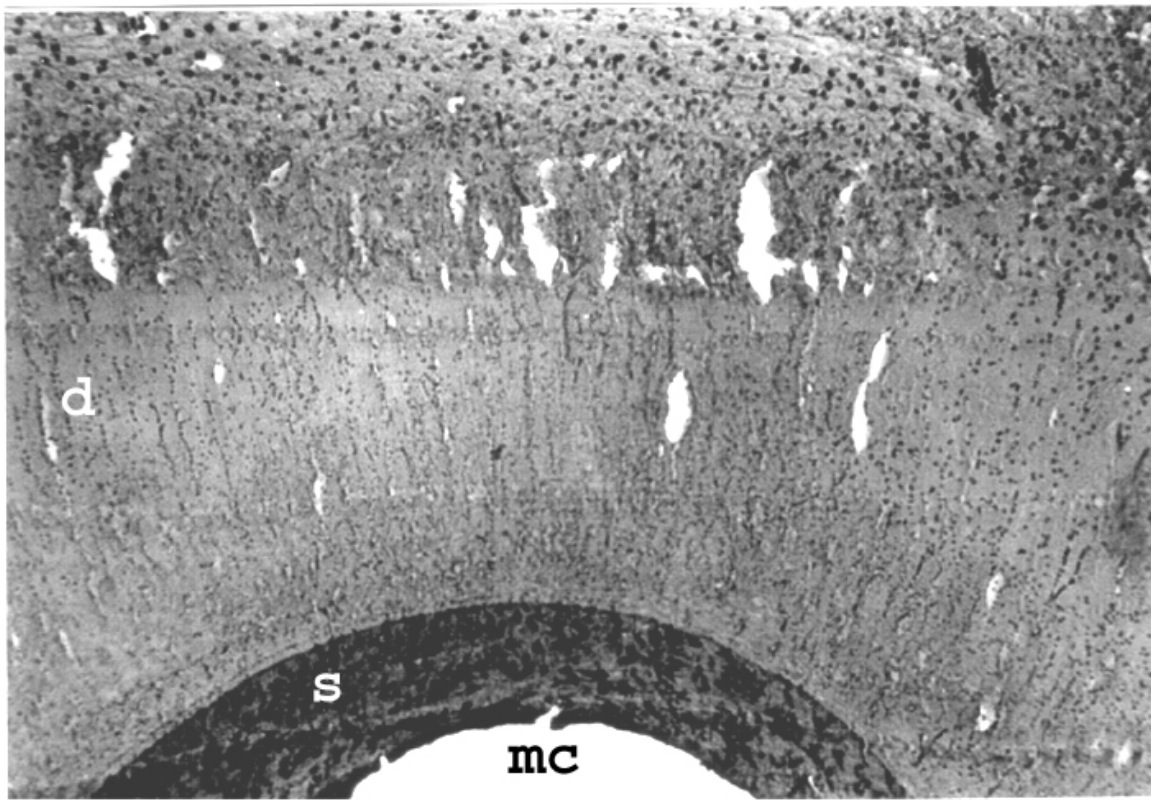
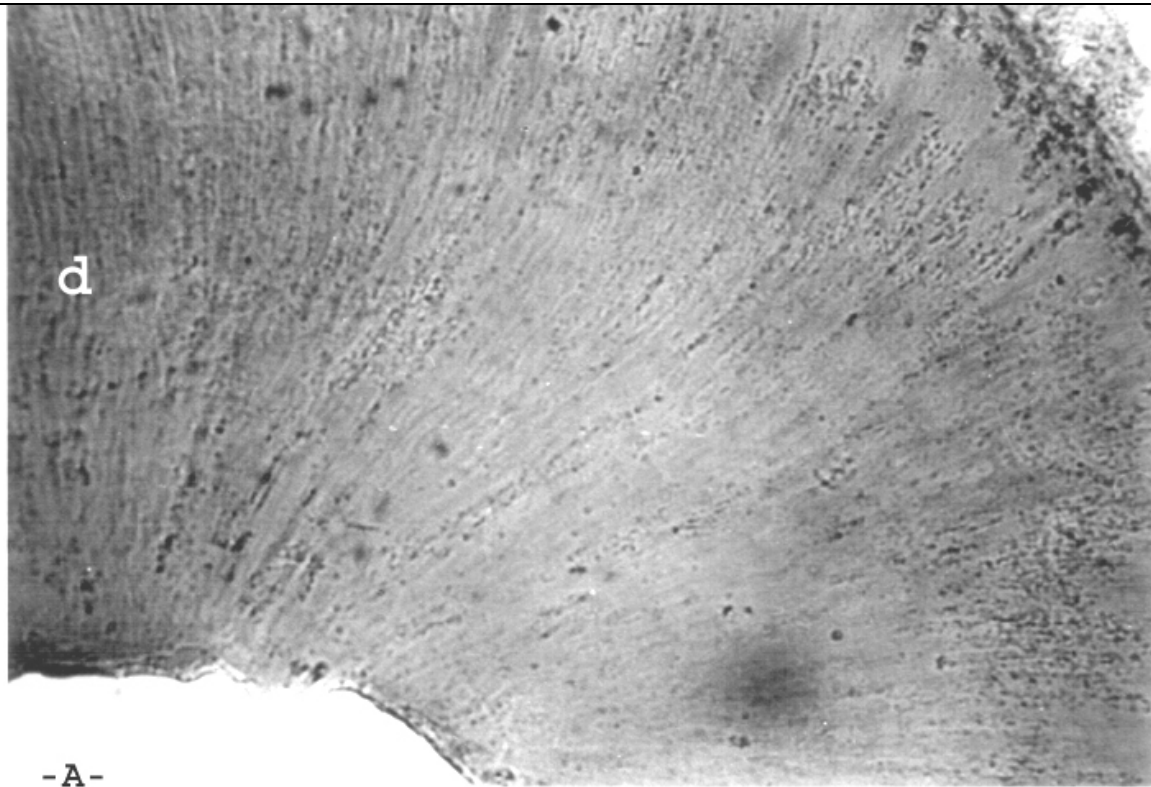


Figure 3 Th microscopic cross sectional views of the (top) nonpolar and (bottom) anodized silver wire treated roots (x40). The penetration depth of the silver was estimated above or equal to 1.38 mm d, Dentin; mc,main canal; s, silver deposits.

The penetration depths of AH26, chloride compounds, Endomethasone, and Sealapex (Kerr, MI) were found to be 1, 0.25 to 0.4, 0.07, and 0 mm, respectively¹⁵. Silver nitrate solution, on the other hand can penetrate into carious dentin to a depth of 0.3 to 1.3 mm¹⁸. In our preliminary study¹⁹ the penetration radius of the anodic silver was found to be 14 mm with the use of 17 to 20 μ A of DC in an agar medium. In addition Becker and Spadaro¹² estimated that anodic silver can penetrate into living bone to an approximate depth of 10 mm. The current results showed that the penetration of anodic silver was deeper (at least 1.38 mm) than that of the above endodontic materials.

Many of the bacteria that may present in an infected root canal flora are

usually proteolytic and obtain energy from protein and amino acid breakdown (e.g. *Prevotella intermedia*, *Veillonella parvula*, *Peptostreptococci*, *Eubacterium* sp, and *Fusobacterium nucleatum*). However, the silver has a strong binding capacity to proteins in a ratio of 97%⁵. A result of the silver anode treatment may produce an ecologic shift of the root canal system unfavorable to proteolytic bacteria.

In conclusion, these results suggest that antibacterial concentrations of silver can be achieved in the root canal system particularly in the middle and lower sites of the root by the silver anode method.

We thank Dr. J.A. Spadaro for valuable comments on the manuscript.

REFERENCES

1. Coslshaw, J. Spadaro, J.A. Becker, R.O. Inhibition of enzyme induction in *E. coli* by anodic silver. *J. Bioelectricity* 3:295-304, 1982.
2. Spadaro, J.A. Berger, T.J. Barranco, S.D. Chapin, S.E. Becker, R.O. *Antimicrob. Agents Chemother.* 6:637-642, 1974
3. Handelman, S.L. MacIntyre, B. Ellis, F.H. Electrophoretic effect of silver ions on oral bacteria. *J. Endodont.* 3:458-462, 1977.
4. Spadaro, J.A. Webster, D.A. Chase S.E. Direct current activation of bacteriostatic silver electrodes. *Trans. Bioelectric Repair Growth Soc.* 3:37-41, 1983
5. Spadaro J.A. *Electrical Silver Antisepsis*. Modern Bioelectricity. Marino, A.A. ed. New York Marcel Dekker, 1988:629-655
6. Tronstad, L. Trope, M, Hammond. B.F. Effect of electric current and silver electrodes on oral bacteria. *Endodont Dent. Traumatol.* 1:112-5, 1985.
7. Berger T.J. Spadaro J.A. Chapin. S.E. Becker, R.O. Electrically generated silver ions: quantitative effects on bacterial and mammalian cells. *Antimicrob. Agents Chemother.* 9:357-358, 1976.
8. Spadaro, J.A. Bone formation and bacterial inhibition with silver and other electrodes. *Reconstr. Surg. Traumatol.* 19:40-50, 1985
9. Spadaro J.A. Electrically enhanced osteogenesis at various metal cathodes. *J. Biomed. Mater. Res.* 16:861-867, 1982
10. Webster, D.A. Spadaro J.A. Becker R.O. Kramer S. Silver anode treatment of chronic osteomyelitis. *Clin. Orthop Rel. Res.* 161:105-111, 1981
11. Tamura K. Some effects of week direct current and silver ions on experimental osteomyelitis and their clinical application. *J. Jpn. Orthop. Assoc.* 57:187-197, 1983.
12. Becker R.O. Spadaro J.A. Treatment of orthopaedic infections with electrically generated silver ions. *J. Bone Joint Surg.* 60A:871-881, 1978.
13. Saha, S. Williams, P.A. Effect of various storage methods on the dielectric properties of compact bone. *Med. Biol. Eng. Comput* 26:199-202, 1988
14. Ackmann J.J. Seit. M.A. Methods of complex impedance measurements in biologic tissue. *Biomed. Eng.* 11:281-291, 1984.
15. Orstavik, D. Antibacterial properties of endodontic materials. *J. Int. Endodont.* 21:161-169, 1988.
16. Spadaro J.A. Chase S.E. Webster, D.A. Bacterial inhibition by electrical activation of percutaneous silver implants. *J. Biomed. Mater. Res.* 20:565-577, 1986.
17. Petering, H.G. Pharmacology and toxicology of heavy metals: silver. *Pharmacol. Ther.* A.1:127-131, 1973.
18. Stephan, R.M. Muntz M.S. Dorfman, A. In vitro studies on sterilization of carious dentin. III. Effective penetration of germicides into carious lesions. *J. Am. Dent. Assoc.* 20:1905-1909, 1943.
19. Aydin, M, Köksal, F, Günay, İ, Serin, M.S. The effect of antibacterial silver electrodes and the nature of ion emission in the outer side of inhibition zone. *Ann. Med. Sci.* 5:52-58, 1996.
20. Sundqvist, G. Ecology of the root canal flora. *J. Endodont.* 18:427-430, 1992.