Original Article / Özgün Araştırma

Effect of Silver Ion Surface Coating on Antimicrobial and Cutting Efficiencies of Nickel-titanium Rotary Files

Gümüş İyon Yüzey Kaplamasının Nikel-titanyum Döner Eğelerin Antimikrobiyal ve Kesme Etkinliği Üzerine Etkisi

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Keywords
Antibacterial activity, cutting efficiency, nickel-titanium files, silver ion coating

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Abstract

Objective: Silver ions are well known to have effective antimicrobial activity against many microorganisms. This study aims to evaluate the antimicrobial and cutting efficiencies of nickel-titanium rotary file surface coated with silver ions by using the dip-coating method.

Materials and Methods: A pilot study was performed before the endodontic file coating. The prepared coating solutions (silan and silver-complex systems) were applied to stainless steel plates by using the spin-coating method. The antibacterial activity of the coatings was researched against *Enterococcus faecalis*, which resulted in the selection of the coating solution with a 20% solid ratio and 2% silver content. Subsequently, the files were assigned to three groups based on the coating speed [group 1, 50 mm/min; group 2, 25 mm/min; and group 3, non-coated files (control)]. The cutting efficiency was evaluated by calculating the weight loss of the transparent acrylic blocks. The file surface and section was evaluated using scanning electron microscopy (SEM) and SEM-energy-dispersive X-ray spectroscopy before and after preparation. Data were statistically analysed with one-way analysis of variance and post-hoc Bonferroni test ($p<0.05$).

Results: Significantly more debris was removed from Group 1. The cutting efficiency was not significantly different between groups 2 and 3. Groups 1 and 2 showed distinctive surface irregularities.

Conclusion: The application of this antimicrobial surface coating was promising for improving antibacterial effectiveness of nickel-titanium rotary files. In addition, the thin film coating did not cause any negative effects on file cutting efficiency.

Öz

Amaç: Gümüş iyonlarının birçok mikroorganizmaya karşı etkili antimikrobiyal aktiviteye sahip olduğu iyi bilinmektedir. Bu çalışmanın amacı, yüzeyleri gümüş iyonları ile kaplanmış nikel-titanyum döner eğelerin antimikrobiyal ve kesme etkinliklerinin daldırma-kaplama yöntemi ile değerlendirilmesidir.

Gereç ve Yöntemler: Endodontik eğe kaplama işlemi yapılmadan önce bir ön çalışma yapıldı. Hazırlanan kaplama solüsyonları (silan ve gümüş-kompleks sistemleri), spin kaplama metodu kullanılarak paslanmaz çelik levhalara uygulandı. Kaplamaların antibakteriyel aktivitesi *Enterococcus faecalis*'e karşı araştırıldı. Sonuç olarak, %20 solit oranı ve %2 gümüş içeriğine sahip kaplama çözeltisi seçildi. Daha sonra eğeler yapılan kaplama hızına göre 3 gruba [grup 1, 50 mm/dk; grup 2, 25 mm/dk; ve grup 3, kaplanmamış eğeler (kontrol)] ayrıldı. Şeffaf akrilik blokların ağırlık kaybı hesaplanarak kesme etkinliği değerlendirildi. Eğe yüzeyi ve kesit değerlendirmesi, yüzey işlemleri öncesi ve sonrasında, taramalı elektron mikroskobu (TEM) ve TEM-energy-dispersive X-ray spektroskopisi ile taranarak yapıldı. Elde edilen veriler, tek yönlü varyans analizi ve post-hoc Bonferroni testi ile istatistiksel olarak analiz edildi (p<0,05).
Bulgular: Grup 1'de anlamlı derecede daha fazla debris uzaklaştırıldı. Grup 2 ve 3 arasında kesme etkinliği açısından anlamlı bir fark gözlenmedi. Grup 1 ve 2 belirgin yüzey düzensizlikleri görüldü.
Sonuç: Bu antimikrobiyal yüzey kaplama uygulanması, nikel-titanyum döner eğelerin antibakteriyel etkinliğinin artırılması için umut vericiydi ve ince film kaplama, eğe kesme etkinliği üzerinde herhangi bir olumsuz etkiye neden olmadı.

Introduction

Many microorganisms play a role in root canal infections, which require complete debridement with sufficient biomechanical instrumentation (1). New applications have been developed to eliminate these microorganisms. Photodynamic therapy, laser-assisted root canal disinfection, ozone, herbal and enzyme alternatives and nanoparticle applications are some of the new techniques (2). Silver or silver ion inorganic materials have broad-spectrum bactericidal effects (3,4) and limited mammalian cell toxicity (5). Several studies have reported that silver nanoparticles have antibacterial effects against endodontic bacteria (6,7).

The bacterial layer on the surface of dental instruments, such as endodontic files, could escape from human defence mechanisms and induce infectious disease. Before the sterilisation procedure, preliminary cleaning of dental materials is mandatory for eliminating disease-causing bacteria (8).

Rotary nickel titanium (NiTi) files provide fast and easy preparation for a root canal and are more efficient than hand files (9). It is well known that various modifications improve mechanical and physical properties of NiTi rotary files by modifying the manufacturing process, microstructures and material properties (10,11). No study has determined the antibacterial effectiveness of NiTi rotary files.

Instead of using different rotary files in each root canal, teeth have more than one canal are usually instrumented with same files. Even if the file surface is cleaned immediately after instrumentation, infected tissue residues and debris can remain. Rotary files having antibacterial surface coating and adequate cutting efficiency may be helpful in this case. The aim of this study is to evaluate the antibacterial and cutting efficiencies of rotary NiTi root canal files coated with silver ions.

Materials and Methods

Coating Materials

The chemical substances utilised were 3-(glycidyloxypropyl) trimethoxy silane (GLYMO) as a SiO_2 source, ethylacetoacetate (HacacOEt) as a complexing agent for aluminium-tri-sec-butoxide [$Al(OsBu)_3$], which is a Lewis acid used to open the epoxy ring of GLYMO; nitric acid solution (0.1 M) as a hydrolysis catalyst, silver nitrate as a silver ion source, N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (DIAMO) as a stabilising agent for the silver ion and isopropyl alcohol (IPA), ethyl alcohol (EtOH) and acetone (Ac) as solvents.

Preparation of Silver Ion Surface Coating

The coating solution was composed of silane and a silver-complex system. To prepare the silane system, GLYMO was hydrolysed with 0.1 M HNO_3 and stirred for 2 h at room temperature. Then, the $Al(OsBu)_3$/HacacOEt complex was prepared. To this end, 1 mol $Al(OsBu)_3$ and 1 mol HacacOEt were mixed and stirred at room temperature for 1 h (12). While the pre-hydrolysed GLYMO solution was stirring, the complex was added drop by drop. Stirring was continued for 1 h after adding the complex. The molar ratios of silane system components were the same, as used in a previous study (13). The solid ratio of the coating solution was adjusted to 35% with solvent. The system was ready for use after stirring for 3 h.

The silver-complex system was generated in another reaction medium. Silver nitrate was dissolved in a solvent mixture (IPA/EtOH/Ac), and DIAMO was added dropwise to the system. The complex mixture, which contained 1-5% silver ions according to the system to be used, was stirred for 2 h. The molar ratios of $AgNO_3$: IPA: EtOH: Ac: DIAMO were 1: 15: 6: 1.5: 5 in the mixture, respectively.

The prepared silver-complex system was added to the silane system. Systems containing 0.5%, 1%, 2% and 3% silver ion were used in experiments.

The coating process was performed on the stainless steel (0.7 mm thickness and 5 × 5 lengths) using the spin coating method. The curing program for the coated plates was set to increase 100 °C in 30 min and remain there for 4 h.

Antibacterial Test Procedure

The solid medium was prepared by using agar, lactose broth, glucose, and 1 L distilled water in an Erlenmeyer flask. The flask and its contents were sterilised by autoclaving at 120 °C for 20 min. The liquid medium was prepared with lactose broth, glucose and 500 mL distilled water. The liquid medium was transferred to capped tubes (5 mL), Erlenmeyer flasks (50 mL) and test tubes (9 mL) and all of them were sterilised like the solid medium. The capped tubes and flasks were used for overnight and daytime inoculation, respectively. The test tubes were used for diluting solutions.

The antibacterial activity of the coatings was investigated against Gram positive *Enterococcus faecalis* (*E. faecalis*). One colony of *E. faecalis* was taken and added to a capped tube, and the capped tube was incubated in shaker incubator for 24 h at 37 °C. A 500 µl aliquot was added to an Erlenmeyer flask, containing 50 mL of liquid medium, and the flask was incubated in shaker incubator for 5.5 h at 37 °C. The incubation times were determined by optical density measurements.

When the daytime inoculation was completed, the bacterial medium was diluted to 10^{-6} cells/mL using the test tubes.

Whole samples were sterilised under UV light for 30 minute (min). A 100 µl aliquot of bacterial medium was taken from the test tube and spread onto a plate containing a sample. The sample was incubated at room temperature for 30 min. After the incubation, the bacterial side of the sample was added to agar medium so the surviving inoculated bacteria could diffuse from the surface into the agar medium. After 30 min, the sample was removed from the surface. This process was applied to all samples. The same amount of bacterial medium was inoculated onto agar medium as colony forming units (CFU). Petri dishes of samples were incubated for 24 h at 37 °C. The growing bacterial colonies were counted, and antibacterial activity of the samples was calculated as a percentage: (CFU colony number - sample colony number)/ CFU colony number × 100.

Coating the Files

To determine coating condition, the coating process was done with solutions of different solid ratios, such as 10%, 15% and 20%. No differences were observed between the samples, so the solution with a 20% solid ratio was used. The coatings were done at two different speeds of 25 and 50 mm/min using the dip-coating method.

Twenty-four ProTaper Universal (Dentsply Maillefer, Ballaigues, Switzerland) file sets (S1, S2, SX, F1, and F2) were used in this study.

Group 1. 8 file sets (40 pieces) coated at 50 mm/min speed.

Group 2. 8 file sets (40 pieces) coated at 25 mm/min speed.

Group 3. 8 file sets (40 pieces) non-coated.

Evaluation of Cutting Efficiency

A total of 120 transparent resin blocks with an artificial root canal were weighed with a high-precision balance (Presica Instruments AG, Dietikon, Switzerland) to 0.0001 g accuracy before preparation. All resin blocks were weighed at once to eliminate the effect of room temperature on accuracy of the measurements. Debris loss was recorded as the cutting efficiency parameter by calculating the difference between the weights before and after preparation of the resin blocks.

Preparation of the Simulated Root Canal Resin Blocks

One clinician prepared five resin blocks with each file set. A total of 40 resin blocks were instrumented in each group. Resin blocks had an 8 mm curvature after a 5 mm flat route. Continuous irrigation was performed during preparation with saline for lubrication and to prevent the accumulation of resin debris. Recapitulation was performed with a size 15 K-file before each file change. The root canal was prepared with a 1:16 reduction contra-angle hand piece, and torque and RPM were adjusted according to the manufacturer's instructions (X-Smart Endodontic Motor; Dentsply Maillefer). The F2 file was selected as the final root canal file.

After preparation, the debris in the blocks was removed with an air-water spray, and the blocks were placed in an ultrasonic bath for 5 min. The moisture in the simulated canals was dried by storing the blocks for 36 h at room temperature and then they were weighed the second time.

Scanning Electron Microscopic Evaluation

Before the image analysis, the file samples were put into an ultrasonic cleaner inside vials containing pure alcohol to remove the surface remnants for 5 min. Then, scanning electron microscopic (SEM) images were obtained. Image samples were classified as; (A) non-coated (control) - before preparation, (B) coated with 25 mm/min - before preparation, (C) coated with 50 mm/min - before preparation, (D) non-coated (control) - after preparation, (E) coated with 25 mm/min - after preparation and (F) coated with 50 mm/min - after preparation.

SEM images were taken from randomly selected F1 and F2 files in each group. Four images were obtained from different levels of each file; (A) the file tip at ×200 magnification, (B) the working part of the file (3-5 mm distance from the file tip) at ×200 magnification, (C) the surface of the file at ×500 magnification and (D) the surface of the file at ×1,000 magnification.

SEM images were analysed according to the surface properties, irregularities, micro cracks and rollings. The thicknesses of the surface coatings obtained with different dipping speeds were measured with SEM. Sectional images of the files were taken from randomly selected specimens from each group. The working parts of the files were sectioned at a distance of 12 mm from the file apex to the inactive file region with a diamond burr under water cooling. Thus, 3 mm long specimens were obtained. SEM images were obtained from edges at ×3000 magnification.

Analysis of File Surfaces with Scanning Electron Microscopic-Energy-Dispersive X-ray Spectroscopy

SEM-energy-dispersive X-ray Spectroscopy (EDS) was used in order to perform an element analysis of specific regions to determine whether the surface contains silver before and after preparation during the SEM imaging of file surfaces. Graphics and numerical data of detected elements were recorded.

Statistical Analysis

The statistical analysis was performed using SPSS software (version 15.0; SPSS Inc., Chicago, IL, USA). One-way analysis of variance and the Bonferroni test were used for the data analysis. A p-value<0.05 was considered significant.

Results

Results of the Bacterial Tests

First Stage Test

Bacterial growth was observed on all non-coated plates and those coated with 0.5% and 1% silver ions. New coatings were added to sterile plates using solutions containing 2%, 3%, 4% and 5% silver ion, and the coatings were tested for different durations (10, 20 and 30 min). No bacterial growth was observed after any of the durations with the 2%, 3%, 4% or 5% silver ion surface coatings. We used the 2% silver ion coating because this was the lowest concentration in which antibacterial effectiveness was achieved (Table 1).

Coating was performed using solutions containing 10%, 15% and 20% solid material to adjust the coating condition. As no significant difference was observed between them, the coatings were performed at two different speeds (25 and 50 mm/min) using the dip-coating method with the solution containing 20% solid.

Weight Loss Following Preparation

A significant weight loss was observed in the resin blocks after preparation of the 50 mm/min group (group 1) compared with that of the 25 mm/min

Table 1. Antibacterial activity of plates with silver ion content in varying percentages after incubation

*	CFU	Non-coated	0.5% Ag	1% Ag	2% Ag	3-5% Ag
Sample 1	31	27	21	25	0	0
Sample 2	25	23	18	23	0	0
Sample 3	42	24	19	26	0	0
Sample 4	54	27	17	17	0	0
Sample 5	30	18	18	19	0	0
Mean	36	24	19	22	0	0

*: Amount of bacteria, CFU: Colony forming unit, Ag: Silver

group (group 2) and the control (group 3) ($p<0.05$) (Table 2). The group 2 files tended to result in more weight loss compared with that of group 3 ($p>0.05$).

Scanning Electron Microscopic Analysis

Machining marks were observed on the surface of the group 3 files when the SEM images of files in each group were evaluated in secondary electron mode before preparation. The group 1 and 2 files showed much more surface roughness than that of the control files. However, the group 1 files exhibited more surface roughness compared with those of group 2 (Figure 1).

Surface structure was almost maintained after preparing the root canal, but blunting and notches were detected in the cutting edges of the group 3 files (Figure 2). The roughness of the coated surface in the grooves of the group 2 files after preparation was less than that observed pre-preparation. More notches were observed compared to blunting on the cutting edges of the group 2 files after preparation. Many cutting edge deformations were observed near the tip region. There was minimal roughness in the grooves or near the tip region in group 1 after preparation. More blunting compared to notches was detected on the cutting edges of these files. There was less roughness near the tip region of the group 1 files, and the cutting edges were more solid than those of the group 2 files (Figure 3).

The deformity in the cutting edges of the group 3 files was clear. The group 1 files exhibited less deformity compared with that of group 3 in terms of the cutting edge after preparation. The group 1 files exhibited the best cutting edge morphology after preparation.

Elemental Analysis of the File Surface

Regions in different shades showing surface roughness were detected on the SEM images before and after preparation. All analysed regions were

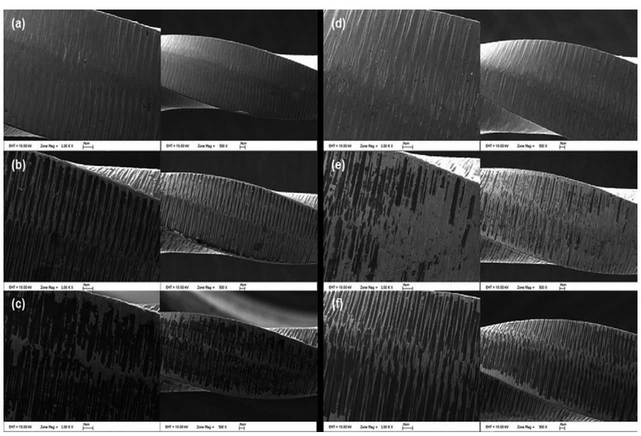


Figure 1. File images before the preparation (original magnification, ×500 and ×1000). (a) group 3-F1 file, (b) group 2-F1 file, (c) group 1-F1 file, (d) group 3-F2 file, (e) group 2-F2 file and (f) group 1-F2 file

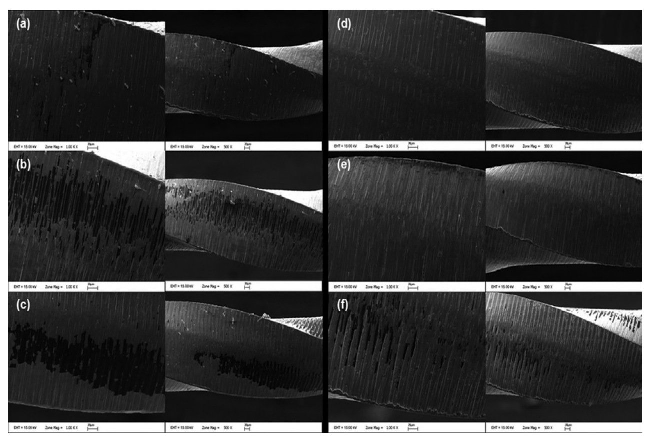


Figure 2. File images after preparation (original magnifications ×500 and ×1,000). (a) group 3-F1 file, (b) group 2-F1 file, (c) group 1-F1 file, (d) group 3-F2 file, (e) group 2-F2 file and (f) group 1-F2 file

Table 2. Weight loss of transparent resin block samples after instrumentation (in grams)

Groups	Mean ± standard deviation	P value	Mean difference	95% CI
Group 1 (coated with 50 mm/min)	0.003915±0.0003034	0.003 ($p<0.05$) (Gr1-Gr2)^{a} 0.000 ($p<0.05$) (Gr1-Gr3)^{a}	0.0002200 0.0003400	0.000062, 0.000378 0.000182, 0.000498
Group 2 (coated with 25 mm/min)	0.003695±0.0003381	0.003 ($p<0.05$) (Gr2-Gr1)^{a} 0.203 ($p>0.05$) (Gr2-Gr3)	-0.0002200 0.0001200	- 0.000378, -0.000062 - 0.000038, 0.000278
Group 3 (non-coated, control)	0.003575±0.0002181	0.000 ($p<0.05$) (Gr3-Gr1)^{a} 0.203 ($p>0.05$) (Gr3-Gr2)	-0.0003400 -0.0001200	- 0.000498, -0.000182 - 0.000278, 0.000038

Gr1: Group 1, Gr2: Group 2, Gr 3: Group 3, Cl: Confidence interval, ^{a}: Statistical value is at significant levels at $\rho<0.05$. *Groups with the same capital superscripts are significantly different p<0.05

coated with silver when analysed by SEM-EDS before and after preparation.

Nickel (Ni), titanium (Ti) and O were detected by the elemental analysis of a randomly selected file from the non-coated file sets. In addition, Ni, Ti and silver (Ag) were detected with an analysis performed in regions with different shades in the coated groups (Figure 4).

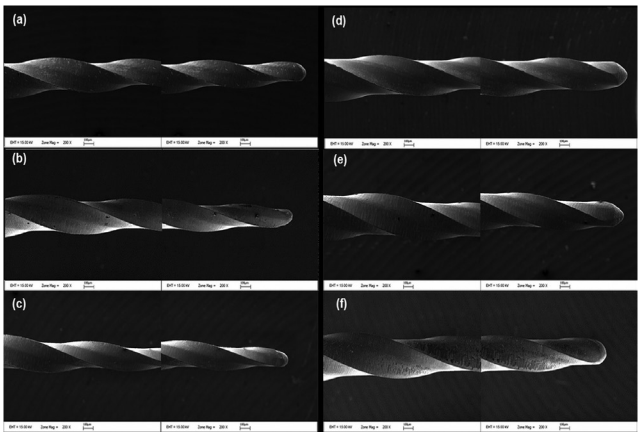
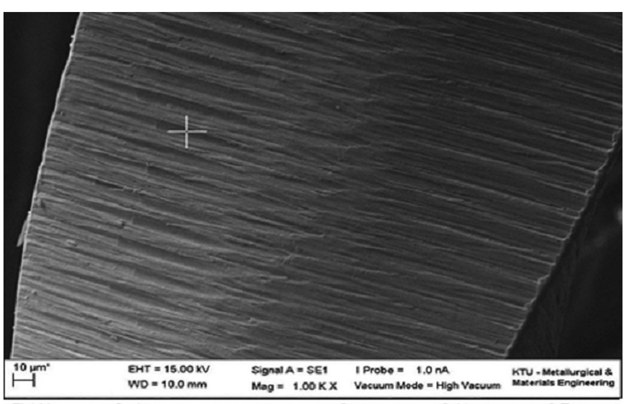


Figure 3. Scanning electron microscopy images at 3-5 mm distance from the file tip after preparation (original magnifications ×500 and ×1000). (a) group 3-F1 file, (b) group 2-F1 file, (c) group 1-F1 file, (d) group 3-F2 file, (e) group 2-F2 file and (f) group 1-F2 file



El AN	Series	unn. [wt.%]	C norm. [wt.%]	C Atom. [at.%]	C Error [%]
Ti 22	K-series	39.71	48.03	53.42	1.2
Ni 28	K-series	41.83	50.60	45.90	1.4
Ag 47	L-series	1.14	1.38	0.68	0.1
	TOTAL	82.67	100.00	100.00	

Figure 4. Ni, Ti and Ag were detected with an elemental analysis performed in regions with a different shade in the coated groups after preparation

Ni: Nickel, Ti: Titanium, Ag: Silver

Determination of Coating Condition

The mean thickness of Group 1 was 17 µm, and the mean coating condition of Group 2 was 11 µm.

Discussion

The aim of this study was to add antimicrobial properties to rotary files and to research the effect of this process on cutting efficiency. The surface of the rotary root canal files was modified to eliminate surface irregularities and increase the resistance against flexural and torsional wear. It has been reported that surface modifications increase surface hardness (14), cutting efficiency (15), resistance against corrosion (16,17) and cyclical fatigue resistance (18). No process that applies antimicrobial surface modifications has the aim of providing antimicrobial properties to root canal files.

In this study, the antimicrobial effect of the coated files on *E. faecalis*, which is the most resistant microorganism seen following unsuccessful root canal procedures, was studied. The bacterium was completely eliminated after contact with the silver ion coating but human tooth studies are needed to determine the degree of this antimicrobial effectiveness during preparation.

Studies based on extracted teeth are closer to a clinical application. However, simulated canals are better for evaluating the standardised conditions of canal shaping and file performance (19). In addition, the hardness and abrasive properties of the resin blocks were not similar to those of root canal dentine. Resin softening due to heat generated by the rotary file prevents resin from mimicking dentin. Using the same file with different surfaces on a standardised canal eliminated the probable variations in root canal morphology.

Yamazaki-Arasaki et al. (20) reported a qualitative analysis in which the surface roughness of the ProTaper file cervical and tip regions was similar before and after preparation. As the effect of roughness on the coating was eliminated, ProTaper Universal files were selected as the most appropriate file for this study.

Different bacterial colonisations were identified in different canals of the same tooth (21). It was also identified that the bacterial composition of different roots of the same tooth in teeth that require retreatment could be less similar, as shown in a polymerase chain reaction study (22). One of the major concerns during preparation of teeth with more than one root canal is causing microbial contamination by transporting the microbial content from one root canal to the other. It may be advisable to use different files in each canal to prevent this transfer. However, in practice, there are questions of cost and difficulties in use. Antibacterial silver ion-coated files may be helpful in this respect.

As most canal files obtained from the manufacturer are not sterilised and have metal burrs, debris and even epithelial cells on their surfaces (23,24), an antibacterial material-coated file is applicable. Thus, the necessity to clean and sterilise the files before first use may be eliminated.

In this study, the evaluation of cutting efficiency according to loss of resin block weight is a method that has been used previously (25,26). In the dip-coating method, it was predicted that the surface coating of the files at 50 mm/min would be thicker than the surface coatings of dipping the files at 25 mm/min and of the control group files. As the thickness of the file increased, it was hoped that more material would be removed from the resin block. The statistical analysis of weight loss in the groups showed that the files coated at 50 mm/min removed significantly more debris than those from groups 2 and 3. Although the thickness of the files in each group was different, this did not cause any file to fracture. The surface coating of rotary files did not have any negative effect on cutting efficiency.

When the most contacted part of the file with dentin was evaluated, SEM images obtained after preparation with ×200, ×500, and ×1000 magnification revealed that the coated files maintained their structure in these regions. This finding shows that the surface coating material had sufficient bond strength. The SEM-EDS analysis and SEM images after preparation demonstrated a complete thin silver ion coating on the file surface.

A partial irregular distribution of coating material was visualised on the file surface from the SEM images obtained before and after preparation. As a result, a more suitable surface coating method is required to improve surface morphology.

The ×3000 magnified images obtained from groups 1-3 were used for the cross-sectional analysis. The coating condition measurement was made at three points of the file section, and the average of the measured values was taken because there were morphological irregularities on the file surface coating.

Conclusion

A silver ion surface coating was effective against *E. faecalis*. Silver ion coating did not cause any negative effects on cutting effeciency of NiTi rotary file. Other coating methods may be applied to produce less surface roughness on the NiTi files. Further studies are needed to investigate antibacterial and cutting efficiency of silver ion-coated files.

Ethics

Ethics Committee Approval: Ethics committee document is not required for this study.

Informed Consent: Patient consent is not required for this study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: K.E., S.C., Design: K.E., S.C., N.K., R.F., H.E., Data Collection or Processing: S.C., B.B., H.E., R.F., Analysis or Interpretation: S.C., N.K., K.E., H.E., T.T., Literature Search: S.C., R.F., Writing: K.E., S.C., T.T.

Conflict of Interest: No conflict of interest was declared by the authors.

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