ORIGINAL RESEARCH

Effect of Sodium Hypochlorite, EDTA, and Chitosan Solution on Corrosion and Quantity of Extruded Nickel Ions Using Two Rotary Instruments (*In Vitro*)

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Abstract

Aim: To compare the effect 2.5% NaOCl, 17% ethylenediaminetetraacetic acid (EDTA), and 0.2% high molecular chitosan solution during root canal preparation using ProTaper Universal and iRace rotary file on corrosion of NiTi instrument and quantity of apically extruded nickel ions.

Materials and methods: Twenty-four extracted human premolars were divided into 4 groups. Group I was the sample with root canal preparation using ProTaper Universal rotary file with 2.5% NaOCl and 17% EDTA, group II was the sample using ProTaper Universal rotary file with 2.5% NaOCl and 0.2% high molecular chitosan solution, group III was the sample using iRace rotary file with 2.5% NaOCl and 17% EDTA, and group IV was the sample using iRace rotary file with 2.5% NaOCl and 0.2% high molecular chitosan solution. After root canal preparation, the quantity of apically extruded nickel ions was evaluated using atomic absorption spectrophotometry (AAS). Instruments were evaluated for corrosion and surface defect using a scanning electron microscope.

Results: A combination of 2.5% NaOCl and 0.2% high molecular chitosan solution caused significantly lower corrosion and surface defect in ProTaper Universal and iRace rotary files and also produced fewer extrusions of nickel ions compared to a combination of 2.5% NaOCl and 17% EDTA. The mean value of corrosion and surface defect scores on iRace was lower and also resulted in less extrusion of nickel ions compared to ProTaper Universal rotary.

Conclusion: 0.2% high molecular chitosan solution can be developed as a chelating material, an alternative to EDTA.

Keywords: 17% EDTA, 0.2% high molecular chitosan solution, 2.5% NaOCl, iRace, ProTaper Universal rotary file.

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Introduction

Chemomechanical preparation of the root canal includes both mechanical instrumentation and antibacterial irrigation and is principally directed toward the elimination of microorganisms from the root canal system.[1] The NiTi instrument was first introduced as a manual instrument in 1988. Considering its properties of superior elasticity and resistance to torsional fracture, Walia introduced nickel titanium endodontic rotary files to the field of endodontics.[2] The specific design characteristics, such as cross-sectional geometry, tip design, taper, rake angle, helix angle, pitch, and radial land, vary and will influence the flexibility, cutting efficiency, and torsional resistance of the instrument.[1]

Some of the rotary files that are widely used today are ProTaper Universal (Dentsply, Maillefer, Ballaigues, Switzerland) and iRace (FKG, La Chaux-de-Fonds, Switzerland) rotary files. ProTaper Universal rotary is a multiple file system with a progressive taper and has a convex triangular cross-section design.[3]

The iRace (instrument reamer with alternating cutting edges) rotary file has been recently introduced as a simplified sequence of the Race system. This instrument has a sharp cutting edge (triangular cross-section) design without radial land, positive rake angle, and electrochemical surface treatment. The surface of new ProTaper and iRace files has an almost similar amount of nickel, titanium, and oxygen components.[2,4]

Sodium hypochlorite (NaOCl) was recommended as an endodontic irrigant by Coolidge in 1919.[5] Various concentrations of NaOCl (0.5–6%) are used for root canal irrigation.[6] It has the unique ability to dissolve necrotic tissue and the organic components of the smear layer, but cannot dissolve inorganic dentin particles and thus cannot prevent the formation of a smear layer during instrumentation.[7] A recognizably efficient protocol for removal of the smear layer is the use of NaOCl at concentrations ranging from 0.5% to 6% and 17% EDTA.[8] EDTA refers to the chelating agent with the formula $(HO_2CCH_2)_2NCH_2CH_2N\ (CH_2CO_2H)_2$. Nygaard-Ostby introduced EDTA to the field of endodontics, in 1957, and recommended using 15% EDTA solution with a pH of 7.3. In endodontics, EDTA 15–17% is used as an irrigation solution.[2,9]

Although the combination of EDTA and NaOCl is the most recommended for optimal irrigation, this combination can cause dentinal erosion to the peritubular and intertubular areas of the dentine. Hulsmann and Hahn reported that the rough surface produced from these two irrigants might become a hiding place

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for bacteria and leads to microleakage. The combination of NaOCl and EDTA can also inhibit the reaction of chloramines from NaOCl. Zaparolli stated that the combination of NaOCl and EDTA could significantly reduce root dentin microhardness.[10–12]

The side effects caused by the use of a combination of NaOCl and EDTA have prompted researchers to look for safer, more biocompatible, and more profitable alternative materials. Among these alternative materials, chitosan has been a very promising irrigant. Chitosan is poly(2-amino-2-deoxy-β(1–4)-D-glucopyranose) with the formula $(C_6H_{11}NO_4)_n$ which can be obtained from chitin deacetylation. High molecular chitosan is chitosan with a molecular weight between 800,000–1,100,000 MW. Chitosan which has a degree of deacetylation of 84.20% and a molecular weight 893,000 MW (high molecular weight) was the first one used in dentistry.[13,14]

Falmini et al. showed that 0.2% chitosan solution was able to remove the smear layer and had almost the same effect as 15% EDTA.[15] Ayu and Trimurni reported that 0.1% chitosan solution was able to remove the smear layer, but there was still a smear plug in the dentinal tubules while 0.2% chitosan solution showed complete removal of the smear layer.[16] Pimenta showed that chitosan solution had chelating properties if used as an irrigant, but it can cause dentinal erosion even though it did not affect intertubular dentin.[17] The development of chitosan as a root canal chelation material is expected to be an alternative irrigant to EDTA.

Even though the benefits of irrigants are essential for chemomechanical preparation, chemical and electrochemical aggressiveness of these solutions may damage the surface of the instruments. Many studies on the susceptibility of endodontic instruments toward corrosion in irrigation solution have been carried out. The corrosion process can be activated during chemomechanical preparation, chemical disinfection, and sterilization.[18] The surface of NiTi instrument mainly consists of oxygen, carbon, and titanium oxides (TiO_2) with smaller amounts of nickel oxides (NiO and Ni_2O_3) and nickel (Ni). Nickel (Ni) may dissolve more easily than titanium (Ti) because its oxide is not so stable.[19]

During the chemomechanical procedure, there is always the possibility of irrigation solution and intracanal debris being extruded beyond the apical foramen even when the working length (WL) is controlled.[20] Glassman reported that apical extrusion of endodontic irrigant routinely occurs *in vivo*.[7] Numerous studies showed that all instrumentation systems produce apical extrusion. However, the type of instrumentation system affects the amount of apical extrusion.[21]

The release of nickel ions caused by corrosion in endodontic instruments extruded to the periradicular region causes unwanted reactions and corresponds to the corrosion rate. Nickel cannot be metabolized in the body and it is known as a haematotoxic, immunotoxic, neurotoxic, genotoxic, reproductive toxic, pulmonary toxic, nephrotoxic, hepatotoxic, and carcinogenic agent.[22] Using instruments and irrigants can minimize the extrusion of nickel ions beyond the apical foramen and will be an advantage for patients and clinicians.

This study aims to compare the effect of 2.5% NaOCl, 17% EDTA, and 0.2% high molecular chitosan solution during root canal preparation using ProTaper Universal and iRace rotary files on corrosion of NiTi instrument and quantity of apically extruded nickel ions.

Materials and Methods

Twenty-four freshly extracted human single-rooted mandibular premolars with mature apices and a curvature of 0°–10° were selected. The teeth were cleaned of debris and soft-tissue remnants and were stored in physiological saline until used in the study. The working length established to 1 mm short of teeth length, and the teeth decoronated to obtain root segments 18 mm long and randomly assigned to four groups. Two coats of nail varnish were applied to the external surface of all roots, except 2 mm apical. Debris and irrigant collection was performed following the Myers and Montgomery method. Teeth was placed inside a glass tube filled with self-curing acrylic resin to obtain a hermetic seal. The teeth and glass tubes were shielded from the operator by a rubber dam during the instrumentation process. A bent 25-gauge needle was forced into the tube's top as a drainage cannula to balance the air pressure inside and outside the tube. These tubes acted as collectors for the debris and irrigant evacuated through the foramen of the root during instrumentation. Endodontic access cavities were prepared with an Endo Access Bur (Dentsply Maillefer) in a high-speed handpiece, and pulpal remnants were extirpated using a broach.

Twenty-four extracted human premolars were divided into 4 groups. Group I was the sample with root canal preparation using ProTaper Universal rotary file with 2.5% NaOCl and 17% EDTA, group II was the sample using ProTaper Universal rotary file with 2.5% NaOCl and 0.2% high molecular chitosan solution, group III was the sample using iRace rotary file with 2.5% NaOCl and 17% EDTA, and group IV was the sample using iRace rotary file with 2.5% NaOCl and 0.2% high molecular chitosan solution. Immediately after instrumentation, the tops with the attached teeth were removed from the collector tubes. The bottles were closed again with new tops. Files were subsequently cleaned in an ultrasonic bath for 400 s, in absolute alcohol 96%, and left for drying on a cotton pellet at room temperature and stored in sterile bottles. Bottles were coded to distinguish one another. Quantity of apically extruded nickel ions was evaluated using AAS and instruments were evaluated for corrosion and surface defect using a scanning electron microscope.

AAS Procedure

To each sample was added 20 mL of concentrated nitric acid and it was heated using an electric heater until the solution was almost dry (±10 mL), then distilled aqua was added to the limit of 15 mL. Making Ni standard solution with concentration 0.1:0.2:0.3:0.4:0.5 ppm and each absorbance was measured by AAS at wavelength (λ) 231.90 nm. The results of absorbance measurements were plotted against concentration to obtain a calibration curve of the Ni standard solution (Fig. 1), then samples were measured by AAS at wavelength (λ) 231.90 nm for Ni analysis.

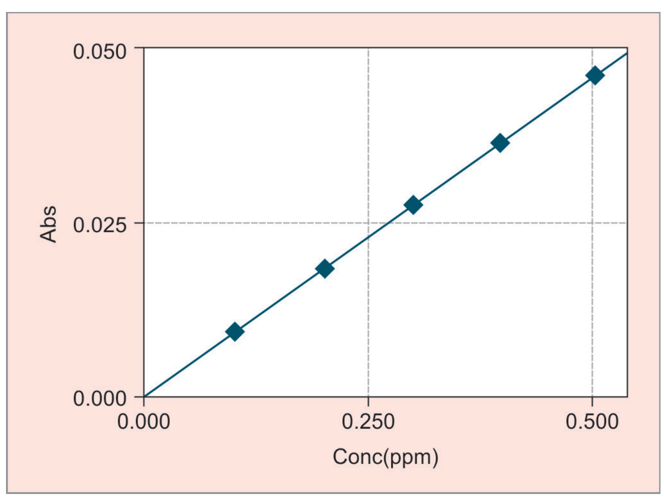


Fig. 1: Calibration curve of Ni standard solution

Scanning Electron Microscopy (SEM) Procedure

The files were cut on the handle to equalize the surface. Files were mounted on SEM specimen mount stubs using double-sided carbon tape. Three files in each group were submitted for SEM evaluation with 1,000× magnification, then the images were compared with a new file.

Results

Analysis of Quantity of Extruded Nickel Ions Using AAS

The mean value of quantity of nickel ions in all groups is presented in Table 1 and Figure 2. In Figure 2, the highest mean value of nickel ions was found in group I (2.415 µg), which was samples using ProTaper Universal rotary file with 2.5% NaOCl and 17% EDTA. The lowest mean value of nickel ions was found in group IV (1.179 µg), which was samples using iRace with 2.5% NaOCl and 0.2% high molecular chitosan solution.

Table 1: Mean value of nickel ions quantity between four groups

Group	*Mean value (µg)*	*Standard deviation (µg)*	*Minimum (µg)*	*Maximum (µg)*
I	2.415	1.875	0.175	5.310
II	2.238	1.383	0.250	4.086
III	1.983	0.746	0.851	2.930
IV	1.179	0.228	0.928	1.458
Total	1.954	1.243	0.175	5.310

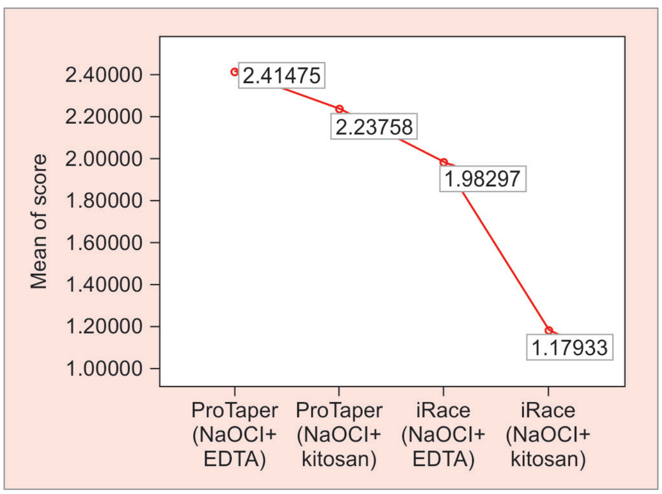


Fig. 2: Mean value based on four groups

Table 2: LSD test of nickel ions quantity

Group	*I*	*II*	*III*	*IV*
I	–			
II	0.805	–		
III	0.549	0.723	–	
IV	0.097	0.151	0.271	–

The next step is the least significance difference (LSD) test. Least significance difference test results are presented in Table 2.

Analysis of Corrosion and Surface Damage of the Instrument Using a SEM

The results of SEM with 1,000× magnification on the surface of new ProTaper Universal and iRace rotary files as a control file are presented in Figure 3. In Figure 3, it can be seen that the surface of the new ProTaper Universal and iRace rotary file did not have defects such as pitting, corrosion, debris, etc. Then, samples A1, A3, A4, B1, B5, B6, C1, C4, C6, D2, D4, and D6 were evaluated using SEM (Figs 4 and 5).

The score for each sample was the sum of all changes noticed on the surface using SEM and assessed with a scoring system from Eggert et al. The Eggert et al. scoring system is presented in Table 3. Scores were given by two observers. Scores of corrosion and surface damage for each sample are presented in Table 4.

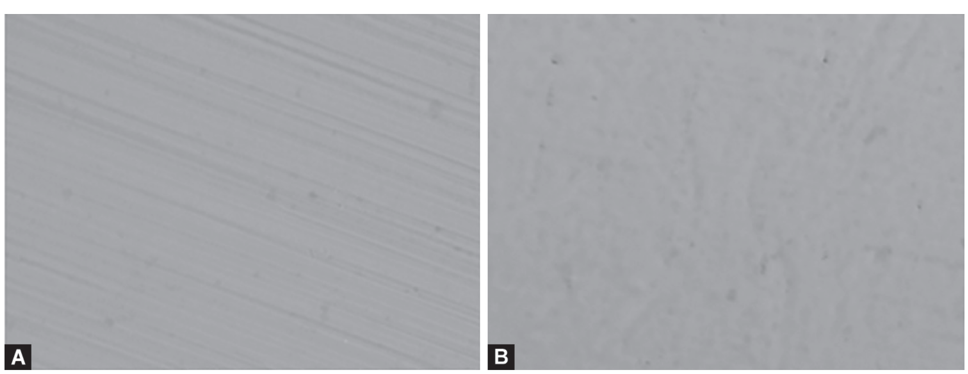
Based on the paired *t* test, there was no difference in the scores between observer 1 and 2 (p value = 0.053 > 0.05), so the data can be taken from observer 1 or 2. Furthermore, the data were taken from the results of observer 1. The mean values of corrosion and surface damage scores in all samples were calculated and are presented in Table 5 and Figure 6.

In Figure 6, the highest mean value of corrosion and surface damage scores was found in group I (36), which was samples using ProTaper Universal rotary file with 2.5% NaOCl and 17% EDTA. The lowest mean value of corrosion and surface damage scores was found in group IV (186,667), which was samples using iRace with 2.5% NaOCl and 0.2% high molecular chitosan solution.

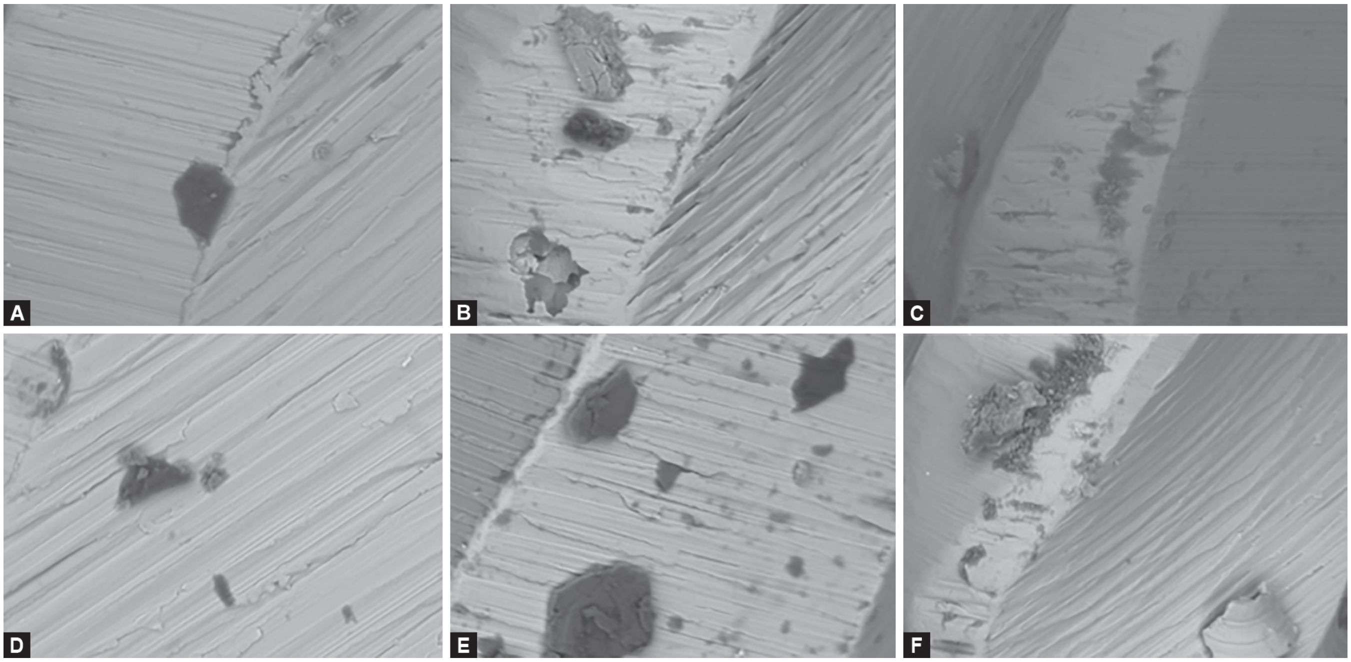
The next step is the LSD test. The results of the LSD test are presented in Table 6.

Discussion

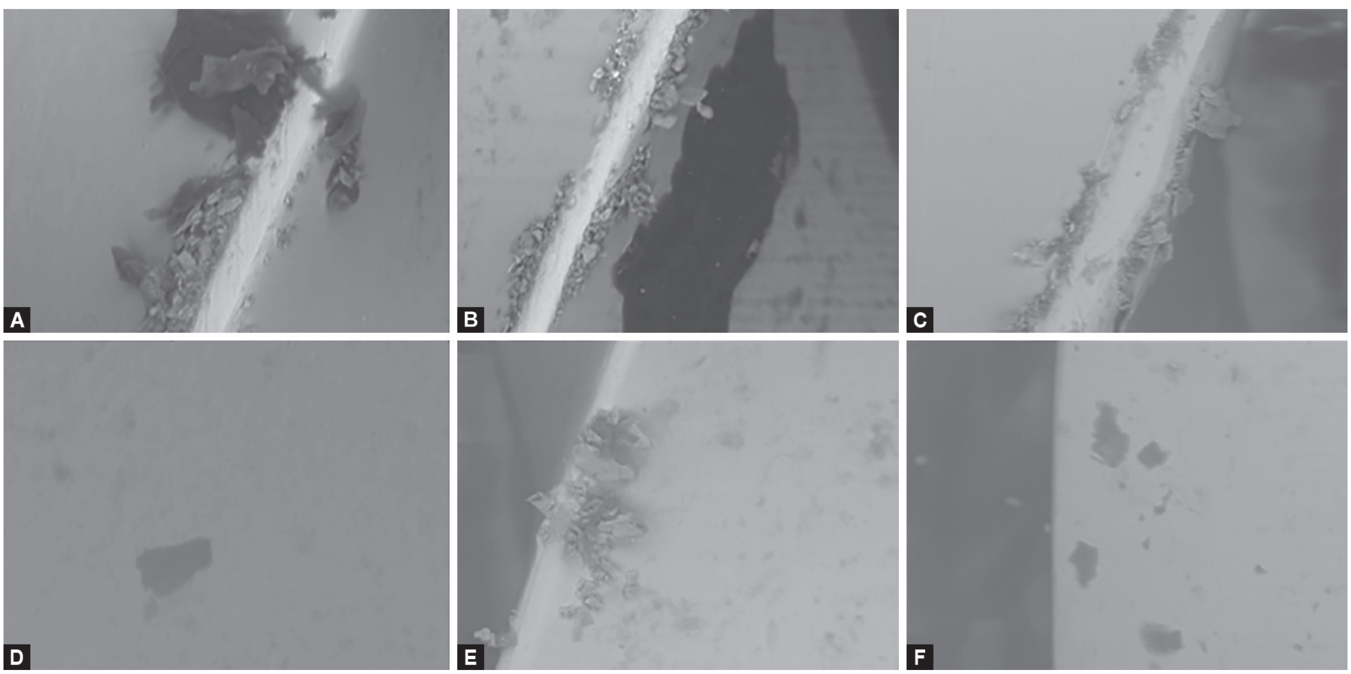
NiTi instruments have a high risk of separation, mainly because of fatigue and torsional shear stress. One factor potentially limiting resistance to fatigue fracture is corrosion that may occur in the presence of irrigation solution.[23] Corrosion patterns of NiTi alloy involved selective removal of nickel from the surface.[24]



Figs 3A and B: (A) New ProTaper Universal rotary; (B) New iRace rotary



Figs 4A to F: (A) SEM results with 1,000× magnification in the sample A1; (B) Sample A3; (C) Sample A4; (D) Sample B1; (E) Sample B5; (F) Sample B6



Figs 5A to F: (A) SEM results with 1,000× magnification in the sample C1; (B) Sample C4; (C) Sample C6; (D) Sample D2; (E) Sample D4; (F) Sample D6

Corrosion is generally associated with specific solutions; so far the most common is chloride. It is known that NaOCl, a chlorine-containing solution, is corrosive to metals. Certain conditions, such as high concentrations of chloride, can interfere with a given alloy's ability to reform a passivating film.[18,24] Popovic et al. indicated that 5.25% NaOCl used as root canal irrigants caused severe corrosion on the surface of the Ni–Ti and stainless steel endodontic files.[18]

In this study, the pH of each irrigant was measured, and the result showed that NaOCl pH was alkaline and EDTA and high molecular chitosan solution pH were neutral. The contact time between the rotary instrument and irrigant on the root canal was 3–5 seconds. The result of this study indicated that the combination of 2.5% NaOCl and 17% EDTA had a significantly higher corrosive effect compared to the combination of 2.5% NaOCl and 0.2% high molecular chitosan solution in ProTaper Universal and iRace rotary file.

This result was in accordance with the results of earlier studies. Ametrano et al. showed that short-term contact with 17% EDTA and 5% NaOCl caused alterations on the surface of ProTaper rotary instruments.[25] Prasad et al. confirmed that short-term contact with 17% EDTA and 5% NaOCl could cause significant surface deterioration of ProTaper and iRace NiTi rotary files.[2] In SES study,

Table 3: Scoring system for corrosion and surface damage (Eggert et al.)

Score	*Criteria*
1	No visible defect
2	Pitting
3	Fretting
4	Micro fractures
5	Complete fracture
6	Metal flash
7	Metal strips
8	Blunt cutting edges
9	Disruption of cutting edge
10	Corrosion
11	Debris

Table 4: Corrosion and surface damage scores for each sample (sum of values for all changes on the surface of the file according to Eggart scoring system)

Sample	*Observer 1*	*Observer 2*
A1	31	27
A3	34	27
A4	43	39
B1	23	23
B5	27	27
B6	30	30
C1	39	36
C4	30	30
C6	27	27
D2	19	19
D4	21	21
D6	16	16

Table 5: Mean value of corrosion and surface damage scores based on 4 groups

Group	*Mean value*	*Standard deviation*	*Minimum*	*Maximum*
I	36	6.245	31	43
II	26.6667	3.51188	23	30
III	32	6.245	27	39
IV	18.6667	2.51661	16	21

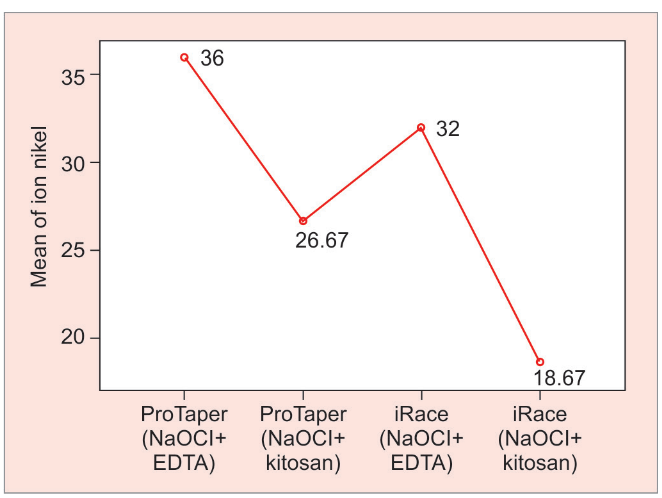


Fig. 6: Mean value of corrosion and surface damage scores based on 4 groups

Table 6: LSD test of corrosion and surface damage score

Group	*I*	*II*	*III*	*IV*
I	–			
II	0.049	–		
III	0.348	0.221	–	
IV	0.003	0.081	0.011	–

immersion with 17% EDTA for 5 minutes caused a significantly increase in surface roughness compared to NaOCl. This may be due to EDTA pH being lower than NaOCl pH.[26] Treacy et al. stated that EDTA acts as a cathodic stimulant inducing high rates of corrosion even in the absence of oxygen and presented that the presence of EDTA in neutral and alkaline solutions accelerates the rate of dissolution of metals.[27]

The result of this study indicated that the combination of 2.5% NaOCl and 0.2% high molecular chitosan solution resulted in significantly lower corrosive and surface damage than the combination of 2.5% NaOCl and 17% EDTA in ProTaper and iRace rotary files. Chitosan has good binding ability with metal ions due to its amine group which is responsible for taking metal cations through the chelation process. Metal cation absorption utilizes the presence of free electron pairs in the –OH and $–NH_2$ groups. The electron pairs in the –OH and $–NH_2$ group will act as ligands that can interact with metal cations through the mechanism of covalent bonding. Chitosan in neutral pH is able to complex dangerous metal ions.[28] Several studies have reported the use of chitosan as a coating to improve surface corrosion resistance in NiTi alloy. Ahmed et al. with elemental analysis stated that NiTi alloys coated with chitosan had less release of nickel ions compared to uncoated NiTi alloys. According to Goryczka et al., coating chitosan did not cause structural changes in the alloy NiTi and maintained the character and the reversibility of the martensitic transformation.[29,30]

Stokes et al. described pitting corrosion of the file surface after 1 hour of immersion in 5.25% NaOCl and speculated that manufacturing factors affect the corrosion of NiTi endodontic instruments. This study used a crown down pressureless technique to prepare the root canal systems and compared between ProTaper Universal and iRace rotary files against corrosion and surface damage scores. The mean values of corrosion and surface damage scores in iRace were lower than those in ProTaper Universal rotary files using either a combination of 2.5% NaOCl and 17% EDTA or 2.5% NaOCl and 0.2% high molecular chitosan solution. This result was in accordance with Sood et al. which showed that ProTaper causes maximum corrosion compared to iRace. This can be due to machining marks on the file surface that act as crevices and initiate corrosion. RaCe files showed relatively less corrosion when compared to ProTaper. This is speculated to be due to grinding and mechanical polishing done during the manufacturing process to remove machining marks. Also, the file coated with silver nitride increases the hardness and is more resistant toward corrosion.[31]

This study also compared the effect of the combination of 2.5% NaOCl and 17% EDTA with 2.5% NaOCl and 0.2% high molecular chitosan solution during the preparation of the root canal system using ProTaper Universal and iRace rotary files on the apical extrusion of nickel ions using AAS. The combination of 2.5% NaOCl and 0.2% high molecular chitosan solution resulted in less extruded nickel ions compared to the combination of 2.5% NaOCl and 17% EDTA on ProTaper Universal and iRace rotary files. This was possible because the combination of 2.5% NaOCl and 0.2%

high molecular chitosan solution caused a lower corrosive effect (according to the mean value of corrosion and surface damage score in this study), resulting in a slight release of nickel ions compared to the combination of 2.5% NaOCl and 17% EDTA. The result of this study was also the same as the result of Anggi and Trimurni which showed that 0.1% and 0.2% chitosan solution resulted in smaller debris extrusion compared to 2.5% NaOCl and 17% EDTA, and the combination of 17% EDTA and 2.5% NaOCl resulted in up to 2 times more extrusion of debris.[32]

In this study, iRace produced less extrusion of nickel ions compared to ProTaper Universal rotary files using either a combination of 2.5% NaOCl and 17% EDTA or 2.5% NaOCl and 0.2% high molecular chitosan solution. This result was the same as Nagaveni et al. which concluded that RaCe extruded the least debris extrusion compared to ProTaper, Hero-Shaper, and K3 rotary systems. RaCe instruments have a nonconvex triangular cross-sectional design, smaller core diameter, and short twisted cutting edges alternating with straight edges which allows more space to carry debris out toward the orifice thus avoiding its compaction in the root canal while ProTaper universal instruments have a convex triangular cross-section and their debris space is smaller than RaCe. ProTaper system leads to a significantly large amount of extruded debris due to faster, aggressive system with its characteristic design features, which removes a substantial amount of dentin in a shorter period of time, and is unable to displace the debris coronally with the same efficiency as it cuts, thus poses an increased risk of debris and irrigant apical extrusion. Also, the long pitch design of the ProTaper instruments might cause a greater amount of debris extrusion.[20,33,34]

In this study, the quantity of extruded nickel ions is still within the threshold that can be tolerated by the body (the estimated maximum nickel that can be received by the body is 200–300 µg per day). But increasing the amount of nickel in the body can also be related to environmental exposure (air), food, drinks, smoking, and skin contact. In addition, nickel ions extruded from the apical foramen will enter the blood vessels in the periapical region that contain many cells such as vascularized periodontal ligaments, then spread through the systemic circulation, and enter the organs, so it is important to minimize the number of extruded nickel ions from the apical foramen when preparing the root canal systems.

Conclusion

According to all benefits found in 0.2% high molecular chitosan solution, 0.2% high molecular chitosan solution can be developed as a chelating material, an alternative to EDTA. Using instruments and irrigants that can minimize apical extrusion will be an advantage both for practitioners and patients.

Clinical Significance

Using instruments and irrigant that can minimize apical extrusion will be an advantage both for practitioners and patients.

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