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Ionic concentration in periradicular medium after dissolution of endodontic file fragments: an in vitro study

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Abstract: The aim of this study was to obtain ionic quantification in periradicular medium after diffusion tests of the solution used inside root canals during the electrochemical dissolution of endodontic file fragments and the NiTi-containing dissolution product via an apical foramen. Thirty single-rooted extracted human teeth had root canals prepared and were attached to Eppendorf tubes filled with sterile saline. The samples were divided into 3 groups (n = 10) according to the solution used inside the root canal during the diffusion tests: Group 1: [NaF 12 g/L + NaCl 1 g/L]; Group 2: [NaF 12 g/L + NaCl 1 g/L + NiTi 0.50 g/L]; Group 3: [NaF 6 g/L + NaCl 0.5 g/L + NiTi 0.25 g/L]. The sample in each Eppendorf tube was then analyzed to assay the ionic quantification in periradicular medium. The groups were compared in relation to ionic quantifications (Kruskal-Wallis and Dunn's tests, $p \leq 0.05$). Group 2 showed significantly higher F^-, Ni and Ti quantities than groups 1 and 3 ($p < 0.05$). Group 3 showed significantly higher Ti and Ni quantities than group 1, where no measurable quantities of Ti and Ni were observed ($p < 0.05$). The conclusions were that a 50% dilution of the NiTi-containing dissolution product resulted in significantly lower F^-, Ni and Ti quantities compared to the undiluted product. The quantifications observed here suggest that irrigation is recommendable during the electrochemical dissolution process to reduce the resultant ion concentrations in both the root canal and the periradicular medium.

Keywords: Diffusion; Electrochemical Techniques; Endodontics; Nickel; Titanium.

Introduction

The removal of fractured endodontic instruments from root canals has been widely reported, and many devices and mechanical methods have been proposed to attain this objective.[1,2,3] Electrochemical dissolution of endodontic instrument fragments has been described as an alternative for its retrieval.[4] The partial dissolution of nickel-titanium (NiTi) and stainless steel instrument fragments has been accomplished in simulated root canals and in root canals of extracted human teeth after electrochemically induced dissolution in NaF- and NaCl-containing solutions for different periods of time.[5-8] This technical procedure allows the reestablishment of the canal path with manual path files.[5,6,8,9]

There is a concern that the dissolution of file fragments inside the root canal could be toxic to periradicular tissues. The Ni ions generated during the dissolution of NiTi endodontic file fragments, for instance, could result in the suppression of cell proliferation in tissues such as the periodontal ligament and alveolar bone.[10-13] During clinical practice, irrigation solutions can be in contact with those tissues and compromise their normal functions due to their cytotoxic potential.[14] Irrigation solutions widely used in endodontics, such as EDTA and NaOCl, can cause tissue damage due to their cytotoxic effects.[14,15] NaF solutions with diluted NiTi fragments were cytotoxic to the periodontal ligament cells after 24 hours of exposure.[12] However, the NaF- and NaCl-containing solution used to induce the electrochemical dissolution of endodontic file fragments and the same solution containing the dissolution product showed lower cytotoxicity than NaOCl in an experimental protocol to assay the viability of human fibroblasts.[13]

Determining the amount of solution likely to be in contact with the periapical tissues is important to evaluate its toxicity. Under in vivo conditions, a restricted area of the periradicular tissues is exposed to irrigation solutions that are diluted within interstitial fluids, which results in a decrease in their concentration.[13,16] The extrusion of endodontic irrigation solution via the apical foramen has been evaluated.[17-21] According to one study, the extrusion of NaOCl was minimal using irrigation with conventional syringes, PUI and the EndoActivator method, with most teeth showing no extrusion of NaOCl.[19] However, others observed that the apical foramen diameter and the methods of irrigation and agitation showed an important influence on the quantity of endodontic irrigation solution extruded via the apical foramen.[17,18,20,21]

In this context, the purpose of the present study was to obtain ionic quantification in periradicular medium after diffusion tests of the solution used inside root canals during electrochemical dissolution of NiTi file fragments and the same solution containing the dissolution product via the apical foramen of extracted human teeth.

Methodology

The study was approved by the local research ethics committee (protocol number 1.373.833). The methods used for the sample preparation and the chemomechanical preparation of the root canals were based on Amaral et al.'s description.[13] The sample size calculation was performed using an alpha of 0.05 and a power of 0.80. Based on these assumptions, the desired sample size was 8 teeth. Thirty single-rooted human teeth were extracted for therapeutic reasons and stored in 10% formalin until utilization. The teeth were then washed for 24 hours under running water to eliminate any trace of formalin. A diamond disk was used to remove the dental crowns, where the root length was standardized at 13 mm. The root canals were prepared using a ProTaper NiTi rotary system (Dentsply Sirona Endodontics, Ballaigues, Switzerland). The cervical and middle thirds of the root canals were shaped using S1 and S2 instruments starting 5 mm short of the apical foramen. The patency length was determined using a size 10 k-file (Dentsply Sirona Endodontics, Ballaigues, Switzerland) inserted up to the apical foramen. The working length was determined to be 1 mm short of the patency length. Foramen standardization was performed with a size 30 NiTi - Flex manual file (Dentsply Sirona Endodontics, Ballaigues, Switzerland) at working length. The apical work was performed with S1, S2, F1, F2 and F3 instruments at the working length. All root canals were irrigated with 20 mL of 5.25% NaOCl during chemomechanical preparation. Final irrigation was performed with 5 mL of 17% EDTA, followed by 5 mL of distilled water. The root canals were dried with F3 ProTaper sterile absorbent paper points (Dentsply Sirona Endodontics, Ballaigues, Switzerland).

The method used here during the diffusion tests was based on the Myers & Montgomery[22] study. According to that method, each sample was attached to a 2.0-mL Eppendorf tube using an individual silicone plug (Figure). The Eppendorf tubes were filled with 0.8 mL of sterile saline solution, while F3 ProTaper absorbent paper points were used in the working length of the root canal. A disposable 27-G needle was inserted into the silicone plug, simulating a cannula, to balance the

internal and external pressures.[22] The samples were randomly divided into 3 groups (n = 10) according to the irrigation solution used inside the root canal during the diffusion tests: Group 1: [NaF 12 g/L + NaCl 1 g/L]; Group 2: [NaF 12 g/L + NaCl 1 g/L + NiTi 0.50 g/L]; Group 3: [NaF 6 g/L + NaCl 0.5 g/L + NiTi 0.25 g/L].

Preparation of solutions

The [NaF 12 g/L + NaCl 1 g/L + NiTi 0.50 g/L] solution was prepared as previously described by Amaral et al.[13] According to that method, the [NaF 12 g/L + NaCl 1 g/L] solution with pH 5.0 was used to prepare the [NaF 12 g/L + NaCl 1 g/L + NiTi 0.50 g/L] solution, where the tips of three ProTaper F1 files (13 mm), corresponding to a mass of 50 mg of NiTi, were used. A three-electrode electrochemical cell was used, containing a saturated calomel electrode as the reference, a platinum electrode as the counter electrode and a NiTi F1 ProTaper file as the working electrode. The electrodes were immersed in 100 mL of the [NaF 12 g/L + NaCl 1 g/L] solution and connected to a computer-controlled AutoLab potentiostat (Metrohm, Herisau, Switzerland). Instrument immersion was limited to 13.0 mm from the tip of the file. A constant anodic potential of +0.7 $V_{(SCE)}$ was applied until the total dissolution of the immersed portion of the ProTaper F1 NiTi file occurred, while the potentiostat registered the anodic current. The assay was performed in triplicate. For each assay, three files were dissolved sequentially in the same solution, and the resulting solution was then stored in two 50-mL conical Falcon tubes. To prepare the [NaF 6 g/L + NaCl 0.5 g/L + NiTi 0.25 g/L] solution, 30 mL of the [NaF 12 g/L + NaCl 1 g/L + NiTi 0.50 g/L] solution was diluted in 30 mL of distilled water. Both solutions were analyzed by atomic absorption spectroscopy in the acetylene gas phase at 232 nm (Shimadzu AA6800, Kyoto, Japan) for further Ni quantification.

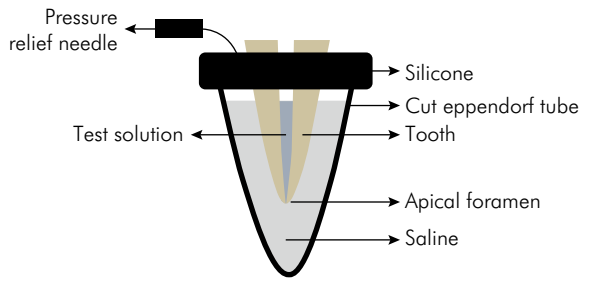

Figure 1. Schematic of the test system. The sample was attached to an Eppendorf tube using an individual silicone plug. A disposable 27-G needle was inserted into the silicone plug to balance the internal and external pressures. The Eppendorf tube was filled with 0.8 mL of sterile saline solution. The root canal was filled with 0.02 mL of the test solution.

Diffusion test

The root canal of each sample was filled with 0.02 mL of the test solution. The solution was maintained in the root canal for two hours. At the end of the test, the root canal was abundantly irrigated with distilled water and dried with ProTaper F3 sterile absorbent paper points (Dentsply Sirona Endodontics, Ballaigues, Switzerland). The root and the silicone plug were removed from the Eppendorf tube, and the solution was analyzed to obtain the F^-, Cl^-, Ni and Ti quantifications. The sample solution of each Eppendorf tube was vortex agitated and diluted 1:100 in type I water to assay the quantification of the ions fluoride and chloride or 1:50 in 2% HNO_3 to assay the quantification of Ni and Ti. An ion chromatograph with a conductivity detector (Dionex, ICS 1000, Sunnyvale, USA) was used to quantify the ions F^- and Cl^-, and an optical emission spectrometer with inductively coupled plasma (Horiba Jobin Yvon, Ultima 2, Quioto, Japan) was used to quantify Ni and Ti. A single dilution of the original sample was measured seven consecutive times to obtain the mean value of each chemical element quantification in each sample.

Statistical analysis

A seven-point calibration curve with external standardization was used to obtain the F^-, Cl^-, Ni and Ti quantifications. The reported expanded measurement uncertainty was established as the combined standard uncertainty multiplied by the coverage k factor. The normality of the data was analyzed by a Shapiro-Wilk test. The nonparametric Kruskal-Wallis test and Dunn's post-test were applied to compare the groups in relation to the F^-, Cl^-, Ni and Ti quantifications. Differences between groups were considered significant at $p \leq 0.05$ (95% confidence interval). All calculations were performed using a commercially available statistical software package (GraphPad Software, Inc., La Jolla, USA).

Results

The atomic absorption spectroscopy analysis of the irrigation solution used inside the root canals during the diffusion tests showed Ni concentration levels of 239.8 and 125.1 mg/L in groups 2 and 3, respectively. Table depicts the results of the F^-, Cl^-, Ti and Ni quantifications in the periradicular medium after diffusion tests, when group 2 showed significantly higher F^- quantity than groups 1 and 3 ($p < 0.05$). Comparison between groups 1 and 3 demonstrated no statistically significant difference (NS). Group 1 showed a significantly higher Cl^- concentration level than group 3 ($p < 0.05$), but no statistically significant difference was observed for the comparison between groups 1 and 2 or groups 2 and 3 (NS). Group 1 displayed no measurable quantities of Ti or Ni, while group 2 showed significantly higher Ti and Ni quantities than group 3 ($p < 0.05$), which showed significantly higher quantities of both elements than group 1 ($p < 0.05$).

Discussion

The aim of the present study was to obtain ionic quantification in periradicular medium after diffusion tests of the solution used in the induced electrochemical dissolution of NiTi file fragments and the same solution containing the dissolution product via an apical foramen. The results showed significantly higher F^-, Ni and Ti quantities in group 2 than in groups 1 and 3. Group 3 showed significantly higher Ti and Ni quantities than group 1, where no measurable quantities of Ti and Ni were observed.

The method used here during the diffusion tests was based on the Myers & Montgomery study.[22] Each sample was attached to an Eppendorf tube using an individual silicone plug, where a needle was inserted to balance the internal and external pressures. Previous studies used different periradicular media during extrusion tests of endodontic irrigants or medications, such as distilled water, ultrapure deionized water, 0.2% agarose gel or even air.[17-20,23,24] Distilled water and ultrapure deionized water are hypotonic media compared to the substances used inside the root canal. Consequently, there is a tendency for ions to migrate from the root canal to the periradicular medium by osmotic pressure. Such an effect is not observed in human tissues that are hypertonic compared to distilled water and ultrapure deionized water. Agarose gel would not enable the methods of analysis used here, and the consistency might not be the same for the periradicular tissues. Conversely, air offers no resistance to irrigant extrusion, which is not comparable to periradicular tissues. In this context, sterile saline solution was used here because it simulates the osmotic conditions present in periradicular tissues and offers some resistance to the free extrusion of irrigants. The saline solution tends to offer less resistance to the extrusion of irrigants than the periradicular tissues, but it guarantees more security in the data interpretation than a medium that offers more resistance than the tissues.

In the present study, the irrigation solutions used inside the root canal during the diffusion tests were selected according to previous studies that tested the active dissolution of endodontic file fragments and its effects on dentine hardness, topography and human fibroblast viability.[6,7,9,13] The NiTi concentration of the irrigation solution of group 2 was determined according to Amaral et al.,[13] who considered the mass of 0.05 mg of dissolved NiTi and the volume of the root canal of 0.1 mL.[12, 25] The irrigation solution of group 3 was the dilution of the solution used in group

Table. Ionic quantifications of fluoride (Fl^-), chloride (Cl^-), titanium (Ti) and nickel (Ni) in periradicular medium.

Group	Fl^- (mg/kg)	Cl^- (mg/kg)	Ti (mg/kg)	Ni (mg/kg)
1	52.76 ± 45.83^{a}	5476.00 ± 115.75^{c}	$< 0.1^{e*}$	$< 0.3^{h**}$
2	98.13 ± 47.54^{b}	$5397.80 \pm 53.11^{c,d}$	3.15 ± 1.92^{f}	4.37 ± 2.41^{i}
3	48.31 ± 19.54^{a}	5331.00 ± 162.84^{d}	1.70 ± 0.68^{g}	2.22 ± 0.85^{i}

*Values represent the mean ± standard error (n = 10). Different letters indicate statistically significant differences between groups ($p < 0.05$). **Quantification limit of the method. Group 1: [NaF 12 g/L + NaCl 1 g/L]; group 2: [NaF 12 g/L + NaCl 1 g/L + NiTi 0.50 g/L]; group 3: [NaF 6 g/L + NaCl 0.5 g/L + NiTi 0.25 g/L].

2 in the same volume of distilled water to simulate the dilution obtained in the root canal by irrigation during the dissolution process. According to the atomic absorption results, the expected Ni concentrations of the irrigation solutions used in groups 2 and 3 were in agreement with the concentration desired for this study.

In the diffusion tests of the present study, the root canal was filled with 0.02 mL of the test solution, which was maintained in the root canal for two hours. This time was used according to Amaral et al.,[8] who observed a significant reduction in the NiTi instrument fragment volume as a consequence of the electrochemical dissolution process in root canals of extracted human maxillary molars using the solution restricted to a small reservoir. According to Amaral et al.'s study, this time is close to what could be considered clinically acceptable and a good starting point to bypass the fragment.[8]

The results presented here showed mean Fl^- concentrations of 52.76, 98.13 and 48.31 mg/kg in groups 1, 2 and 3, respectively. These values are lower than those in a range from 12.3 to 22.6 g/L, which have been largely applied in dentistry to inhibit dental structure demineralization.[26,27,28] Consequently, the amount of Fl^- ions in the periradicular medium does not appear to be relevant during the dissolution process in clinical practice. The results presented here showed mean Cl- concentrations of 5476.0, 5397.8 and 5331.00 mg/kg in groups 1, 2 and 3, respectively. These concentrations are close to the Cl^- concentration of 5460 mg/L present in the saline solution used as periradicular medium during the diffusion tests. Consequently, the amount of Cl^- ions in the periradicular medium also does not appear to be relevant during the process.

In the present study, the results showed mean Ni concentrations of 4.37 and 2.22 mg/kg in groups 2 and 3, respectively, which corresponded to diffusions of 7% and 6.7% of the Ni present inside the root canal to the periradicular medium. Although the test used here may not be considered an extrusion test but a diffusion test, if we consider that the root canals were filled with 0.1 ml of the irrigant, approximately 0.007 mL of the irrigant would have extruded to the periradicular medium. This value is compatible with Reis et al.[20], who observed irrigant extrusion ranging from 0.00067 to 0.01719 mL using different final agitation techniques in immature teeth. Rodríguez-Figueroa et al.[19] observed volume values of sodium hypochlorite extrusion ranging from 0.001 to 0.026 mL using different final irrigation techniques, which is also compatible with our results. However, our results are discrepant from Vidas et al.,[21] who observed values ranging from 0.2 to 2.3 g of extruded irrigant that correspond to approximately 0.2 to 2.3 mL. This discrepancy may be related to the different methods used by those authors, with air as the periradicular medium and consequently no resistance to irrigant extrusion.

A normal cellular response could be observed at Ni concentrations between 0.4 and 0.9 mg/L, but Ni concentrations between 4.0 and 9.0 mg/L caused cellular function impairment and cell proliferation inhibition.[10,13] Consequently, the values observed here in group 2 can result in cellular function impairment and cell proliferation inhibition. However, these values can be attained only if the dissolution of the entire fragment is obtained without irrigation during the process. The irrigation performed during the dissolution process removes the Ni ions from the root canals and consequently reduces their concentration. In addition, only partial dissolution of the fragments was achieved in the studies where the dissolution method was applied.[5-9] According to the values observed in group 3, the dissolution of half of the fragment generates Ni ion concentrations lower than the range where cellular function impairment and cell proliferation inhibition occur,[10,13] even if no irrigation is performed during dissolution.

These considerations are partially in agreement with Amaral et al.,[13] who used diffusion tests of [NaF 12 g/L + NaCl 1 g/L], [NaF 12 g/L + NaCl 1 g/L + NiTi] and 5.25% sodium hypochlorite solutions via the apical foramen of human teeth to obtain extracts from the periradicular medium to evaluate its effect on human fibroblasts. They observed that pure extracts of all solutions killed more than 90% of the cells. At 50% and 25% dilutions, the [NaF 12 g/L + NaCl 1 g/L + NiTi] extract killed approximately 80% and 20% of the cells, respectively. The extracts of all test solutions were nontoxic at a 12.5% dilution.[13] Even

with partially discrepant results, they observed that the reduction in the solution concentration reduced its cytotoxicity. In addition, they concluded that the tested solutions demonstrate lower cytotoxicity than NaOCl, supporting the potential clinical use of the [NaF 12 g/L + NaCl 1 g/L] solution to remove endodontic file fragments by electrochemical dissolution.[13] However, our considerations are discrepant from Mitchell et al.,[12] who observed that NaF solution and artificial saline containing NiTi were cytotoxic to periodontal ligament cells after 2 and 24 hours. This outcome could be due to the longer exposure time, sample concentration, or distinctiveness of the method, as previously described.[13] However, the more relevant aspect is that they used a solution with a Ni concentration equal to 3.96×10^3 mg/L, which is much higher than the values of 4.37 and 2.22 mg/kg observed here.

The results presented here showed mean Ti concentrations of 3.15 and 1.70 mg/kg in groups 2 and 3, respectively. As previously described, a Ti concentration of 13.00 mg/L is required to induce the significant cytotoxicity of gingival epithelial cells.[29] Consequently, the amount of Ti ions observed here does not appear to be relevant during the dissolution process. However, cytotoxicity is significantly increased after exposure of gastrointestinal tract cells to Ti concentrations higher than 0.045 mg/L.[30] In part, these results are in agreement with Amaral et al.[12], who observed that the [NaF 12 g/L + NaCl 1 g/L + NiTi] extract required dilution to 12.5% to become nontoxic. This reinforces the importance of irrigation during the dissolution process to remove the resultant ions from the root canals and consequently reduce their concentration.

Conclusion

The F^-, Ni and Ti quantities in the periradicular medium were significantly lower after diffusion tests using the [NaF 12 g/L + NaCl 1 g/L] solution inside the root canal than the NiTi-containing dissolution product. A 50% dilution of the NiTi-containing dissolution product resulted in significantly lower F^-, Ni and Ti quantities compared to the undiluted product. Cl^- quantification in the periradicular medium does not appear to be relevant in any test. The quantifications observed here suggest that root canal irrigation is strongly recommendable during the induced electrochemical dissolution of endodontic file fragments to reduce the resultant ion concentration in both the root canal and the periradicular medium.

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