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ÁREA DE CONCENTRAÇÃO EM ENDODONTIA

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AVALIAÇÃO MICROSCÓPICA DAS PAREDES DENTINÁRIAS APÓS DISSOLUÇÃO ELETROQUÍMICA DE INSTRUMENTOS DE NÍQUEL-TITÂNIO FRATURADOS EM DENTES HUMANOS EXTRAÍDOS

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Dissertação apresentada ao Programa de Pós-Graduação em Odontologia da Pontifícia Universidade Católica do Paraná, como parte dos requisitos para obtenção do título de Mestre em Odontologia, Área de Concentração em Endodontia.

Orientador: Prof. Dr. Everdan Carneiro.

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ARTIGO EM PORTUGUÊS

Avaliação microscópica das paredes dentinárias após dissolução eletroquímica de instrumentos de níquel-titânio fraturados em dentes humanos extraídos

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Resumo

Objetivo: Avaliar as paredes dentinárias por microscopia óptica (MO) e microscopia eletrônica de varredura (MEV) após o processo de dissolução eletroquímica de instrumentos de níquel-titânio fraturados em dentes humanos extraídos variando a concentração de cloreto de sódio das soluções testadas. **Material e Método**: Quarenta e cinco incisivos inferiores foram submetidos ao processo de dissolução eletroquímica com soluções fluoretadas, uma saturada em cloreto de sódio e outra não: Solução 1 - NaF 12g/L + NaCl 1g/L, pH = 5,0 (n = 15); Solução 2 – NaF 12g/L + NaCl 180g/L, pH = 5,0 (n = 15). Utilizou-se água destilada como controle (n = 15). Trinta dentes foram analisados para cada solução (n = 10) em MO. Quinze para cada solução (n = 5) em MEV. Para MO, os dentes passaram pelo processo de descalcificação em EDTA 5% durante 10 meses. Em seguida foram realizados seis cortes transversais semiseriados de 5µm, processados e corados com hematoxilina e eosina. Para avaliação em MEV, 15 raízes foram clivadas longitudinalmente e irrigadas com EDTA 17% por 1 minuto para remover a smear layer. Cada hemisecção foi analisada totalizando 10 paredes dentinárias para cada solução. **Resultados**: Uma vez que a variável dependente apresentou o resultado de estrutura inalterada, em todos os grupos, conclui-se que não existe diferença estatisticamente significante na percentagem de sucesso entre os grupos. O teste qui-quadrado indicou P = 1. **Conclusão**: A estrutura dentinária manteve-se inalterada e estruturada, sem danos decorrentes do método de dissolução eletroquímica.

Palavras-chave: Endodontia. Dissolução Eletroquímica. Níquel-Titânio. Microscopia. Dentina.

Introdução

A fratura de instrumentos de níquel-titânio no interior do canal radicular pode resultar em um desafio para o profissional, principalmente quando é constatada uma infecção prévia, e a limpeza e desinfecção do sistema de canais radiculares tornam-se inviáveis (Souter & Messer 2005). A procura de métodos conservadores para remoção de instrumentos endodônticos fraturados como a dissolução eletroquímica tem sido pesquisados.

Pesquisas abrangendo a dissolução eletroquímica de instrumentos endodônticos envolvem o uso de soluções fluoretadas. Alterações na concentração dessas soluções foram propostas e os resultados demonstraram uma significativa diminuição do tempo de aplicação da corrente elétrica até que a ultrapassagem do fragmento por um instrumento de pequeno calibre fosse obtida. (Aboud et al., 2014, Ormiga et al., 2015, Kowalczuck et al., 2017).

Em 2017, Kowalczuck et al., realizaram um experimento confrontando os valores da corrente elétrica obtidos no processo de dissolução eletroquímica empregando duas soluções: uma fluoretada com baixa concentração de cloreto de sódio (NaF 12g/L + NaCl 1g/L, pH = 5,0), e outra fluoretada saturada com cloreto de sódio (NaF 12g/L + NaCl 180 g/L, pH = 5,0). Após o experimento foi quantificada a dissolução dos instrumentos fraturados no terço apical em incisivos inferiores humanos. Os resultados evidenciaram que a saturação da solução fluoretada com cloreto de sódio implicou em aumento dos valores de corrente elétrica, e em maiores reduções de comprimento de fragmentos de instrumentos fraturados em canais radiculares humanos submetidos ao processo de dissolução eletroquímica.

A permeabilidade da dentina poderia ser um fator negativo caso as soluções fluoretadas testadas implicassem em algum dano à estrutura das paredes. O pH 5.0 dessas soluções também é um fator a ser considerado. Embora a acidificação da solução favoreça o processo de corrosão e assim permita a fragilização da liga exposta à solução, uma possível ação nociva à estrutura dentinária limitaria o uso de substâncias de pH ácido (Aboud et al., 2014, Kowalczuck et al., 2017).

O presente estudo teve como objetivo avaliar por meio da microscopia eletrônica de varredura (MEV) e microscopia óptica (MO) se as soluções

fluoretadas saturadas ou não com cloreto de sódio, após o processo de dissolução eletroquímica de instrumentos de níquel-titânio fraturados em dentes humanos extraídos, podem causar algum tipo de dano na estrutura da dentina radicular. A hipótese nula é que as soluções fluoretadas, com baixa concentração ou saturadas, não alterem a dentina radicular após o experimento de dissolução eletroquímica.

Material e Método

Quarenta e cinco incisivos inferiores tiveram limas ProTaper Universal F1 fraturadas intencionalmente a 3mm da ponta na porção apical dos canais radiculares. Cada canal foi submetido ao processo de dissolução eletroquímica com potencial de + 1.5 V para a solução 1 (NaF 12g/L + NaCl 1 g/L, pH = 5,0) e + 0,3 V para a solução 2 (NaF 12g/L + NaCl 180 g/L, pH = 5,0) durante 30 minutos. Ao término do processo, as amostras foram preparadas para avaliação microscópica das paredes dentinárias.

Para ambas as avaliações foram analisadas a presença ou ausência de danos à estrutura dentinária na superfície do canal radicular ou nos túbulos dentinários de cada raiz de acordo com os seguintes critérios:

1 = Erosão, reabsorção ou alterações nos túbulos dentinários.

2 = Estrutura inalterada.

1.1. MICROSCOPIA ELETRÔNICA DE VARREDURA

Três grupos foram testados: Solução 1 (n = 5), com baixa concentração de cloreto de sódio; Solução 2 (n = 5), saturada e água destilada (n = 5), grupo controle. Os espécimes foram desgastados longitudinalmente em profundidade nas paredes vestibular e lingual com um disco diamantado de dupla-face. Com o auxílio de um mini formão e um martelo cirúrgico realizou-se a clivagem das raízes em duas porções. As hemisecções radiculares foram irrigadas com ácido etilenodiaminotetracético a 17% (EDTA) por 1 minuto afim de remover a smear layer, sem contudo comprometer a integridade dentinária. As raízes foram submetidas a microscopia eletrônica de varredura (MEV).

(Vega 3 SEM – Analytical Scanning Electron Microscope, TESCAN, Brno, República Tcheca). Em ampliações de 250X e 500X.

1.2. MICROSCOPIA ÓPTICA

Os trinta dentes separados para a avaliação histológica foram divididos em três grupos: Solução 1 com baixa concentração (n = 10), solução 2 saturada (n = 10) e grupo controle, água destilada (n = 10). Esses dentes foram submetidos ao processo de desmineralização em EDTA 5% durante 10 meses. Após desmineralização total, os dentes foram clivados e submetidos ao processamento histotécnico. A partir disso foram realizados seis cortes semiseriados de 5µm cada, processados para análise microscópica e corados com hematoxilina e eosina.

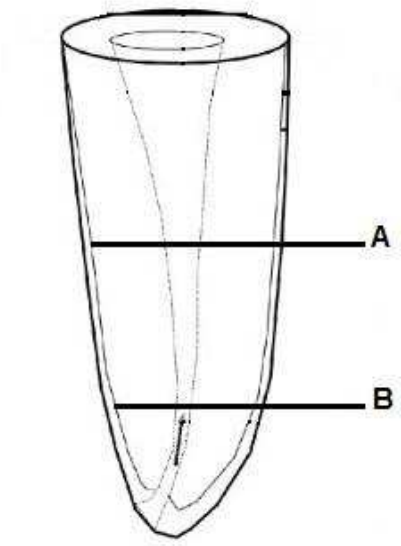

Figura 1. Áreas dos cortes semiseriados para análise em M.O

Resultados

As paredes dentinárias radiculares foram avaliadas a fim de observar a estrutura das mesmas após o experimento de dissolução eletroquímica, sugerindo que as soluções não geraram alteração na estrutura dentinária, tanto para o MEV (Figura 2) como para microscopia óptica (Figura 3). Uma vez que a variável dependente apresentou resultado de estrutura inalterada em todos os

grupos, conclui-se que não existe diferença estaticamente significante na percentagem de sucesso entre os grupos. O teste qui-quadrado indicou P = 1.

Microscopia Eletônica de Varredura

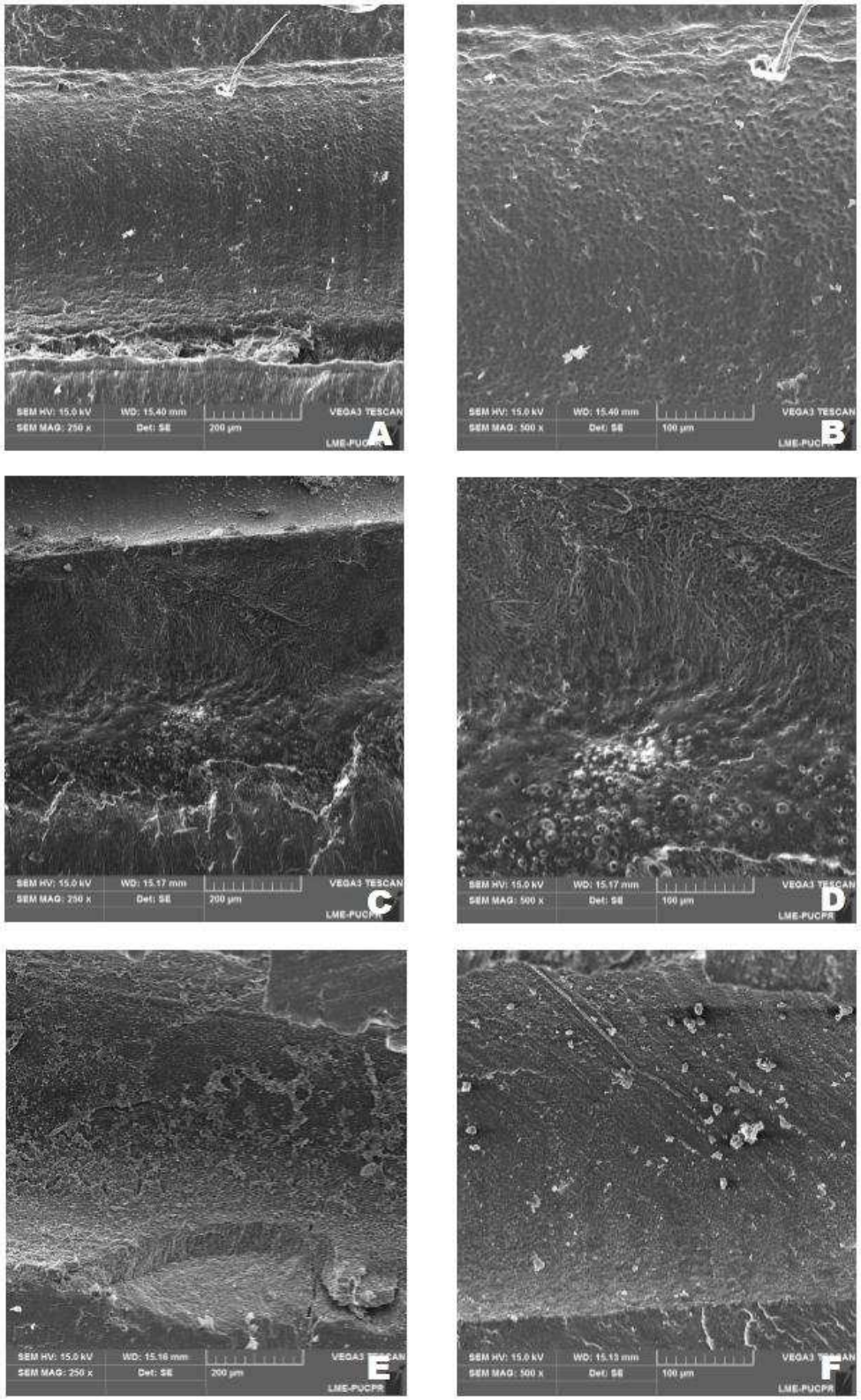

Figura 2. Eletromicrografias de varredura da dentina radicular submetida ao experimento de dissolução eletroquímica. (A e B) solução fluoretada não saturada por cloreto de sódio, em 250 e 500X, respectivamente; (C e D) solução fluoretada saturada por cloreto de sódio, em 250 e 500X, respectivamente; (E e F) água destilada, em 250 e 500X, respectivamente.

Microscopia Óptica

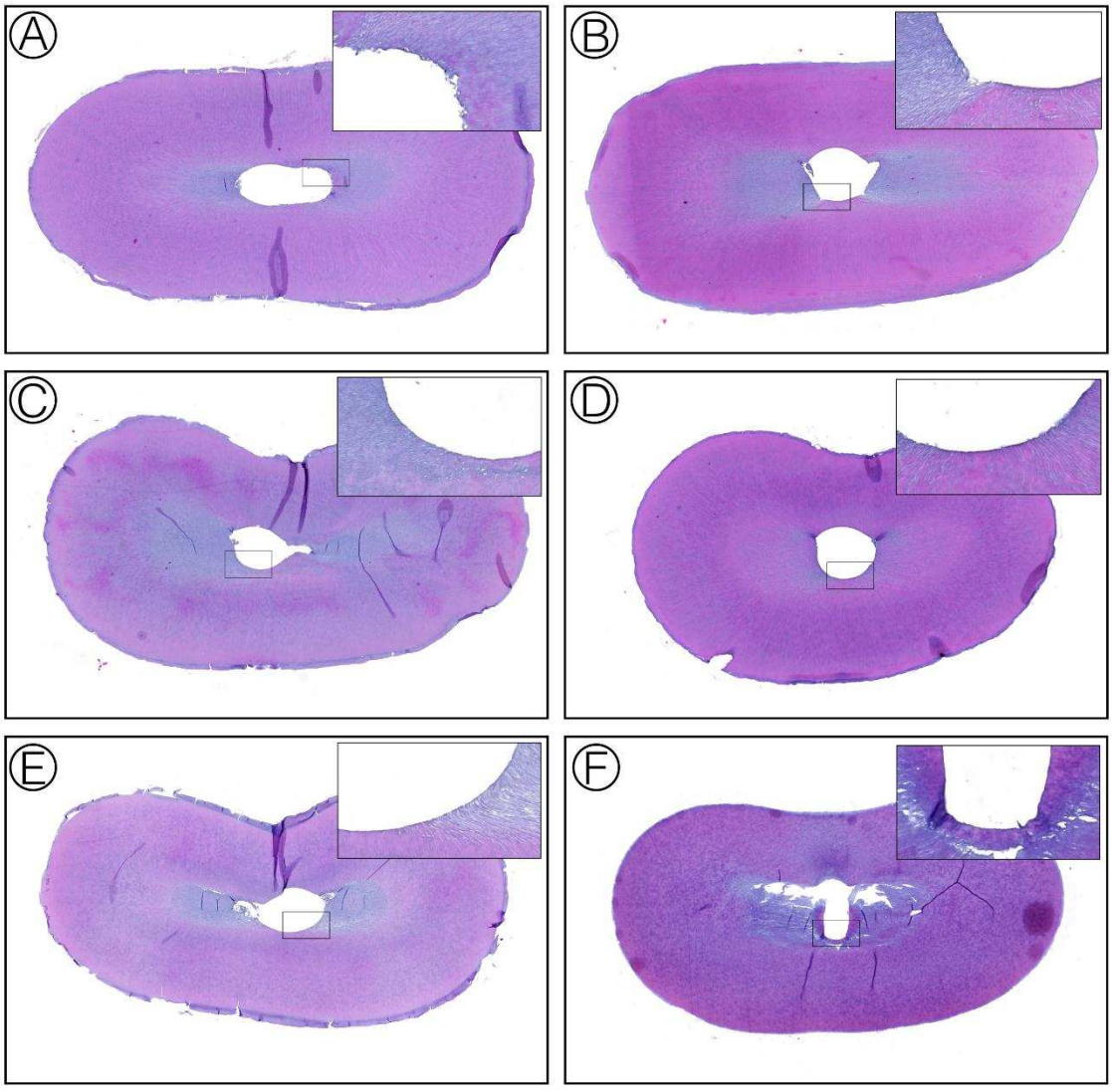

Figura 3. Microscopia óptica das raízes submetidas ao processo de dissolução eletroquímica. (A e B) solução fluoretada não saturada por cloreto de sódio; (C e D) solução fluoretada saturada por cloreto de sódio; (E e F) água destilada.

Discussão

A redução em comprimento de instrumentos de níquel-titânio fraturados em dentes humanos foi constatada por Kowalczuck et al., (2017). Os autores utilizaram o método da dissolução eletroquímica e constataram que a solução fluoretada saturada com cloreto de sódio apresentou um aumento da corrente elétrica e os melhores resultados quando comparado aos tamanhos iniciais e finais dos instrumentos fraturados. Para que novos avanços no estudo envolvendo dissolução eletroquímica e soluções fluoretadas sejam promissores, a integridade dentinária radicular após o experimento deve ser constatada.

Os métodos escolhidos para observar a integridade dentinária foram a microscopia eletrônica de varredura (MEV) (Torabinejad et al*., 2003,* Foschi et al., 2004, Prati et al., 1994) e o exame microscópico dentinário dos espécimes (Siqueira Jr et al., 2017). Nesses métodos pode-se observar a estrutura dentinária tanto no sentido longitudinal como transversal da dentina radicular intracanal. Tendo em vista que o sucesso do procedimento de dissolução eletroquímica deve ser considerado juntamente com a integridade da estrutura dentinária, o presente estudo é o primeiro a investigar as paredes dentinárias após o experimento de dissolução eletroquímica, possibilitando assim avanços nesta área.

A saturação do cloreto de sódio favorece o processo de dissolução da liga de níquel-titânio, já que a presença de íons cloreto e de íons fluoreto potencializam o processo de corrosão por agirem sinergicamente (Li et al., 2007). Baseado no estudo de Kowalczuck et al., (2017), três grupos foram utilizados para verificar a integridade radicular após o processo de dissolução eletroquímica: Duas soluções fluoretadas com NaF 12g/L^{-1}, variando a concentração do cloreto de sódio, uma saturada, outra não saturada e uma com água destilada, utilizada como controle. Os resultados demonstraram que a estrutura dentinária não sofreu alteração nos três grupos estudados. O pH de 5.0 e a alta concentração de flúor não demonstraram alterações na dentina radicular intracanal, quando comparado à água destilada.

Nos incisivos inferiores humanos estudados não existem áreas de desmineralização por cárie ou ácidos na dentina radicular intracanal, então o efeito remineralizador do flúor na dentina não pode ser considerado (Cury &

Tenuta 2009, Tenuta & Cury 2010). As soluções fluoretadas de 12g/L^{-1} contém 12.000 ppm de flúor. Essa alta concentração de flúor em contato com a parede dentinária radicular pode não ser absorvida pela presença da smear layer. Essa camada é composta por resíduos de matriz colágena mineralizada (Pashley et al., 1981) que pode impedir a difusão bacteriana, de irrigantes do canal radicular e de cimentos endodônticos (Kouvas et al., 1998). Questiona-se se a smear layer pode representar uma barreira, protegendo as paredes do canal radicular (Violich & Chandler 2010). Buchalla et al., 2007 avaliaram se a smear layer afeta a absorção de flúor dentinário na concentração de 12.000 ppm com um pH 4.0. Os resultados indicaram que a presença ou ausência da camada de smear layer em espécimes dentinários bovinos não demonstraram interferência na estrutura e absorção do flúor.

Em relação ao pH, o efeito tampão da dentina também pode ser destacado. Macedo et al., 2014 testaram diferentes concentrações de hipoclorito de sódio e diferente pHs. Os autores sugeriram que a dentina pode exercer um efeito tampão quando a mesma foi submetida à solução de NaOCl com pH 5,0 durante 1 hora.

A hipótese nula do presente estudo foi aceita. As soluções testadas mantiveram a estrutura dentinária inalterada e estruturada, sem danos decorrentes do método de dissolução eletroquímica. Baseado nos resultados da presente pesquisa, novas perspectivas de estudo se abrem neste campo ainda pouco explorado.

Conclusão

Após as análises em microscopia observaram-se que as paredes dentinárias não sofreram alterações após o processo de dissolução eletroquímica.

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ARTIGO EM INGLÊS

Microscopic evaluation of the dentinal walls of extracted human teeth following electrochemical dissolution of fragmented nickel-titanium instruments

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Abstract

Aim: To evaluate the integrity of the dentinal walls by means of optical microscopy (OM) and scanning electron microscopy (SEM) after an electrochemical dissolution process for fragmented nickel-titanium instruments in extracted human teeth.

Methodology: Forty-five mandibular incisors subjected to the electrochemical dissolution process using either sodium chloride-saturated (NaF 12 g/L + NaCl 180 g/L, pH = 5.0), non-saturated fluoridated solutions (NaF 12 g/L + NaCl 1 g/L, pH = 5.0), or distilled water were evaluated. For each group (n = 15), it was randomly selected 10 teeth for OM and 5 for the SEM evaluation. The teeth analyzed using OM underwent a 10-month long decalcification process in 5% EDTA. Six semi-serialized 5-µm cross-sections were processed and stained with hematoxylin and eosin. For the SEM evaluation, the roots were sectioned longitudinally followed by irrigation with EDTA (17%) for 1 minute. Each hemi-section was analyzed.

Results: Since the dependent variable presented a favorable outcome in all the groups, (i.e., no structural change in the dentinal structure), it was concluded that there was no statistically significant difference in the percentage of success among the groups. The chi-square test yielded a P = 1.

Conclusion: The dentinal structure remained unchanged following the electrochemical dissolution method.

Keywords: Endodontics. Electrochemical Dissolution. Nickel-Titanium. Microscopy. Dentine.

Introduction

Professionals in endodontics may face challenges posed by the fragmentation of nickel-titanium (NiTi) instruments inside the root canal, particularly in cases with a history of prior infection and those in which the cleaning and disinfection of the root canal system are difficult (Souter & Messer 2005). Thus, there is a demand and an ongoing search for conservative methods to remove fragmented endodontic instruments, such as electrochemical dissolution.

Research on the electrochemical dissolution of endodontic instruments involves the use of fluoridated solutions. Different concentrations of these solutions have been proposed (Aboud et al., 2014, Ormiga et al., 2015, Kowalczuck et al*.,* 2017). In a previous study, our research group (Kowalczuck et al., 2017) compared the electrical current values obtained in an electrochemical dissolution process by using two fluoridated solutions with different sodium chloride concentrations: a low (NaF 12 g/L + NaCl 1 g/L, pH = 5.0), and a saturating concentration (NaF 12 g/L + NaCl 180 g/L, pH = 5.0). Then, the dissolution of fragmented instruments in the apical third of human lower incisors was quantified after the experiment. The results showed that the sodium chloride-saturated fluoridated solution was more effective, ledding to an increase in electric current values and subsequent reductions in the lengths of the fractured instruments in the root canals.

In addition to solution concentration, the low pH of those experimental solutions (5.0) is one important factor. Although the acidification of the solutions favors the corrosion process, allowing the weakening of the alloy exposed to the solution, a potentially harmful action on the dentinal structure might limit the use of these materials (Aboud et al., 2014, Kowalczuck et al*.,* 2017).

The aim of this study was to assess whether saturated and non-saturated fluoridated sodium chloride solutions caused some type of damage to the structure of the radicular dentine following an electrochemical dissolution process of fragmented NiTi instruments ex vivo, by using optical microscopy (OM) and scanning electron microscopy (SEM). The null hypothesis was that the

electrochemical dissolution process do not alter the structure of the radicular dentin.

Materials and Methods

It was collected all forty-five teeth that had undergone an electrochemical dissolution process of NiTi instruments from a previous research (Kowalczuck et al., 2017). The teeth were divided in 3 groups (n = 15):

LC Group - low concentration NaCl solution (NaF 12 g/L + NaCl 1 g/L, pH = 5.0)

SC Group - saturating NaCl concentration solution (NaF 12 g/L + NaCl 180 g/L, pH = 5.0).

Control group - distilled water

For each group, it was randomly selected five teeth for the SEM and the remaining 10 for the optical microscopy (OM) evaluation. The presence or absence of damage to the dentin structure on the surface of the root canal or in the dentinal tubules of each root was analyzed according to the following criteria:

1 = Erosion, resorption, or alterations of the dentinal tubules

2 = Unaltered structure

1.1 Optical microscopy

All the teeth considered for histologic evaluation underwent a decalcification process in 5% EDTA for 10 months. After total decalcification, six semi-serialized cuts into 5-μm-thick sections were processed for histological examination using hematoxylin-eosin stain.

1.2 Scanning electron microscopy

The selected teeth for SEM were longitudinally sheared using double-sided diamond blades. The roots were hemi-sectioned using a mini-chisel and a surgical hammer, and irrigated with 17% EDTA for 1 minute to remove the smear

layer, without compromising the dentinal integrity. The roots were analyzed by means of scanning electron microscopy (SEM; Vega 3 SEM – Analytical Scanning Electron Microscope, TESCAN, Brno, Czech Republic) at 250×, 500× and 5000× magnifications.

Results

The evaluation of the dentinal walls of the roots of all groups showed no signs of erosion, resorption, or alterations of the dentinal tubules. The dependent variable showed a favorable outcome (no change in the dentinal structure) in all the groups (chi-square test $P = 1$).

Representative samples analyzed by OM are shown in Figure 2, and SEM analyze is represented in Figures 1. The present results suggest that the solutions used for the electrochemical dissolution process did not alter the dentin structure.

Scanning Electron Microscopy

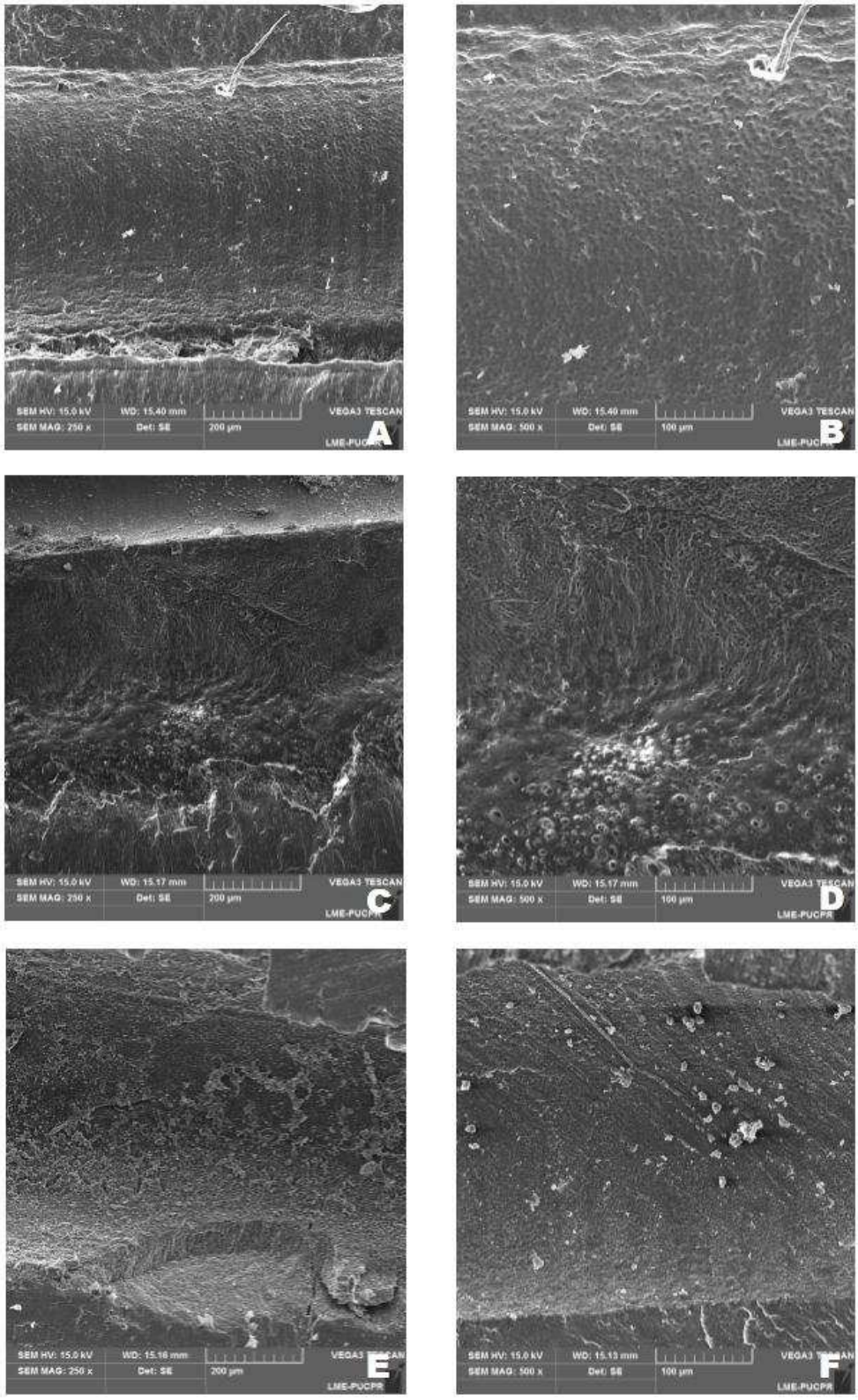

Figure 1. Scanning electron micrographs of the dentin roots subjected to the electrochemical dissolution experiment. (A and B) fluoridated, non-saturated

sodium chloride solution, at 250 and 500X, respectively; (C and D) fluoridated, sodium chloride-saturated solution, at 250 and 500X, respectively; (E and F) distilled water solution, at 250 and 500X, respectively.

Optical Microscopy

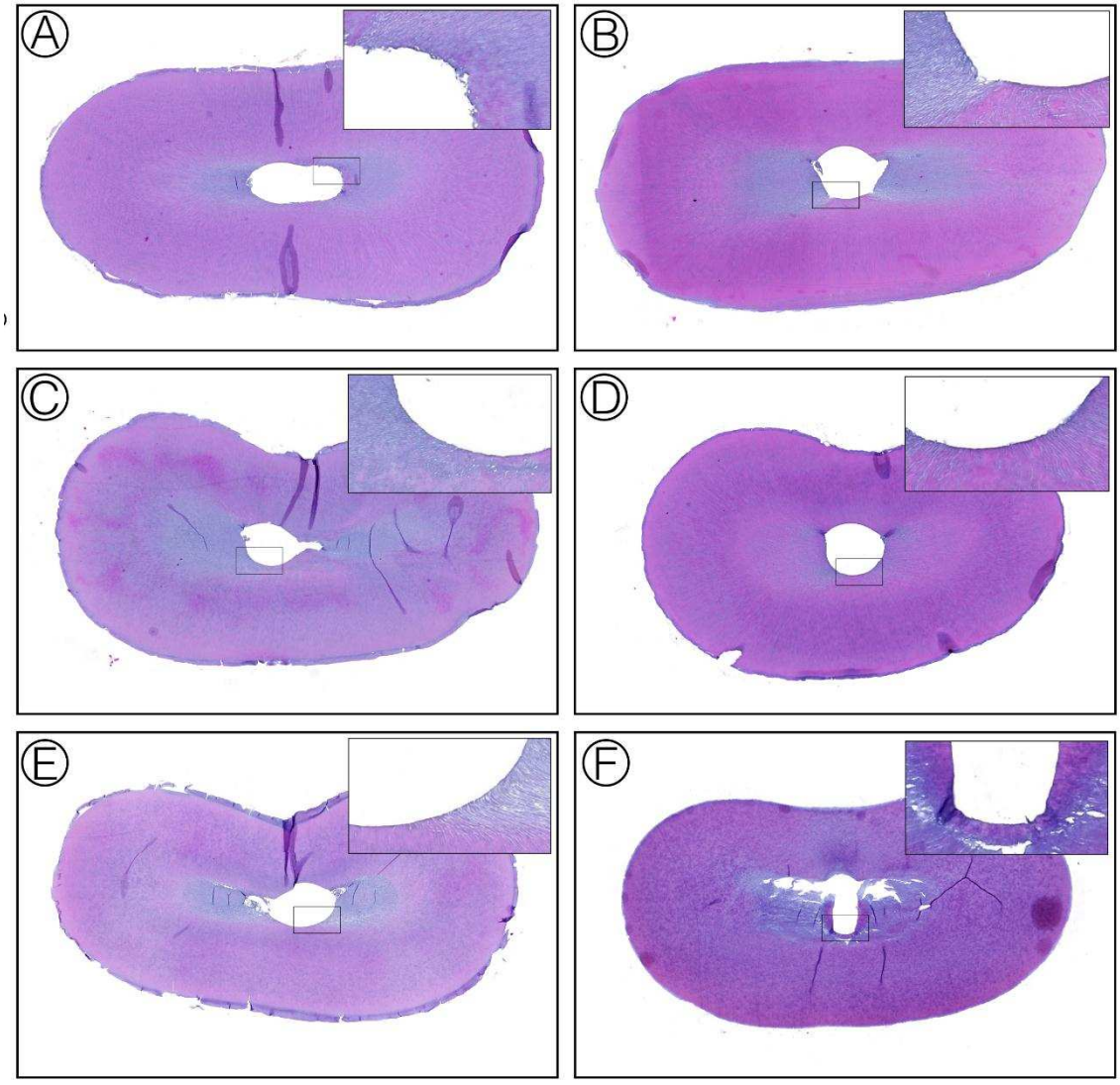

Figure 2. Optical microscopy of dentin roots subjected to the electrochemical dissolution process. (A and B) fluoridated, non-saturated sodium chloride solution; (C and D) fluoridated, sodium chloride-saturated solution; (E and F) distilled water.

Discussion

A reduction in the length of the fragmented NiTi instruments in human teeth subjected to electrochemical dissolution was established ex vivo by Kowalczuck et al., 2017. The authors found that saturation of fluoride solution with sodium chloride led to an increase in electrical current and better results when compared to the initial and final sizes of the fragmented instruments. Nevertheless, it is extremely important to evaluate the dentinal integrity following any experiment with electrochemical dissolution and fluoride solutions to ensure that the future of this method (Aboud et al., 2014).

In the present study, the methods chosen to evaluate dentinal integrity included the microscopic examination of the dentin by means of OM (Siqueira Jr et al., 2017) and SEM (Torabinejad et al*., 2003,* Foschi et al., 2004, Prati et al., 1994). These methods allowed the lengthwise and crosswise observation of the dentinal structure and intra-canal radicular dentin, respectively. The success of an electrochemical dissolution procedure must be considered in conjunction with the integrity of the dentinal structure; in this context, to the best of our knowledge, the present study was the first to investigate the dentinal walls after an electrochemical dissolution experiment, thus showing that the results may play an important role for the future studies in this matter.

The saturation of the solution with sodium chloride favors the NiTi dissolution process, as chloride and fluoride ions act synergistically to promote the corrosion (Li et al., 2007). Following the Kowalczuck et al., 2017, electrochemical dissolution experiment, the present study evaluated the radicular integrity after the use of two fluoridated solutions containing NaF 12 g/L and different concentrations of sodium chloride (one saturated and one unsaturated) and also a control group which consisted solely of distilled water. The results showed that the dentin structure remained inalterated in all the three groups, regardless the pH value of 5.0 and high concentration of fluoride of the solutions used for the electrochemical dissolution.

A possible bias could be the remineralizing effect of fluoride on dentin could not be considered (Cury & Tenuta 2009, Tenuta & Cury 2010). However, in the present study it was not observed any demineralized areas induced by

cavities or acid in the intracanal radicular dentin. Althought the solutions used in the electrochemical dissolution (Kowalczuck et al., 2017) presented a high concentration of fluoride (12,000 ppm) and the fluoride was in contact with the dentin walls for 30 minutes, its absorption could had been prevented by the presence of the smear layer. The smear layer usually contains mineralized collagen matrix residues (Pashley et al., 1981) that prevent the spread of bacteria, root canal irrigants, and endodontic sealers (Kouvas et al., 1998). It may be hypothesized that the smear layer acts as a barrier, protecting the root canal walls (Violich & Chandler 2010). Buchalla et al., 2007 evaluated whether the smear layer affected the dentinal fluoride absorption at a concentration of 12,000 ppm and a pH of 4.0. Their results indicated that the presence or absence of the smear layer in bovine dentinal specimens showed no interference in their structure or the absorption of fluoride. Regarding the pH, the buffer effect of dentine should also be emphasized. Macedo et al., 2014, tested different sodium hypochlorite concentrations at different pH values and suggested that the dentine might exert a buffer effect upon exposure to a pH 5.0 NaOCl solution for 1 hour.

Thus, the null hypothesis of the present study was accepted. The test solutions did not alter the dentinal structure, and apparently no damage was caused by the electrochemical dissolution method proposed in the earlier investigation (Kowalczuck et al., 2017). Therefore, future research may be considered in this field, which is rather yet to be explored in detail.

Conclusion

Microscopic analyses revealed that the dentinal walls were not altered following the experimental electrochemical dissolution process ex vivo.

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ANEXO

Normas para publicação

2. ETHICAL GUIDELINES

International Endodontic Journal adheres to the below ethical guidelines for publication and research.

2.1. Authorship and Acknowledgements

Authors submitting a paper do so on the understanding that the manuscript has been read and approved by all authors and that all authors agree to the submission of the manuscript to the Journal.

International Endodontic Journal adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE, authorship criteria should be based on 1) substantial contributions to conception and design of, or acquisiation of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3.

Acknowledgements: Under acknowledgements please specify contributors to the article other than the authors accredited. Please also include specifications of the source of funding for the study and any potential conflict of interests if appropriate. Please find more information on the conflict of interest form in section 2.6.

2.2. Ethical Approvals

Experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical AssociationDeclaration of Helsinki (version 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study. The authors MUST upload a copy of the ethical approval letter when submitting their manuscript. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

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The International Endodontic Journal asks that authors submitting manuscripts reporting from a clinical trial to register the trials in any of the following public clinical trials registries: www.clinicaltrials.gov, https://www.clinicaltrialsregister.eu/, http://isrctn.org/. Other primary registries if named in the WHO network will also be considered acceptable. The clinical trial registration number and name of the trial register should be included in the Acknowledgements at the submission stage.

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2.3.2 Epidemiological observational trials

Submitting authors of epidemiological human observations studies are required to review and submit a 'strengthening the reporting of observational studies in Epidemiology' (STROBE) checklist and statement. Compliance with this should be detailed in the materials and methods section. (www.strobe-statement.org)

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Abbreviations: International Endodontic Journal adheres to the conventions outlined in Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and Authors. When non-standard terms appearing 3 or more times in the manuscript are to be abbreviated, they should be written out completely in the text when first used with the abbreviation in parenthesis.

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All manuscripts submitted to *International Endodontic Journal* should include Title Page, Abstract, Main Text, References and Acknowledgements, Tables, Figures and Figure Legends as appropriate

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• **Results**: Give the main results of the study, including the outcome of any statistical analysis.

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Abstract for Mini Review Articles should be non-structured of no more than 250 words, including a clear research question, details of the literature search strategy and clear conclusions.

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When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort.
Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.
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Discussion: may usefully start with a brief summary of the major findings, but repetition of parts of the abstract or of the results section should be avoided. The Discussion section should progress with a review of the methodology before discussing the results in light of previous work in the field. The Discussion should end with a brief conclusion and a comment on the potential clinical relevance of the findings. Statements and interpretation of the data should be appropriately supported by original references.

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It is the policy of the Journal to encourage reference to the original papers rather than to literature reviews. Authors should therefore keep citations of reviews to the absolute minimum.

We recommend the use of a tool such as EndNote or Reference Manager for reference management and formatting. The EndNote reference style can be obtained upon request to the editorial office (iejeditor@cardiff.ac.uk). Reference Manager reference styles can be searched for here: www.refman.com/support/rmstyles.asp

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DuPont B (1974) Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. Proceedings of the Third Annual Meeting of the International Society for Experimental Rematology; pp. 44-46. Houston, TX, USA: International Society for Experimental Hematology.

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7 Guidelines for reporting of DNA microarray data

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