PONTIFÍCIA UNIVERSIDADE CATÓLICA DO PARANÁ

ESCOLA DE CIÊNCIAS DA VIDA
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO CLÍNICA ODONTOLÓGICA INTEGRADA

LUIZA GIACOMET CASSOL

AVALIAÇÃO DA SOLUÇÃO DE HIPOCLORITO DE SÓDIO NA DISSOLUÇÃO ELETROQUÍMICA DE INSTRUMENTOS DE NÍQUEL-TITÂNIO TRATADOS TERMICAMENTE (GOLD).

Curitiba
2018

LUIZA GIACOMET CASSOL

AVALIAÇÃO DA SOLUÇÃO DE HIPOCLORITO DE SÓDIO NA DISSOLUÇÃO ELETROQUÍMICA DE INSTRUMENTOS DE NÍQUEL-TITÂNIO TRATADOS TERMICAMENTE (GOLD).

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 Clínica Odontológica Integrada (Ênfase em Endodontia).

Orientador: Prof. Dr. Ulisses Xavier da Silva Neto
Coorientador: Prof. Dr. Alexandre Kowalczuck

Curitiba
2018

PUCPR
GRUPO MARISTA

Pontifícia Universidade Católica do Paraná
Escola de Ciências da Vida
Programa de Pós-Graduação em Odontologia

TERMO DE APROVAÇÃO

LUIZA GIACOMET CASSOL

AVALIAÇÃO DE DUAS SOLUÇÕES, NA DISSOLUÇÃO ELETROQUÍMICA DE INSTRUMENTOS DE NÍQUEL TITÂNIO TRATADOS TERMICAMENTE (GOLD)

Dissertação apresentada ao Programa de Pós-Graduação em Odontologia da Pontifícia Universidade Católica do Paraná, como parte dos requisitos parciais para a obtenção do Título de **Mestre em Odontologia**, Área de Concentração em **Clínica Odontológica Integrada com Ênfase em Endodontia**.

Orientador(a): Prof. Dr. Ulisses Xavier da Silva Neto
Programa de Pós-Graduação em Odontologia, PUCPR

Prof. Dr. Everdan Carneiro
Programa de Pós-Graduação em Odontologia, PUCPR

Prof. Dr. Alexandre Kowalczuck
Curso de Odontologia, PUCPR

Curitiba, 29 de novembro de 2018.

AGRADECIMENTO ESPECIAL

Ao meu orientador, Prof. Dr. Ulisses Xavier da Silva Neto, pelo apoio, confiança e disponibilidade em me orientar. Por todos os ensinamentos transmitidos desde a graduação. Muito obrigada!

Ao meu coorientador, Prof. Dr, Alexandre Kowalczuck, por me inspirar e apoiar a fazer parte desta linha pesquisa, e compartilhar a experiência sobre o assunto. Sua participação foi de extrema importância neste estudo.

Ao Prof. Dr. Carlos Laurindo, por disponibilizar seu tempo em me auxiliar nos ensaios de corrosão.

À mestranda Jéssica, por compartilhar seus conhecimentos sobre corrosão de metais e executar as imagens do microscópio eletrônico de varredura.

Ao Prof. Dr. Sérgio Ignácio, pela sua competência em realizar a análise estatística.

À todos os professores de Engenharia Elétrica e Mecânica que procurei durante o mestrado para esclarecer minhas dúvidas sobre a pesquisa.

Ao farmacêutico-bioquímico, Claudio Katayama, pelo profissionalismo na elaboração das soluções.

AGRADECIMENTOS

À Deus, por iluminar meu caminho e me manter com fé em todos os momentos.

Aos meus pais, Silvia e Jesur, por sempre me incentivarem a seguir o caminho da educação. Pelos seus valores, por tudo que fizeram e ainda fazem por mim. Amo vocês!

Ao meu irmão, Pedro, pelo apoio e parceria. Sou grata por ter você em minha vida.

Ao meu namorado, Nathan, pelo amor, amizade e companheirismo sempre.

À Profª Dra. Vânia Portela Dietzel Westphalen, ao Prof Dr. Luiz Fernando Fariniuk, ao Prof. Dr. Everdan Carneiro, ao Prof. Dr. Ulisses Xavier da Silva Neto pela competência e dedicação para com o ensino da Endodontia. Por me inspirarem a seguir esta especialidade. Admiro muito todos vocês.

À todos os meus colegas de mestrado, Ana Carolina Mastriani, Adriane Antoniw, André Segatto, Helington Kruger, Luana Lazarotto, Marcos Felipe Iparraguirre Nuñovero, Nicole Baumeier, Thomaz Pessoa, pela ajuda, parceria e amizade.

Aos funcionários da Clínica Odontológica da PUC-PR.

À todos que contribuíram para minha formação profissional: Professores, pacientes, funcionários e amigos.

LISTA DE ABREVIATURAS E SIGLAS

NiTi	Níquel-Titânio
OCP	Potencial de circuito aberto
NaF	Fluoreto de sódio
g/L	Gramas por litro
NaCl	Cloreto de sódio
NaF	Fluoreto de sódio
NaOCl	Hipoclorito de sódio
PUCPR	Pontifícia Universidade Católica do Paraná
mV/s	Milivolt por segundo
+	Positivo
V	Volt
mL	Mililitro
PTU F1	Instrumento rotatório ProTaper Universal F1
WOGS	Instrumento WaveOne Gold Small
mm	Milímetro
s	Segundo
min	Minuto
%	Por cento
±	Sinal de mais ou menos
X	Aumentos
MEV	Microscopia Eletrônica de Varredura
mA	Miliampére
ddp	Diferença de potencial

SUMÁRIO

ARTIGO EM PORTUGUÊS

Página título

Avaliação da solução de hipoclorito de sódio na dissolução eletroquímica de instrumentos de níquel-titânio tratados termicamente (GOLD).

AUTORES:

Luiza Giacomet Cassol[a], DDS

Alexandre Kowalczuck[a], DDS, MS, PhD

Carlos Augusto Henning Laurindo[b], MS, PhD

Everdan Carneiro[a], DDS, MS, PhD

Vânia Portela Ditzel Westphalen[a], DDS, MS, PhD

Ulisses Xavier da Silva Neto[a], DDS, MS, PhD

[a]Departamento de Endodontia, Escola de Ciências da Vida, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil

[b]Departamento de Engenharia Mecânica, Escola Politécnica, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil

Resumo

Objetivo: Comparar a influência de duas soluções, uma fluoretada e outra de hipoclorito de sódio, ambas saturadas com cloreto de sódio, durante a dissolução eletroquímica de instrumentos com liga convencional de níquel-titânio, e instrumentos de níquel-titânio tratados termicamente (GOLD).

Material e métodos: Duas soluções foram avaliadas (solução NaF - NaF 12g/L + NaCl 180 g/L, solução NaOCl – NaOCl 2,5% + NaCl 180g/L) pelo teste de polarização dos instrumentos Protaper Universal F1 (PTU F1) e Wave One Gold Small (WOGS), com a amostra de 48 instrumentos. Os potenciais de corrente elétrica estabelecidos foram de 0,5 V e 5 V para a solução NaF e NaOCl, respectivamente. A célula eletroquímica composta de três eletrodos foi utilizada para o teste de polarização dos instrumentos PF1 e WOGS, que tiveram 6 mm da ponta imersos nas soluções testadas. O registro da corrente elétrica ocorreu durante 540 segundos (s). Caso a dissolução completa da porção imersa do instrumento ocorresse em tempo inferior ao previsto, o experimento era considerado como finalizado. Foram mensurados as variações de tempo (em segundos) dos instrumentos na solução NaF, NaOCl e água destilada. A avaliação dos padrões de corrosão dos instrumentos ocorreu por meio de microscopia eletrônica de varredura (MEV). Os dados foram submetidos a análise estatística por meio dos testes de Mann Whitney, Kruskal Wallis e Dunn.

Resultados: As soluções NaF e NaOCl apresentaram diferença estatisticamente significante ($p<0,05$) em relação ao tempo de dissolução do instrumento (em segundos), com média de 12,9 s e 83,6 s, respectivamente. Não houve diferença estatisticamente significante ($p>0,05$) em relação ao tempo na comparação da dissolução entre os instrumentos PTU F1 e WOGS.

Conclusão: Ambas as soluções possuem capacidade de dissolução eletroquímica dos instrumentos PTU F1 E WOGS. No entanto, a solução NaF promoveu dissolução dos instrumentos em menor tempo.

Palavras-chave: Endodontia, Dissolução, Instrumentos de níquel-titânio, Hipoclorito de sódio.

Introdução

Os instrumentos de níquel-titânio (NiTi) foram introduzidos a odontologia, há três décadas (Walia, *et a*l. 1988). Tornaram-se importante para realização do tratamento endodôntico, pois esta liga possui adequada flexibilidade e resistência à fadiga cíclica para se utilizar em instrumentos mecanizados, que proporcionam um formato de preparo que permite o adequado saneamento do sistema de canais radiculares em menor tempo quando comparado à instrumentos manuais. (Cheung & Liu 2009, Schäfer & Bürklein 2012)

A liga NiTi possui a característica relevante de efeito térmico de memória de forma, que provém das fases martensita e austenita. Visando o aprimoramento desta característica que novas tecnologias com tratamentos termomecânicos de fabricação vêm sendo desenvolvidos. O tratamento térmico GOLD foi criado para aumentar a superelasticidade e a resistência à fadiga cíclica do instrumento (Webber *et al.* 2015).

Entretanto, apesar das novas tecnologias e dos avanços conquistados, a fratura de instrumentos pode ocorrer, especialmente em canais radiculares com curvaturas severas. Muitos métodos e técnicas foram propostos para remoção dos instrumentos fraturados dentro do canal radicular, porém ainda não há nenhuma técnica padronizada que não cause danos à estrutura dentária. (Shen *et al.* 2004)

Recentemente, foi apresentado um método que minimize o desgaste das paredes do canal radicular (Ormiga *et al.* 2010). As pesquisas ainda estão nos estágios iniciais, mas os resultados obtidos são promissores. Este método consiste na imersão de dois eletrodos em um eletrólito, em que um funcionará como ânodo e o outro como cátodo. O contato entre o instrumento fraturado e o eletrodo usado como ânodo é necessário, pois a dissolução do metal é o objetivo do processo. A diferença de potencial entre os dois eletrodos, resulta na migração de elétrons do ânodo para o cátodo, consequentemente, há liberação dos íons metálicos para a solução (Ormiga *et al.* 2010).

O hipoclorito de sódio é comumente utilizado como solução irrigadora no tratamento endodôntico. Quando presente na solução eletrolítica, aumenta a dissolução do NiTi, por meio da desestruturação da camada de passivação que protege o metal contra corrosão e oxidação (Sarkar *et al.* 1983). O mesmo ocorre com soluções fluoretadas, em que os íons flúor atuam sobre o titânio

potencializando o processo de dissolução (Shen 2004). Além disso, a concentração de solução fluoretada saturada com cloreto de sódio (NaF 12g/L + NaCl 180 g/L), implica no aumento dos valores da corrente elétrica, consequentemente, maior dissolução dos instrumentos fraturados em menor tempo (Kowalczuck *et al.* 2017).

As pesquisas sobre dissolução eletroquímica de instrumentos endodônticos utilizaram a liga convencional de níquel titânio, estudos envolvendo a liga de níquel-titânio tratada termicamente (GOLD) não estão relatados na literatura até o presente momento (Ormiga *et al*. 2015, Kowalczuck *et al.* 2017). Considerando os recentes avanços da metalurgia na fabricação de instrumentos endodônticos, e a aceitação e incorporação dos instrumentos NiTi GOLD na prática clínica cotidiana, é pertinente elucidar o comportamento desta liga metálica no processo de dissolução eletroquímica.

O intuito deste trabalho foi comparar a influência de duas soluções: uma fluoretada saturada com cloreto de sódio, e outra solução de hipoclorito sódio saturada com cloreto de sódio, em relação ao processo de dissolução eletroquímica de instrumentos ProTaper Universal e WaveOne Gold Small.

Material e Métodos

A amostra contendo 48 instrumentos Protaper Universal F1 (PTU F1) e Wave One Gold Small (WOGS) foi testada por meio do ensaio de polarização. Foram avaliados os comportamentos de três soluções: Solução NaF – NaF 12g/L + NaCl 180 g/L, Solução NaOCl – NaOCl (2,5%) + NaCl 180g/L e solução 3 – água destilada (grupo controle). Inicialmente foi determinado o potencial de circuito aberto (OCP) de cada uma das soluções. Uma célula eletroquímica composta de três eletrodos foi utilizada. Um eletrodo de calomelano saturado foi utilizado como eletrodo de referência, um eletrodo de platina como contra-eletrodo, e um eletrodo plano de NiTi como eletrodo de trabalho. A célula eletroquímica foi acoplada a um potenciostato (IviumStat, Ivium Technologies B. V. Eindhoven, Holanda) e os testes foram realizados a uma velocidade de varredura de 1 mV/s, partindo-se do potencial a circuito aberto até o potencial de +10 V. Foram utilizados 200mL da solução em cada teste, sendo que em cada experimento a solução foi completamente renovada. O teste foi repetido três vezes para cada solução. Com o intuito de determinar os potenciais a partir dos quais haveria a dissolução ativa do níquel-titânio para cada uma das soluções, o teste de polarização potenciodinâmica foi procedido. Utilizando de uma célula eletroquímica com a mesma configuração descrita anteriormente, foram aplicados os potenciais iniciais obtidos no teste para determinação de OCP, até +10 V para cada solução.

Polarização de instrumentos ProTaper Universal F1 (PTU F1) e Wave One Gold Small (WOGS)

A mesma célula eletroquímica descrita previamente foi utilizada. Entretanto, o eletrodo plano de NiTi foi substituído pelas limas PTU F1 ou WOGS utilizadas como eletrodos de trabalho. Os instrumentos PTU F1 ou WOGS, tiveram os 6,0 milímetros (mm) da ponta imersos nas soluções testadas. Partindo do potencial capaz de causar dissolução para cada solução, o registro da corrente elétrica ocorreu durante 540 segundos (s). Os potenciais de corrente elétrica estabelecidos foram de 0,5 V e 5 V para a solução NaF e NaOCl, respectivamente. A solução 3 não obteve resultados compatíveis com a dissolução das ligas de NiTi. Caso a dissolução completa da porção imersa do instrumento ocorresse em tempo inferior ao previsto, o experimento era considerado finalizado. Os valores de tempo em que a dissolução ocorreu, foram compilados em uma tabela e submetidos à análise

estatística por meio dos testes Mann Whitney, Kruskal Wallis e Dunn. A avaliação dos padrões de corrosão dos instrumentos ocorreu por meio de microscopia eletrônica de varredura (MEV) (Vega 3 SEM – Analytical Scanning Electron Microscope, TESCAN, Brno, República Tcheca) em ampliação de 125X.

Resultados

Polarização de instrumentos ProTaper Universal F1 (PTU F1) e Wave One Gold Small (WOGS)

As soluções NaF e NaOCl apresentaram diferença estatisticamente significante (p<0,05) em relação ao tempo de dissolução dos instrumentos, demonstraram média de 12,96 segundos e 83,63 segundos, respectivamente. (Tabela 1).

Tabela 1. Estatística descritiva do tempo (em segundos) de dissolução das soluções.

				Intervalo de confiança de 95%	
Solução	N	Média	Desvio Padrão	Limite Inferior	Limite Superior
NaF	24	12,96	4,08	11,24	14,68
NaOCl	24	83,63	65,77	55,85	111,40

Os instrumentos PTU F1 e WOGS não apresentaram diferença estatisticamente significante (p>0,05) em relação ao tempo de dissolução, demonstraram média de 50,13 segundos e 46,46 segundos, respectivamente. (Tabela 2).

Tabela 2. Estatística descritiva do tempo (segundos) de dissolução entre os instrumentos.

Instrumento	N	Média	Desvio Padrão	Intervalo de confiança de 95% Limite Inferior	Limite Superior
PTU F1	24	50,13	67,00	21,83	78,42
WOGS	24	46,46	59,51	25,55	67,37

Ao comparar as médias de tempo entre as amostras, levando em consideração a hipótese nula, houve apenas diferença estatisticamente significante entre as soluções NaF e NaOCl. (Tabela 3).

Tabela 3. Comparação múltiplas não paramétricas de Dunn.

	Diferença de Posto Médio	Estatística de Teste	Valor de p
PTU F1 + Sol NaF WOGS + Sol NaF	-1,08	-0,19	0,84
PTU F1 + Sol NaF PTU F1 + Sol NaOCl	-23,25	-4,07	0,00
PTU F1 + Sol NaF WOGS + Sol NaOCl	-25,83	-4,52	0,00
WOGS + Sol NaF PTU F1 + Sol NaOCl	22,16	3,88	0,00
WOGS + Sol NaF WOGS + Sol NaOCl	-24,75	-4,33	0,00
PTU F1 + Sol NaOCl WOGS + Sol NaOCl	-2,58	-0,45	0,65

Nível de significância de 0,05

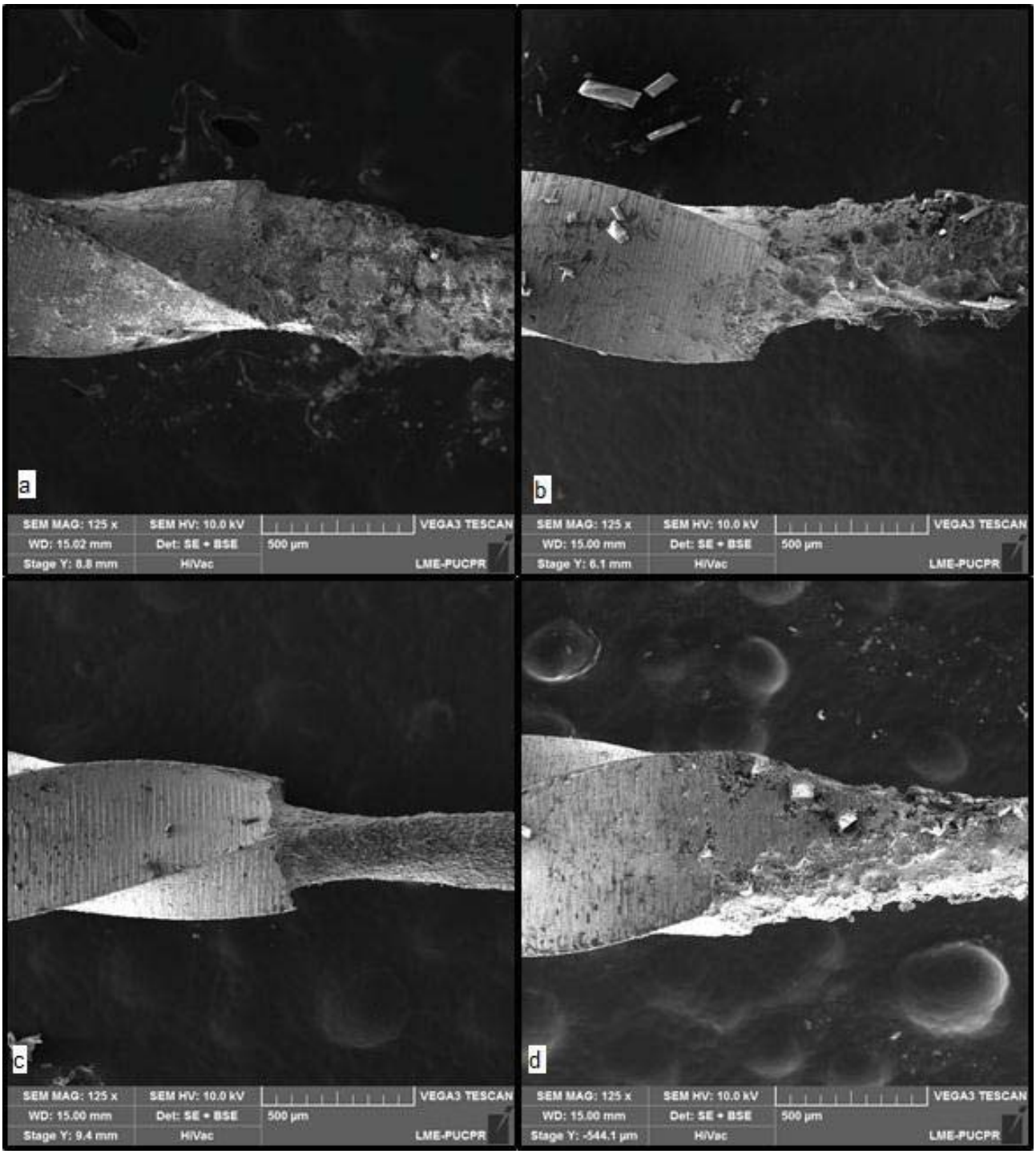


Figura 1. Eletromicrografias de varredura de fragmentos de instrumentos submetidos ao experimento de dissolução eletroquímica. **(A)** Fragmento do instrumento PTU F1 submetido à solução NaOCl em 125X. **(B)** Fragmento do instrumento PTU F1 submetido à solução NaF em 125X. **(C)** Fragmento do instrumento WOGS submetido à solução NaOCl em 125X. **(D)** Fragmento do instrumento WOGS submetido à solução NaF em 125X.

Na imagem (A) observa-se a corrosão por pites da porção em que o metal esteve em contato com o eletrólito. A porção do instrumento que não teve contato com a solução, não houve danos à estrutura. Na imagem (B) há a característica de corrosão por pites da porção do instrumento PTU F1 imerso na solução NaF. A imagem (C) a corrosão é do tipo uniforme da porção do instrumento WOGS imerso na solução NaOCl. Imagem (D) Corrosão do tipo alveolar da porção do instrumento WOGS imerso na solução NaF.

Discussão

A utilização da dissolução eletroquímica em endodontia é um método proposto para dissolver instrumentos endodônticos quando fraturados no interior do canal radicular, pela polarização da liga metálica em um eletrólito, por meio de dois eletrodos (Ormiga *et al.* 2010). A solução de fluoreto de sódio com cloreto de sódio, vêm sendo utilizada como eletrólito nos estudos sobre dissolução eletroquímica pois há a interação sinérgica dos íons flúor e cloreto na corrosão da liga de NiTi (Ormiga *et al*. 2010, Kowalczuck *et al* 2017, Li *et al*. 2007, Amaral *et al.* 2018, Ormiga *et al.* 2011).

O hipoclorito de sódio começou a ser amplamente utilizado como agente desinfetante a partir do final do século 19, quando estudos laboratoriais começaram a ser publicados (Zehnder 2006). Possui propriedades antimicrobianas, capacidade de dissolver tecido orgânico (Harrison & Hand 1981) fácil acesso, boa relação custo benefício, e estas características o tornaram o principal irrigante no preparo de canais radiculares (Estrela *et al.* 2002).

O presente estudo, é o primeiro que utiliza o hipoclorito de sódio associado ao cloreto de sódio como solução eletrolítica. Esta solução demonstrou ter capacidade de dissolver instrumentos endodônticos, com média de 83 segundos. A saturação da solução com cloreto de sódio aumenta o valor da corrente elétrica transmitida, e consequentemente a dissolução do instrumento em menor tempo (Kowalczuck *et al.* 2017). Durante o experimento observou-se o escurecimento da solução eletrolítica pela liberação de íons metálicos presentes na liga de NiTi. Estes íons metálicos, podem atuar resultando em uma barreira entre o eletrodo anódico e o instrumento, dificultando a dissolução do fragmento e supostamente podem até ser tóxicos aos tecidos periapicais se não forem eliminados, como em casos de estudos *in vivo*. Uma alternativa para minimizar o possível escurecimento da solução, seria a constante renovação do eletrólito. Além disso, o níquel (presente na liga de NiTi) é conhecido como um dos catalizadores na reação de decomposição do hipoclorito de sódio, e o resultado é uma reação exotérmica e que libera oxigênio, fato este que foi observado no presente estudo durante os ensaios de polarização, houve o aumento de temperatura da solução eletrolítica (Stitt *et al.* 2003).

No estudo *ex vivo,* que utilizou a solução fluoretada [NaF 12g/L + NaCl 1g/L] como solução eletrolítica e a solução de hipoclorito de sódio a 5,25% como agente irrigante do preparo endodôntico químico-mecânico, a solução de hipoclorito de sódio demonstrou ter maior citotoxicidade do que a solução fluoretada (Amaral *et al.* 2018). Porém, devemos considerar que as células cultivadas (fibroblastos) são mais sensíveis à toxicidade induzida por agentes químicos, do que aquelas encontradas nos tecidos periapicais (Bajrami D. *et al.* 2014). Além disso, em estudos *in vivo* a solução estaria restrita ao canal radicular, o que pode ser comparado ao uso do hipoclorito de sódio como irrigante no preparo dos canais radiculares. Este irrigante é considerado citotóxico, porém em raras circunstâncias é extravasado além do ápice radicular e entra em contato com os tecidos periapicais (Amaral *et al.* 2018). Uma possível adversidade proveniente da liberação do níquel seria uma ocorrência destes íons atingirem acidentalmente os tecidos periapicais em pacientes alérgicos.

O teste de potencial de circuito aberto (OCP) é utilizado para verificar o comportamento eletroquímico das soluções, pois cada solução apresenta o potencial de corrente elétrica capaz de causar a corrosão dos instrumentos de NiTi. (Ormiga *et al.* 2011). A solução fluoretada saturada com cloreto de sódio (solução NaF) demonstrou melhores resultados em relação ao tempo, quando comparado ao estudo que se utilizou a mesma solução, porém, com diferente potencial de corrente elétrica (Kowalczuck *et al.* 2017). No estudo que utilizou o potencial de corrente elétrica de 0,3 V, a média de tempo de dissolução foi de 540 s. No presente estudo, foi utilizado o 0,5 V e a média de tempo de dissolução foi de 12 segundos. Pode-se inferir que o potencial de corrente elétrica e o tempo são inversamente proporcionais, ou seja, quanto maior o valor de corrente elétrica, menor será o tempo de dissolução dos instrumentos.

A aplicação de corrente elétrica sobre tecidos vivos pode apresentar riscos à integridade dos mesmos dependendo da intensidade aplicada. Porém, a dentina e o cemento presentes no dente, são considerados isolantes de corrente elétrica. (Nekoofar *et al.*, 2006). Consequentemente, o circuito elétrico se torna restrito ao dente há a possibilidade de aplicar o método *in vivo*. Além disso*,* os valores de tensão utilizados no presente estudo, não são capazes de gerar danos ao corpo humano (IEC/TS 60479-1).

O tratamento GOLD é uma tecnologia baseada no tratamento térmico da liga e lentamente resfriado, o que causa a modificação das temperaturas de transformação da fase inicial e final austenítica ,e confere a aparência dourada aos instrumentos WOGS (Webber *et al.,* 2015). Os tratamentos térmicos comumente aplicados à liga de NiTi são: solubilização, têmpera e recozimento. Estes tratamentos podem causar diferentes reações no estado sólido: precipitação, recristalização e transformação estrutural de fase. A solubilização é o tratamento que utiliza altas temperaturas para que ocorra a dissolução dos precipitados. O recozimento, pelo contrário, é realizado em temperatura baixa, para haver a formação de precipitados em dimensões reduzidas, em condições controladas que aumentam a resistência da matriz à deformação. A têmpera constitui-se no resfriamento do material (Miller & Lagoudas 2001, Huang & Liu 2001). Através deste tratamento térmico, ocorre a formação dos precipitados de Ni_4Ti_3, que são responsáveis por aumentar a superelasticidade e a memória de forma da liga e também por afetar as características da transformação martensítica na formação da fase R (Khalil-Allafi, *et al*. 2002). Diferentemente das ligas de NiTi tratadas termicamente, na liga convencional não ocorre a formação destes precipitados (Otsuka & Ren 2005). Havia a hipótese de diferentes comportamentos no processo de dissolução eletroquímica dos instrumentos, por apresentarem diferenças estruturais. No entanto, o presente experimento apresentou resultado semelhante para as ligas de NiTi convencional e NiTi GOLD.

Não houve diferença estatisticamente significante no tempo de dissolução entre ambos instrumentos. Este resultado, pode ser pelo fato de que ambas as ligas testadas são compostas do mesmo material, o NiTi, apesar de apresentarem diferença na forma de fabricação. A diferença de potencial causada por elementos químicos eletronegativos, como o flúor e cloro, é o que resulta na corrosão da liga metálica de NiTi (Li *et al.* 2007). Além disso, a superfície de contato do instrumento e de solução eletrolítica disponível em estudos *in vitro* é maior, do que quando realizados estudos *ex vivo,* e consequentemente o resultado poderia ser alterado.

Conclusão

Ambas as soluções demonstraram ter capacidade de dissolução dos instrumentos PTU F1 e WOGS. A solução NaF demonstrou ter resultados superiores em relação ao tempo.

Referências

1. Amaral CCF, Ormiga F, Boldrini LC, *et al*. (2018) Evaluation of the effects of the solution used for eletrochemical dissolution of nickel-titanium endodontic files on dentine structure, microhardness and cell viability. *International Endodontic Journal.* **51**, 1424-1445.
2. Bajrami D, Hoxha V, Gorduysus O, et al (2014) Cytotoxic effect of endodontic irrigants *in vitro*. *Medical Science Monitor Basic Research.* **20**, 22-26.
3. Cheung GPS, Liu CSY (2009) A retrospective study of endodontic treatment outcome between nickel-titanium rotary and stainless steel hand filing techniques. *Journal of Endodontics.* **35**, 938-943.
4. Estrela C, Estrela CRA, Barbin EL (2002) Mechanism of action of sodium hypochlorite. *Brazilian Dental Journal.* **13**, 113-117.
5. Harrison JW, Hand RE (1981) The effect of dilution and organic matter on the antibacterial property of 5.25% sodium hypochlorite. *Journal of Endodontics.* **7**, 128-132.
6. Huang X, Liu Y (2001) Effect of annealing on the transformation behavior and superelasticity of NiTi shape memory alloy. *Scripta Materialia.* **45**, 153-160.
7. IEC/TS 60479-1 (2005) Effects of current on human beings and livestock – Part 1: General aspects.
8. Khalil-Allafi J, Dlouhy A, Eggeler G (2002) Ni4Ti3 precipitation during aging of NiTi shape memory alloys and its influence on martensitic phase transformations. *Acta Materialia.* **50**, 4255-4274.
9. Kowalczuck A, Silva Neto UX, Fariniuk LF, *et al*. (2017) Electrochemical dissolution of fractured nickel-titanium instruments in human extracted teeth. *International Endodontic Journal.* **50**, 578-585.
10. Li X, Wang J, Han EH *et al.* (2007) Influence of fluoride and chloride on corrosion behavior of NiTi orthodontic wires. *Acta Biomaterialia.* **3**, 807-815.
11. Miller DA, Lagoudas DC (2001) Influence of cold work and heat treatment on the shape memory effect and plastic strain development of NiTi. Materials Science and Engineering A. **308**, 161-175.

12. Nekoofar MH, Ghandi MM, Hayes SJ *et al.* (2006) The fundamental operating principles of electronic root canal length measurement devices. *International Endodontic Journal*. **39**, 595-609.
13. Ormiga F, Da Cunha PGJA, de Araújo MCP (2010) Dissolution of nickel-titanium endodontic files via na electrochemical process: a new concept for future retrieval of fractured files in root canals. *Journal of Endodontics*. **36**, 717-720.
14. Ormiga F, da Cunha PGJA, de Araújo MCP *et al*. (2011) An initial investigation of the electrochemical dissolution of fragments of nickel-titanium endodontic files. *Journal of Endodontics.* **37**, 526-530.
15. Ormiga F, Aboud LRL, Gomes JACP (2015) Electrochemical-induced dissolution of nickel-titanium endodontic instruments with different designs. *International Endodontic Journal.* **48**, 342-350.
16. Otsuka K, Ren X (2005) Physical metallurgy of Ti-Ni-based shape memory alloys. *Progress in Materials Science.* **50**, 511-678.
17. Sarkar NK, Redmond W, Schwaninger B (1983) The chloride corrosion behavior of four orthodontic wires. *Journal of Oral Rehabilitation*. **10**, 121-128.
18. Schafer E, Burklein S (2012) Impact of nickel-titanium instrumentation of root canal on clinical outcomes: A focused review. *Odontology.* **100**, 130-136.
19. Shen Y, Peng B, Cheung GSP (2004) Factors associated with the removal of fractured NiTi instruments from root canal systems. *Oral Surgey, Oral Medicine, Oral Pathology, and Oral Radiology.* **98**, 605-610.
20. Stitt EH, Hancock FE, Peeling RH *et al.* (2003) Experimental reactor development for a gas evolving catalytic decomposition reaction. *Catalysis Today.* **79-80**, 125-138.
21. Walia H, Brantley WA, Gerstein H (1988) An initial investigation of the bending and torsional properties of nitinol root canal files. *Journal of endodontics.* **14**, 346-351.
22. Webber J (2015) Shaping canals with confidence: Wave One GOLD single-file. *International Dentistry - African edition.* **6**, 34-40.

23. Zehnder M (2006) Root canal irrigants. *Journal of endodontics.* **32**, 389-398.

ARTIGO EM INGLÊS

Title page

Evaluation of the sodium hypochlorite solution in the electrochemical dissolution of thermally treated nickel-titanium instruments (GOLD).

AUTHORS:

Luiza Giacomet Cassol[a], DDS

Alexandre Kowalczuck[a], DDS, MS, PhD

Carlos Augusto Henning Laurindo[b], MS, PhD

Everdan Carneiro[a], DDS, MS, PhD

Vânia Portela Ditzel Westphalen[a], DDS, MS, PhD

Ulisses Xavier da Silva Neto[a], DDS, MS, PhD

[a]Department of Endodontics, School of Life Sciences, Pontifícia Universidade Católica do Paraná. Curitiba, Brazil.

[b]Department of Mechanical Engineering, Polytechnic School, Pontifícia Universidade Católica do Paraná, Curitiba, Brazil.

Abstract

Aim: To compare the influence of two solutions, fluoride solution and sodium hypochlorite both saturated with sodium chloride, during the electrochemical dissolution of instruments with conventional nickel-titanium alloy and thermally treated nickel-titanium (GOLD).

Methodology: Two solutions were evaluated (NaF solution - NaF 12g / L + NaCl 180 g / L, NaOCl solution- NaOCl 2.5% + NaCl 180g / L) by the polarization test of the Protaper Universal F1 instruments (PTU F1) and Wave One Gold Small (WOGS), with the sample of 48 instruments. The electrical current potentials were 0.5 V and 5 V for the NaF and NaOCl solution, respectively. The electrochemical cell composed of three electrodes for the polarization test of the PF1 and WOGS instruments, which had 6 mm of the tip immersed in the solutions tested. The recording of electric current occurred for 540 seconds (s). If the complete dissolution of the immersed portion of the instrument occurred in less than expected time, the experiment was considered finished. The time variations (in seconds) of the instruments in the NaF, NaOCl and distelled water solution were measured. The evaluation of the corrosion patterns of the instruments occurred by scanning electron microscopy (SEM). Data were submitted to statistical analysis using the Mann Whitney, Kruskal Wallis and Dunn tests.

Results: NaF and NaOCl solutions presented a statistically significant difference ($p < 0.05$) in relation to the dissolution time of the instrument (in seconds), with a mean of 12.9 s and 83.6 s, respectively. There was no statistically significant difference ($p > 0.05$) in relation to the time in the dissolution comparison between the PTU F1 and WOGS instruments.

Conclusions: Both solutions have electrochemical dissolution capacity of PTU F1 and WOGS instruments. However, the NaF solution promoted dissolution of the instruments in less time.

Keywords: Endodontics, Dissolution, Nickel-titanium instruments, Sodium hypochlorite.

Introduction

Nickel-titanium (NiTi) instruments were introduced to dentistry three decades ago (Walia, et al., 1988). They have become important for endodontic treatment, since this alloy has adequate flexibility and resistance to cyclic fatigue to be used in mechanized instruments, which provide a preparation format that allows the adequate sanitation of the root canal system in a shorter time when compared to instruments. (Cheung & Liu 2009, Schäfer & Bürklein 2012)

The NiTi alloy has the relevant thermal memory effect characteristic, which comes from the martensite and austenite phases. Aiming the improvement of these characteristic new technologies with thermomechanical treatments of manufacturing have been developed. The GOLD heat treatment was created to increase the superelasticity and resistance to cyclic fatigue of the instrument (Webber 2015).

However, despite new technologies and advances, instrument fracture can occur, especially in root canals with severe curvatures. Many methods and techniques have been proposed for the removal of fractured instruments within the root canal, but there is still no standardized technique that does not cause damage to the dental structure. (Shen *et al*. 2004)

Recently, a method has been presented that minimizes root canal wall wear (Ormiga *et al.* 2010). Research is still in the early stages, but the results are promising. This method consists in the immersion of two electrodes in an electrolyte, in which one will function as anode and the other as cathode. The contact between the fractured instrument and the electrode used as the anode is necessary, since the dissolution of the metal is the objective of the process. The potential difference between the two electrodes results in the migration of electrons from the anode to the cathode, consequently, there is release of the metal ions into the solution (Ormiga *et al.* 2010).

The Sodium hypochlorite is commonly used as an irrigating solution in endodontic treatment. When present in the electrolytic solution, the dissolution of NiTi increases by disintegrating the passivation layer that protects the metal against corrosion and oxidation (Sarkar *et al.* 1983). The same occurs with fluoride solutions, in which fluoride ions act on the titanium potentiating the dissolution process (Shen *et al.* 2004). In addition, the concentration of fluoride solution

saturated with sodium chloride (NaF 12g / L + NaCl 180 g / L) implies an increase in the values of the electric current, consequently, greater dissolution of the fractured instruments in a shorter time (Kowalczuck *et al*. 2017).

The research on electrochemical dissolution of endodontic instruments using conventional nickel titanium alloys, studies involving thermally treated nickel-titanium alloys (GOLD) have not been reported in the literature until now (Ormiga *et al.* 2015, Kowalczuck *et al*., 2017). Considering the recent advances in metallurgy in the manufacture of endodontic instruments, and the acceptance and incorporation of NiTi GOLD instruments into daily clinical practice, it is pertinent to elucidate the behavior of this metal alloy in the electrochemical dissolution process.

The purpose of this study was to compare the influence of two solutions: a fluoride saturated with sodium chloride and another solution of sodium hypochlorite saturated with sodium chloride, in relation to the electrochemical dissolution process of ProTaper Universal and Wave One Gold Small instruments.

Materials and methods

The sample containing 48 instruments Protaper Universal F1 (PTU F1) and Wave One Gold Small (WOGS) was tested by means of the polarization test. The behavior of three solutions was evaluated: NaF - NaF solution 12g / L + NaCl 180 g / L, NaOCl - NaOCl solution (2.5%) + NaCl 180g / L and solution 3 - distilled water (control group). Initially, the open circuit potential (OCP) of each solution was determined. An electrochemical cell composed of three electrodes was used. A saturated calomel electrode was used as reference electrode, a platinum electrode as a counter electrode, and a NiTi flat electrode as the working electrode. The electrochemical cell was coupled to a potentiostat (IviumStat, Ivium Technologies BV Eindhoven, The Netherlands) and the tests were performed at a scanning speed of 1 mV / s, starting from the open circuit potential up to +10 V potential. 200mL of the solution was used in each test, and in each experiment the solution was completely renewed. The test was repeated three times for each solution. In order to determine the potential from which the active dissolution of the nickel-titanium for each solution would take place, the potentiodynamic polarization test was carried out. Using an electrochemical cell with the same configuration previously described, the initial potentials obtained in the OCP determination test were applied up to +10 V for each solution.

Polarization of instruments ProTaper Universal F1 (PTU F1) and Wave One Gold Small (WOGS)

The same electrochemical cell described previously was used. However, the NiTi flat electrode was replaced by the PTU F1 or WOGS files used as the working electrode. The PTU F1 or WOGS instruments had the 6.0 mm (mm) tip immersed in the solutions tested. Starting from the potential capable of causing dissolution for each solution, the recording of the electric current occurred during 540 seconds (s). The electrical current potentials were 0.5 V and 5 V for the NaF and NaOCl solution, respectively. Solution 3 did not obtain results compatible with the dissolution of the NiTi alloys. If the complete dissolution of the immersed portion of the instrument occurred in less than expected time, the experiment was considered finished. The time values at which the dissolution occurred were compiled into a table and submitted to statistical analysis using the Mann Whitney, Kruskal Wallis and Dunn tests.

Results

Polarization of instruments ProTaper Universal F1 (PTU F1) and Wave One Gold Small (WOGS)

The NaF and NaOCl solutions presented a statistically significant difference ($p < 0.05$) in relation to the dissolution time of the instruments, showing an average of 12.96 seconds and 83.63 seconds, respectively. (Table 1).

Table 1. Descriptive statistics of the dissolution time (in seconds) of the solutions.

				Confidence interval 95%	
Solution	N	Mean	Standard Deviation	Lower Bound	Uper Bound
NaF	24	12,96	4,08	11,24	14,68
NaOCl	24	83,63	65,77	55,85	111,40

The PTU F1 and WOGS instruments did not present a statistically significant difference ($p > 0.05$) in relation to dissolution time, showed a mean of 50.13 seconds and 46.46 seconds, respectively.
(Table 2).

Table 2. Descriptive statistics of dissolution time (seconds) between instruments.

				Confidence interval 95%	
Instrument	N	Mean	Standard Deviation	Lower Bound	Upper Bound
PTU F1	24	50,13	67,00	21,83	78,42
WOGS	24	46,46	59,51	25,55	67,37

When comparing the mean time between samples, taking into account the null hypothesis, there was only a statistically significant difference between the NaF and NaOCl solutions. (Table 3).

Table 3. Multiple non-parametric Dunn comparison.

	Mean position difference	Test statistic	P value
PTU F1 + Sol NaF WOGS + Sol NaF	-1,08	-0,19	0,84
PTU F1 + Sol NaF PTU F1 + Sol NaOCl	-23,25	-4,07	0,00
PTU F1 + Sol NaF WOGS + Sol NaOCl	-25,83	-4,52	0,00
WOGS + Sol NaF PTU F1 + Sol NaOCl	22,16	3,88	0,00
WOGS + Sol NaF WOGS + Sol NaOCl	-24,75	-4,33	0,00
PTU F1 + Sol NaOCl WOGS + Sol NaOCl	-2,58	-0,45	0,65

Level of significance 0,05.

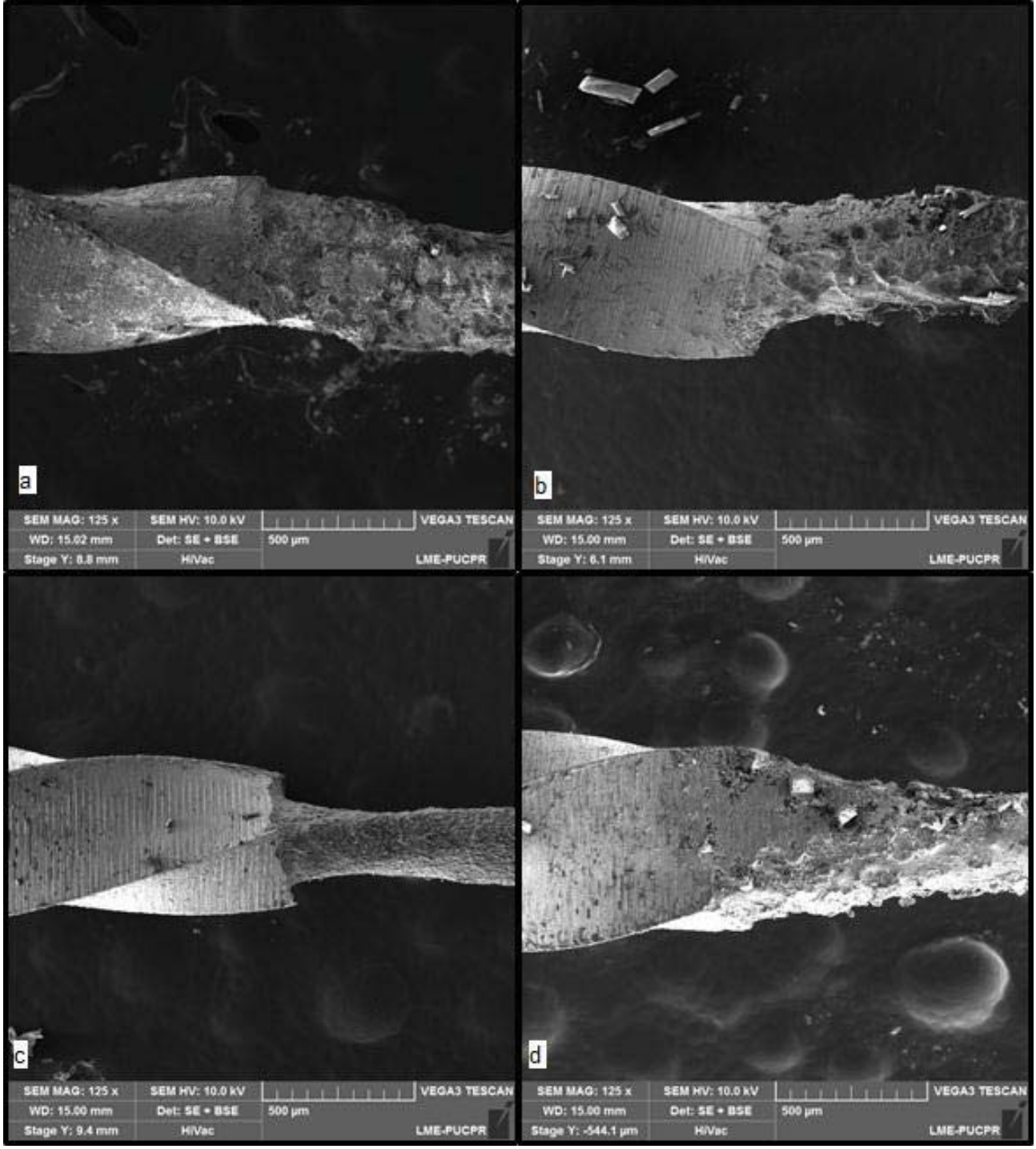


Figure 1. Scanning electromicrographs of instrument fragments submitted to the electrochemical dissolution experiment. **(A)** Fragment of the PTU F1 instrument submitted to the NaOCl solution in 125X. **(B)** Fragment of the PTU F1 instrument submitted to the NaF solution in 125X. **(C)** Fragment of the WOGS instrument submitted to the NaOCl solution in 125X. **(D)** Fragment of the WOGS instrument submitted to the NaF solution in 125X.

In the image (A) the corrosion by pites of the portion in which the metal was in contact with the electrolyte is observed. The portion of the instrument that had no contact with the solution, there was no damage to the structure. In the image (B) there is the pitting corrosion characteristic of the portion of the PTU F1 instrument immersed in the NaF solution. The corrosion image (C) is of the uniform type of portion of the WOGS instrument immersed in the NaOCl solution. Image (D) Alveolar corrosion of the portion of the WOGS instrument immersed in the NaF solution.

Discussion

The use of electrochemical dissolution in endodontics is a proposed method to dissolve endodontic instruments when fractured inside the root canal by the polarization of the metal alloy in an electrolyte using two electrodes (Ormiga *et al.* 2010). The solution of sodium fluoride with sodium chloride has been used as electrolyte in the studies on electrochemical dissolution because there is the synergic interaction of fluorine and chloride ions in the corrosion of the NiTi alloy (Ormiga *et al.* 2010, Kowalczuck *et al* 2017, Li *et al*. 2007, Amaral *et al.* 2018, Ormiga *et al.* 2011). Sodium hypochlorite began to be widely used as a disinfectant agent from the late 19th century when laboratory studies began to be published (Zehnder 2006). It has antimicrobial properties, ability to dissolve organic tissue (Harrison & Hand 1981), easy access, cost-effective, and are these characteristics that have made it the main irrigant in the preparation of root canals (Estrela *et al*., 2002). The present study is the first to use sodium hypochlorite associated with sodium chloride as an electrolyte solution. This solution demonstrated the ability to dissolve endodontic instruments, averaging 83 seconds. The saturation of the solution with sodium chloride increases the value of the electric current, and consequently the dissolution of the instrument in less time (Kowalczuck *et al*., 2017). During the experiment, the darkening of the electrolytic solution was observed by the release of metallic ions present in the NiTi alloy. These metal ions can act as a barrier between the anodic electrode and the instrument, making it difficult to dissolve the fragment and may even be toxic to periapical tissues if not eliminated, as in in vivo studies. An alternative to minimize possible browning of the solution would be the constant renewal of the electrolyte. In addition, nickel (present in the NiTi alloy) is known as one of the catalysts in the sodium hypochlorite decomposition reaction, and the result is an exothermic reaction and releases oxygen, a fact that was observed in the present study during the tests of polarization, the temperature of the electrolytic solution increased. (Stitt *et al.,* 2003).

In the ex vivo study, which used the fluoridated solution [NaF 12g/L + NaCl 1g/L] as the electrolytic solution and 5.25% sodium hypochlorite solution as the irrigating agent of the chemical-mechanical endodontic preparation, sodium

hypochlorite solution was shown to have greater cytotoxicity than the fluoride solution (Amaral *et al*., 2018). However, we must consider that cultured cells (fibroblasts) are more sensitive to chemical-induced toxicity than those found in periapical tissues (Bajrami D. *et al.* 2014). In addition, in vivo studies the solution would be restricted to the root canal, which can be compared to the use of sodium hypochlorite as an irrigant in the preparation of root canals. This irrigant is considered cytotoxic, but in rare circumstances it is extravasated beyond the root apex and comes into contact with the periapical tissues (Amaral *et al*. 2018). A possible adversity arising from the release of nickel would be an occurrence of these ions accidentally reaching the periapical tissues in allergic patients.

The open circuit potential test (OCP) is used to verify the electrochemical behavior of the solutions, since each solution presents the potential of electric current capable of causing the corrosion of NiTi instruments (Ormiga *et al.,* 2011). The fluoridated solution saturated with sodium chloride (NaF solution) showed better results in relation to time, when compared to the study that used the same solution, but with different electrical current potential (Kowalczuck *et al*., 2017). In the study that used the potential of 0.3 V electrical current, the average dissolution time was 540 s. In the present study, 0.5 V was used and the mean dissolution time was 12 seconds. It can be inferred that the electric current potential and the time are inversely proportional, that is, the higher the electric current value, the shorter the dissolution time of the instruments. The application of electric current to living tissue can present risks to the integrity of the same depending on the intensity applied. However, dentin and cement present in the tooth are considered electrical current insulation (Nekoofar *et al.,* 2006). Consequently, the electric circuit becomes restricted to the tooth, it is possible to apply the in vivo method. In addition, the voltage values used in the present study are not capable of causing damage to the human body (IEC / TS 60479-1).

The GOLD treatment is a technology based on heat treatment of the alloy and slowly cooled, which causes the modification of the transformation temperatures of the initial and final austenitic phase, and gives the golden appearance to WOGS instruments (Webber 2015). The heat treatments commonly applied to the NiTi alloy are: solubilization, quenching and annealing. These treatments can cause different reactions in the solid state: precipitation,

recrystallization and structural transformation of phase. Solubilization is the treatment that uses high temperatures for the dissolution of the precipitates to occur. The annealing, on the other hand, is carried out at low temperature, so that precipitates are formed in reduced dimensions, under controlled conditions that increase the resistance of the matrix to deformation. The annealing is the cooling of the material (Miller & Lagoudas 2001, Huang & Liu 2001). This thermal treatment produces the Ni4Ti3 precipitates, which are responsible for increasing the superelasticity and shape memory of the alloy and also for (Khalil-Allafi, *et al*., 2002). Unlike thermally treated NiTi alloys, the formation of these precipitates does not occur in the conventional alloy (Otsuka & Ren 2005). There was the hypothesis of different behaviors in the process of electrochemical dissolution of the instruments, because they present structural differences. However, the present experiment presented similar results for NiTi and NiTi GOLD alloys. There was no statistically significant difference in the dissolution time between the two instruments. This result can be due to the fact that both alloys tested are composed of the same material, NiTi, although they present differences in the form of fabrication. The potential difference caused by electronegative chemical elements, such as fluorine and chlorine, is what results in the corrosion of NiTi metal alloy (Li *et al.,* 2007). In addition, the contact surface of the instrument and electrolyte solution available in in vitro studies is greater than when performing ex vivo studies, and consequently the result could be altered.

Conclusion

Both solutions have demonstrated the ability to dissolve PTU F1 and WOGS instruments. The NaF solution has been shown to have superior results over time

References

1. Amaral CCF, Ormiga F, Boldrini LC, *et al*. (2018) Evaluation of the effects of the solution used for eletrochemical dissolution of nickel-titanium endodontic files on dentine structure, microhardness and cell viability. *International Endodontic Journal.* **51**, 1424-1445.
2. Bajrami D, Hoxha V, Gorduysus O, et al (2014) Cytotoxic effect of endodontic irrigants *in vitro*. *Medical Science Monitor Basic Research.* **20**, 22-26.
3. Cheung GPS, Liu CSY (2009) A retrospective study of endodontic treatment outcome between nickel-titanium rotary and stainless steel hand filing techniques. *Journal of Endodontics.* **35**, 938-943.
4. Estrela C, Estrela CRA, Barbin EL (2002) Mechanism of action of sodium hypochlorite. *Brazilian Dental Journal.* **13**, 113-117.
5. Harrison JW, Hand RE (1981) The effect of dilution and organic matter on the antibacterial property of 5.25% sodium hypochlorite. *Journal of Endodontics.* **7**, 128-132.
6. Huang X, Liu Y (2001) Effect of annealing on the transformation behavior and superelasticity of NiTi shape memory alloy. *Scripta Materialia.* **45**, 153-160.
7. IEC/TS 60479-1 (2005) Effects of current on human beings and livestock – Part 1: General aspects.
8. Khalil-Allafi J, Dlouhy A, Eggeler G (2002) Ni4Ti3 precipitation during aging of NiTi shape memory alloys and its influence on martensitic phase transformations. *Acta Materialia.* **50**, 4255-4274.
9. Kowalcuzuck A, Silva Neto UX, Fariniuk LF, *et al*. (2017) Electrochemical dissolution of fractured nickel-titanium instruments in human extracted teeth. *International Endodontic Journal.* **50**, 578-585.
10. Li X, Wang J, Han EH *et al.* (2007) Influence of fluoride and chloride on corrosion behavior of NiTi orthodontic wires. *Acta Biomaterialia.* **3**, 807-815.
11. Miller DA, Lagoudas DC (2001) Influence of cold work and heat treatment on the shape memory effect and plastic strain development of NiTi. Materials Science and Engineering A. **308**, 161-175.

12. Nekoofar MH, Ghandi MM, Hayes SJ *et al.* (2006) The fundamental operating principles of electronic root canal length measurement devices. *International Endodontic Journal*. **39**, 595-609.
13. Ormiga F, Da Cunha PGJA, de Araújo MCP (2010) Dissolution of nickel-titanium endodontic files via na electrochemical process: a new concept for future retrieval of fractured files in root canals. *Journal of Endodontics*. **36**, 717-720.
14. Ormiga F, da Cunha PGJA, de Araújo MCP *et al*. (2011) An initial investigation of the electrochemical dissolution of fragments of nickel-titanium endodontic files. *Journal of Endodontics.* **37**, 526-530.
15. Ormiga F, Aboud LRL, Gomes JACP (2015) Electrochemical-induced dissolution of nickel-titanium endodontic instruments with different designs. *International Endodontic Journal.* **48**, 342-350.
16. Otsuka K, Ren X (2005) Physical metallurgy of Ti-Ni-based shape memory alloys. *Progress in Materials Science.* **50**, 511-678.
17. Sarkar NK, Redmond W, Schwaninger B (1983) The chloride corrosion behavior of four orthodontic wires. *Journal of Oral Rehabilitation*. **10**, 121-128.
18. Schafer E, Burklein S (2012) Impact of nickel-titanium instrumentation of root canal on clinical outcomes: A focused review. *Odontology.* **100**, 130-136.
19. Shen Y, Peng B, Cheung GSP (2004) Factors associated with the removal of fractured NiTi instruments from root canal systems. *Oral Surgey, Oral Medicine, Oral Pathology, and Oral Radiology.* **98**, 605-610.
20. Stitt EH, Hancock FE, Peeling RH *et al.* (2003) Experimental reactor development for a gas evolving catalytic decomposition reaction. *Catalysis Today.* **79-80**, 125-138.
21. Walia H, Brantley WA, Gerstein H (1988) An initial investigation of the bending and torsional properties of nitinol root canal files. *Journal of endodontics.* **14**, 346-351.
22. Webber J (2015) Shaping canals with confidence: Wave One GOLD single-file. *International Dentistry - African edition.* **6**, 34-40.

23. Zehnder M (2006) Root canal irrigants. *Journal of endodontics.* **32**, 389-398.

ANEXOS

Análise estatística

Primeiramente o teste de normalidade foi realizado, pois a amostra é menor que trinta e a variável dissolução é numérica. O resultado dos testes de Komogorov-Smirnov e Shapiro-Wilk foi de distribuição não normal, apesar do grupo Wave One Gold Small com a solução 1 apresentar normalidade (p> 0,05) (Tabela 1). Desta forma a comparação entre instrumento e solução foi feita utilizando o teste não paramétrico U de Mann Whitney (Tabela 2, 3, 4, 5, 6, 7 e 8) enquanto a comparação considerando simultaneamente instrumento e solução foi realizada utilizando o teste não paramétrico de Kruskal Wallis (Tabela 9 e 10). O teste de Kruskal Wallis indicou diferença entre pelo menos dois tratamentos (instrumento versus solução) a comparação dois a dois foi feita utilizando o teste não paramétrico de Dunn (Tabela 11).

Tabela 1. Teste de Normalidade – Dissolução (Segundos)

Instrumento X Solução	**Kolmogorov-Smirnov**			**Shapiro-Wilk**		
	Estatística	df	Valor p	Estatística	df	Valor p
PTU F1/Solução 1	0,309	12	0,002	0,741	12	0,002
PTU F1 /Solução 2	0,299	12	0,004	0,670	12	0,000
WOGS/ Solução 1	0,178	12	,200	0,877	12	0,081
WOGS/ Solução 2	0,359	12	0,000	0,638	12	0,000

Tabela 2. Descritivos.

Dissolução (segundos) Instrumento	N	Média	Desvio Padrão	Erro Padrão	Intervalo de confiança de 95% para a média		Mínimo	Máximo
					Limite inferior	Limite superior		
PTU F1	24	50,13	67,009	13,678	21,83	78,42	9	250
WOGS	24	46,46	49,515	10,107	25,55	67,37	8	200
Total	48	48,49	58,314	8,417	31,36	65,22	8	250

Tabela 3. Descritivos.

Dissolução (segundos) Solução	N	Média	Desvio Padrão	Erro Padrão	Intervalo de confiança de 95% para a média		Mínimo	Máximo
					Limite inferior	Limite superior		
Solução 1	24	12,96	4,080	0,833	11,24	14,68	8	25
Solução 2	24	83,63	65,779	13,427	55,85	111,40	35	250
Total	48	48,29	58,314	8,417	31,36	65,22	8	250

Tabela 4. Descritivos

Dissolução (segundos) Instrumento X Solução	N	Média	Desvio Padrão	Erro Padrão	Intervalo de confiança de 95% para a média		Mínimo	Máximo
					Limite inferior	Limite superior		
PTU F1/Sol. 1	12	12,50	3,334	0,965	10,38	14,62	9	22
PTU F1/Sol. 2	12	87,75	79,302	22,892	37,36	138,14	35	250
WOGS/Sol.1	12	13,42	4,814	1,390	10,36	16,48	8	25
WOGS/Sol.2	12	79,50	52,165	15,059	46,36	112,64	44	200
Total	48	48,29	58,314	8,417	31,36	65,22	8	250

Tabela 5. Postos

Variável	Instrumento	N	Posto médio	Soma das classificações
Dissolução (Segundos)	PTU F1	24	23,58	566,000
	WOGS	24	25,42	610,000
	Total	48		

Tabela 6.

Estatística de teste[a]	
Teste	
U- Mann Whitney	266,000
Z	-4,454
Valor p	0,64949

a. Variável de agrupamento: Instrumento

Por meio do teste U-Mann Whitney foi obtido o valor de p 0,64949 (Tabela 6) quando comparado a média de tempo de dissolução dos instrumentos. O valor p> 0,05 não há diferença estatística siginificante entre Protaper Universal F1 e Wave One Gold Small. Na tabelas 7 e 8, o valor de p obtido foi de 0,000 quando comparado o valor da média de tempo de dissolução entre a solução 1 e 2, que demonstra que houve diferença estatisticamente significante (valor p < que 0,05).

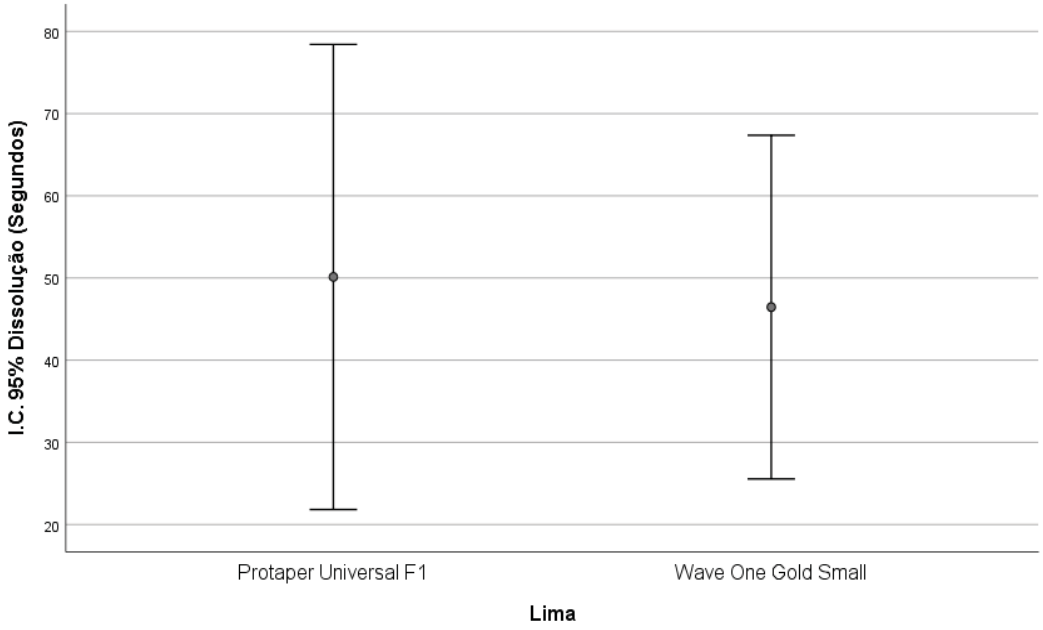


Tabela 7. Postos

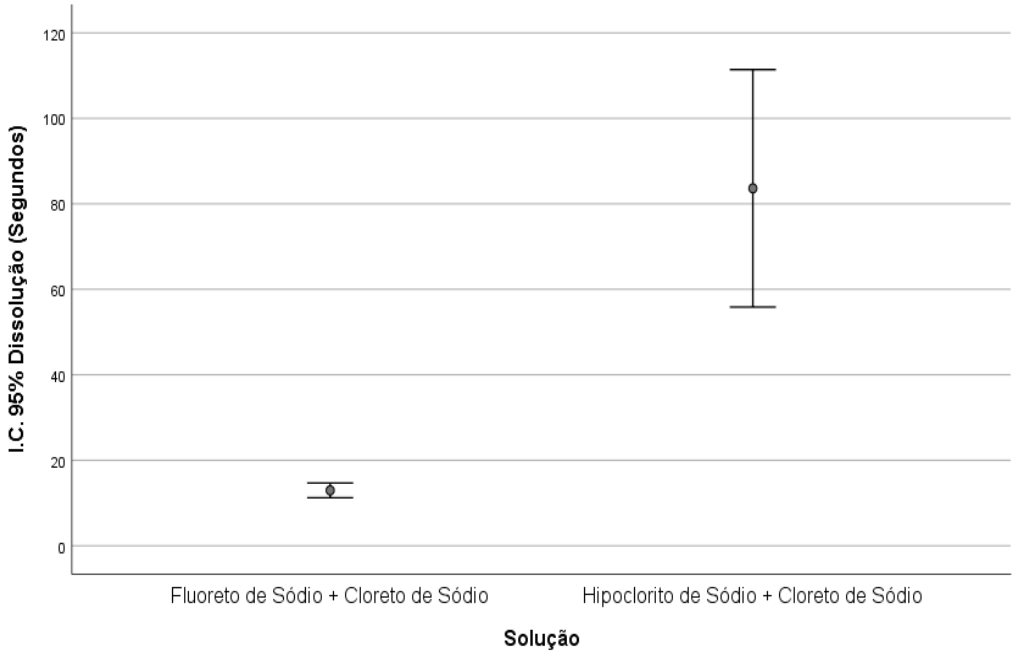
Variável	Solução	N	Posto médio	Soma das classificações
Dissolução	1	24	12,50	300,000
(Segundos)	2	24	36,50	876,000
	Total	48		

Tabela 8.

Estatística de teste[a]

Teste	Dissolução (segundos)
U de Mann-Whitney	0,000
Z	-5,949
Valor p	0,00000

a. Variável de agrupamento: Solução



O teste não paramétrico de Kruskal-Wallis substitui o Anova quando a distribuição é não normal. O resultado foi estatisticamente significante com valor de p 0,00.

Teste não paramétrico de Kruskal-Wallis

Tabela 9.

Postos	Lima X Solução	N	Posto médio
Variável	PTU F1/Solução 1	12	11,96
Dissolução	PTU F1/Solução 2	12	35,21
(Segundos)	WOGS/ Solução 1	12	13,04
	WOGS/ Solução 2	12	37,79
	Total	48	

Tabela 10.

Estatística de teste[a,b]

Teste	Dissolução (segundos)
H de Kruskal-Wallis	35,637
gl	3
Valor p	0,0000

a. Teste de Kruskal Wallis

b. Variável de agrupamento: Lima X Solução

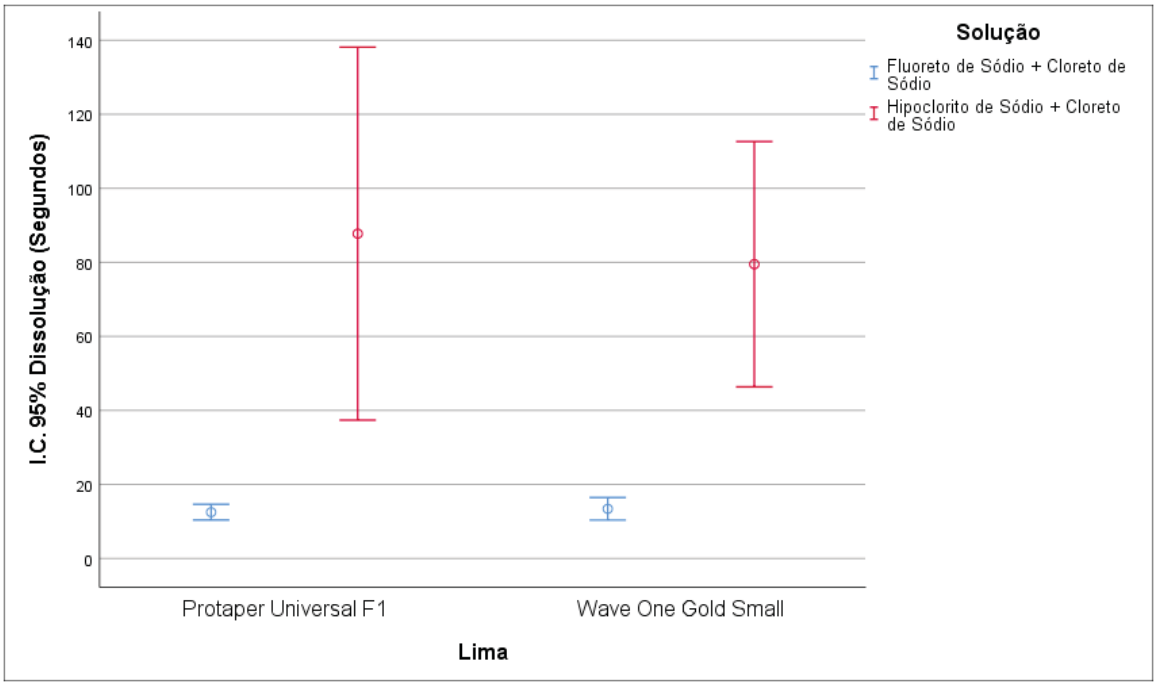
Tabela 11. Comparações múltiplas não paramétricas de Dunn

Amostra 1-Amostra 2	Diferença de posto médio	Estatística de teste	Valor p
PTUF1/Solução 1 + WOGS/Solução 1	-1,083	-0,190	0,849
PTUF1/Solução 1 + PTUF1/Solução 2	-23,250	-4,075	0,000
PTUF1/Solução 1 + WOGS/Solução 2	-25,833	-4,528	0,000
WOGS/Solução 1			

+	22,167	3,886	0,000
PTUF1/Solução 2			
WOGS/Solução 1			
+	-24,750	-4,338	0,000
WOGS/Solução 2			
PTUF1/Solução 2			
+	-2,583	-0,453	0,651
WOGS/Solução 2			

Cada linha testa a hipótese nula em que as distribuições da Amostra 1 e Amostra 2 são iguais. As significâncias são exibidas. O nível de significância é 0,05.

No teste de comparação múltiplas não paramétricas de Dunn, o valor p 0,00 demonstrou diferença estatisticamente significante quando a solução 2 foi utilizada.



Metodologia complementar

Células eletroquímicas

Inicialmente foram realizados os testes de circuito aberto para a solução1, 2 e 3. A célula eletroquímica era composta por três eletrodos: eletrodo de calomelano saturado (eletrodo de referência), eletrodo de platina (contra-eletrodo) e eletrodo plano de NiTi (eletrodo de trabalho). (Figura 1).

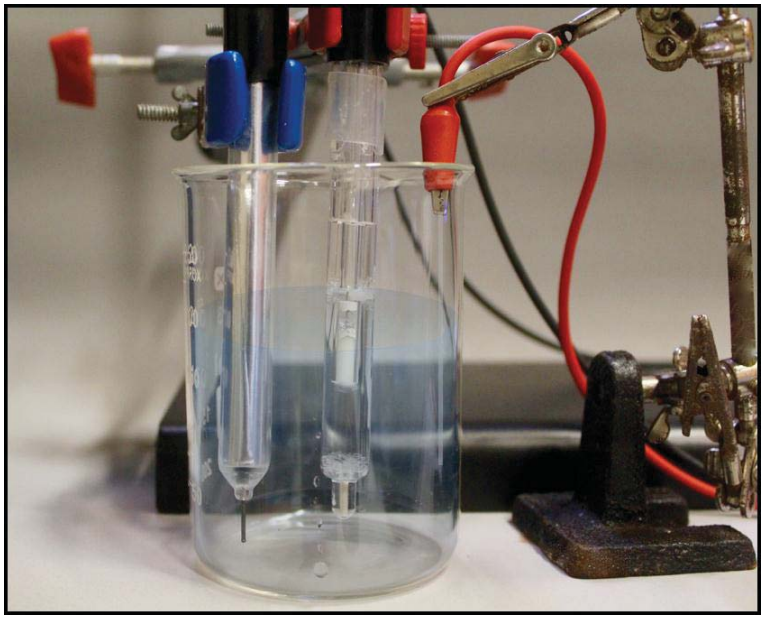


Figura 1. Célula eletroquímica composta por três eletrodos. Contra-eletrodo, eletrodo de referência e eletrodo de trabalho (da esquerda para a direita). Imagem disponibilizada pelo Prof. Dr. Alexandre Kowalczuck.

Para os testes de polarização potenciodinâmica, o eletrodo de trabalho foi substituído pelos instrumentos Wave One Gold Small (WOGS) e Protaper Universal F1 (PF1) com 6mm imersos nas soluções (Figura 2).

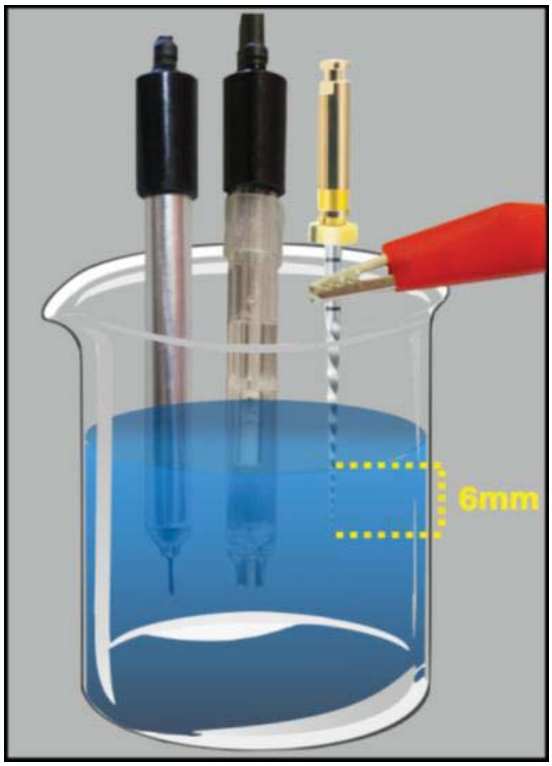


Figura 2. Esquema representativo da célula eletroquímica com os 6mm apicais dos instrumentos PF1 e WOGS imersos nas soluções. Disponibilizado pelo Prof. Dr. Alexandre Kowalczuck.

Figura 3. Instrumentos utilizados: Protaper Universal F1 (PTU F1) e Wave One Gold Small (WOGS)

A célula eletroquímica era acoplada ao potenciostato (IviumStat, Ivium Technologies B. V. Eindhoven, Holanda), e um gráfico era formado por meio de um computador. Foi utilizado um cursor de borracha e uma régua milimetrada para

delimitar os 6mm da ponta do instrumento que ficaria em contato com a solução. (Figura 4 e 5).

Figura 4. Potenciostato. (IviumStat, Ivium Technologies B. V. Eindhoven, Holanda)

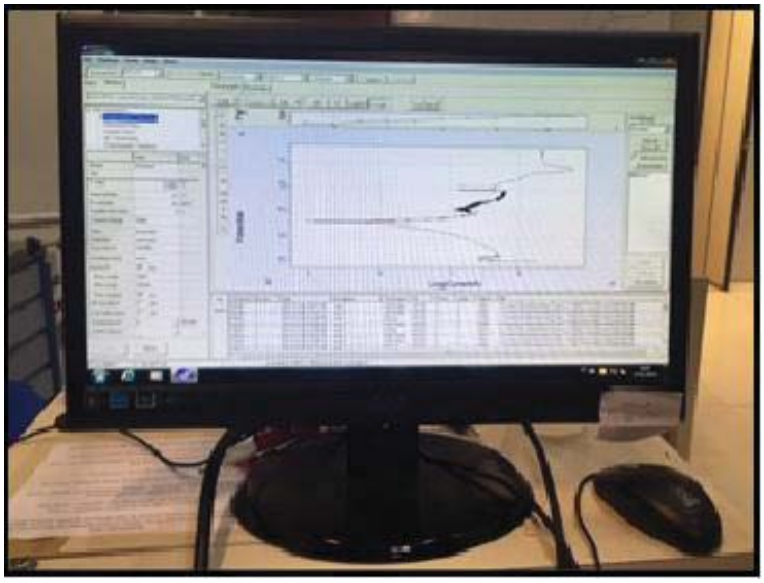


Figura 5. Imagem gerada em um gráfico por meio de um computador acoplado ao Potenciostato.

Normas para publicação – International Endodontic Journal

Author Guidelines

1. GENERAL

International Endodontic Journal publishes original scientific articles, reviews, clinical articles and case reports in the field of Endodontology; the branch of dental sciences dealing with health, injuries to and diseases of the pulp and periradicular region, and their relationship with systemic well-being and health. Original scientific articles are published in the areas of biomedical science, applied materials science, bioengineering, epidemiology and social science relevant to endodontic disease and its management, and to the restoration of root-treated teeth. In addition, review articles, reports of clinical cases, book reviews, summaries and abstracts of scientific meetings and news items are accepted.

Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in *International Endodontic Journal*. Authors are encouraged to visit Wiley Author Services for further information on the preparation and submission of articles and figures.

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 Association Declaration 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 and a separate English translation. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

2.3 Clinical Trials

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.

2.3.1 Randomised control clinical trials

Randomised control clinical trials should be reported using the guidelines available at www.consort-statement.org. A CONSORT checklist and flow diagram (as a Figure) should also be included in the submission material.

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)

2.4 Systematic Reviews

Systematic reviews should be reported using the PRISMA guidelines available at http://prisma-statement.org/. A PRISMA checklist and flow diagram (as a Figure) should also be included in the submission material.

2.5 DNA Sequences and Crystallographic Structure Determinations

Papers reporting protein or DNA sequences and crystallographic structure determinations will not be accepted without a Genbank or Brookhaven accession number, respectively. Other supporting data sets must be made available on the publication date from the authors directly.

2.6 Conflict of Interest and Source of Funding

International Endodontic Journal requires that all authors (both the corresponding author and co-authors) disclose any potential sources of conflict of interest. Any interest or relationship, financial or otherwise that might be perceived as influencing an author's objectivity is considered a potential source of conflict of interest. These must be disclosed when directly relevant or indirectly related to the work that the authors describe in their manuscript. Potential sources of conflict of interest include but are not limited to patent or stock ownership, membership of a company board of directors, membership of an advisory board or committee for a company, and consultancy for or receipt of speaker's fees from a company. If authors are unsure whether a past or present affiliation or relationship should be disclosed in the manuscript, please contact the editorial office at iejeditor@cardiff.ac.uk. The existence of a conflict of interest does not preclude publication in this journal.

The above policies are in accordance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals produced by the International Committee of Medical Journal Editors (http://www.icmje.org/).

It is the responsibility of the corresponding author to have all authors of a manuscript fill out a conflict of interest disclosure form, and to upload all forms individually (do not combine the forms into one file) together with the manuscript on submission. The disclosure statement should be included under Acknowledgements. Please find the form below:

Conflict of Interest Disclosure Form

2.7 Appeal of Decision

The decision on a paper is final and cannot be appealed.

2.8 Permissions

If all or parts of previously published illustrations are used, permission must be obtained from the copyright holder concerned. It is the author's responsibility to obtain these in writing and provide copies to the Publishers.

2.8 Copyright Assignment

If your paper is accepted, the author identified as the formal corresponding author for the paper will receive an email prompting them to login into Author Services; where via the Wiley Author Licensing Service (WALS) they will be able to complete the license agreement on behalf of all authors on the paper. Your article cannot be published until this has been done.

For authors choosing OnlineOpen

If the OnlineOpen option is selected the corresponding author will have a choice of the following Creative Commons License Open Access Agreements (OAA):
Creative Commons Attribution License OAA
Creative Commons Attribution Non-Commercial License OAA
Creative Commons Attribution Non-Commercial - No Derivs License OAA

To preview the terms and conditions of these open access agreements please visit the Copyright FAQs hosted on Wiley Author Services http://exchanges.wiley.com/authors/faqs---copyright-_301.html and visit http://www.wileyopenaccess.com/details/content/12f25db4c87/Copyright--License.html.

If you select the OnlineOpen option and your research is funded by certain funders [e.g. The Wellcome Trust and members of the Research Councils UK (RCUK) or the Austrian Science Fund (FWF)] you will be given the opportunity to publish your article under a CC-BY license supporting you in complying with Wellcome Trust and Research Councils UK requirements. For more information on this policy and the Journal’s compliant self-archiving policy please visit: http://www.wiley.com/go/funderstatement.

2.9 OnlineOpen

OnlineOpen is available to authors of primary research articles who wish to make their article available to non-subscribers on publication, or whose funding agency requires grantees to archive the final version of their article. With OnlineOpen, the author, the author's funding agency, or the author's institution pays a fee to ensure that the article is made available to

non-subscribers upon publication via Wiley Online Library, as well as deposited in the funding agency's preferred archive. For the full list of terms and conditions, see http://wileyonlinelibrary.com/onlineopen#OnlineOpen_Terms

Any authors wishing to send their paper OnlineOpen will be required to complete the payment form available from our website at: https://authorservices.wiley.com/bauthor/onlineopen_order.asp

Prior to acceptance there is no requirement to inform an Editorial Office that you intend to publish your paper OnlineOpen if you do not wish to. All OnlineOpen articles are treated in the same way as any other article. They go through the journal's standard peer-review process and will be accepted or rejected based on their own merit.

3. MANUSCRIPT SUBMISSION PROCEDURE

Manuscripts should be submitted electronically via the online submission site http://mc.manuscriptcentral.com/iej. The use of an online submission and peer review site enables immediate distribution of manuscripts and consequentially speeds up the review process. It also allows authors to track the status of their own manuscripts. Complete instructions for submitting a paper is available online and below. Further assistance can be obtained from iejeditor@cardiff.ac.uk.

3.1. Getting Started

• Launch your web browser (supported browsers include Internet Explorer 5.5 or higher, Safari 1.2.4, or Firefox 1.0.4 or higher) and go to the journal's online Submission Site: http://mc.manuscriptcentral.com/iej

• Log-in, or if you are a new user, click on 'register here'.

• If you are registering as a new user.

- After clicking on 'register here', enter your name and e-mail information and click 'Next'. Your e-mail information is very important.

- Enter your institution and address information as appropriate, and then click 'Next.'

- Enter a user ID and password of your choice (we recommend using your e-mail address as your user ID), and then select your areas of expertise. Click 'Finish'.

• If you are registered, but have forgotten your log in details, please enter your e-mail address under 'Password Help'. The system will send you an automatic user ID and a new temporary password.

• Log-in and select 'Author Centre '

3.2. Submitting Your Manuscript

• After you have logged into your 'Author Centre', submit your manuscript by clicking on the

submission link under 'Author Resources'.
• Enter data and answer questions as appropriate. You may copy and paste directly from your manuscript and you may upload your pre-prepared covering letter.
• Click the 'Next' button on each screen to save your work and advance to the next screen.
• You are required to upload your files.
- Click on the 'Browse' button and locate the file on your computer.
- Select the designation of each file in the drop down next to the Browse button.
- When you have selected all files you wish to upload, click the 'Upload Files' button.
• Review your submission (in HTML and PDF format) before completing your submission by sending it to the Journal. Click the 'Submit' button when you are finished reviewing.

3.3. Manuscript Files Accepted

Manuscripts should be uploaded as Word (.doc) or Rich Text Format (.rft) files (not write-protected) plus separate figure files. GIF, JPEG, PICT or Bitmap files are acceptable for submission, but only high-resolution TIF or EPS files are suitable for printing. The files will be automatically converted to HTML and PDF on upload and will be used for the review process. The text file must contain the abstract, main text, references, tables, and figure legends, but no embedded figures or Title page. The Title page should be uploaded as a separate file. In the main text, please reference figures as for instance 'Figure 1', 'Figure 2' etc to match the tag name you choose for the individual figure files uploaded. Manuscripts should be formatted as described in the Author Guidelines below.

3.4. Blinded Review

Manuscript that do not conform to the general aims and scope of the journal will be returned immediately without review. All other manuscripts will be reviewed by experts in the field (generally two referees). International Endodontic Journal aims to forward referees´ comments and to inform the corresponding author of the result of the review process. Manuscripts will be considered for fast-track publication under special circumstances after consultation with the Editor.
International Endodontic Journal uses double blinded review. The names of the reviewers will thus not be disclosed to the author submitting a paper and the name(s) of the author(s) will not be disclosed to the reviewers.
To allow double blinded review, please submit (upload) your main manuscript and title page as separate files.
Please upload:
• Your manuscript without title page under the file designation 'main document'
• Figure files under the file designation 'figures'
• The title page and Acknowledgements where applicable, should be uploaded under the file

designation 'title page'
All documents uploaded under the file designation 'title page' will not be viewable in the html and pdf format you are asked to review in the end of the submission process. The files viewable in the html and pdf format are the files available to the reviewer in the review process.

3.5. Suspension of Submission Mid-way in the Submission Process

You may suspend a submission at any phase before clicking the 'Submit' button and save it to submit later. The manuscript can then be located under 'Unsubmitted Manuscripts' and you can click on 'Continue Submission' to continue your submission when you choose to.

3.6. E-mail Confirmation of Submission

After submission you will receive an e-mail to confirm receipt of your manuscript. If you do not receive the confirmation e-mail after 24 hours, please check your e-mail address carefully in the system. If the e-mail address is correct please contact your IT department. The error may be caused by some sort of spam filtering on your e-mail server. Also, the e-mails should be received if the IT department adds our e-mail server (uranus.scholarone.com) to their whitelist.

3.7. Manuscript Status

You can access ScholarOne Manuscripts any time to check your 'Author Centre' for the status of your manuscript. The Journal will inform you by e-mail once a decision has been made.

3.8. Submission of Revised Manuscripts

To submit a revised manuscript, locate your manuscript under 'Manuscripts with Decisions' and click on 'Submit a Revision'. Please remember to delete any old files uploaded when you upload your revised manuscript.

4. MANUSCRIPT TYPES ACCEPTED

Original Scientific Articles: must describe significant and original experimental observations and provide sufficient detail so that the observations can be critically evaluated and, if necessary, repeated. Original Scientific Articles must conform to the highest international standards in the field.

Review Articles: are accepted for their broad general interest; all are refereed by experts in the field who are asked to comment on issues such as timeliness, general interest and

balanced treatment of controversies, as well as on scientific accuracy. Reviews should generally include a clearly defined search strategy and take a broad view of the field rather than merely summarizing the authors´ own previous work. Extensive or unbalanced citation of the authors´ own publications is discouraged.

Clinical Articles: are suited to describe significant improvements in clinical practice such as the report of a novel technique, a breakthrough in technology or practical approaches to recognised clinical challenges. They should conform to the highest scientific and clinical practice standards.

Case Reports: illustrating unusual and clinically relevant observations are acceptable but they must be of sufficiently high quality to be considered worthy of publication in the Journal. On rare occasions, completed cases displaying non-obvious solutions to significant clinical challenges will be considered. Illustrative material must be of the highest quality and healing outcomes, if appropriate, should be demonstrated.

Supporting Information: *International Endodontic Journal* encourages submission of adjuncts to printed papers via the supporting information website (see submission of supporting information below). It is encouraged that authors wishing to describe novel procedures or illustrate cases more fully with figures and/or video may wish to utilise this facility.

Letters to the Editor: are also acceptable.

Meeting Reports: are also acceptable.

5. MANUSCRIPT FORMAT AND STRUCTURE

5.1. Format

Language: The language of publication is English. It is preferred that manuscript is professionally edited. A list of independent suppliers of editing services can be found at http://authorservices.wiley.com/bauthor/english_language.asp. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication

Presentation: Authors should pay special attention to the presentation of their research

findings or clinical reports so that they may be communicated clearly. Technical jargon should be avoided as much as possible and clearly explained where its use is unavoidable. Abbreviations should also be kept to a minimum, particularly those that are not standard. The background and hypotheses underlying the study, as well as its main conclusions, should be clearly explained. Titles and abstracts especially should be written in language that will be readily intelligible to any scientist.

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.

5.2. Structure

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

Title Page: The title page should bear: (i) Title, which should be concise as well as descriptive; (ii) Initial(s) and last (family) name of each author; (iii) Name and address of department, hospital or institution to which work should be attributed; (iv) Running title (no more than 30 letters and spaces); (v) No more than six keywords (in alphabetical order); (vi) Name, full postal address, telephone, fax number and e-mail address of author responsible for correspondence.

Abstract for Original Scientific Articles should be no more than 300 words giving details of what was done using the following structure:

• **Aim**: Give a clear statement of the main aim of the study and the main hypothesis tested, if any.
• **Methodology**: Describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and statistical tests.
• **Results**: Give the main results of the study, including the outcome of any statistical analysis.
• **Conclusions**: State the primary conclusions of the study and their implications. Suggest areas for further research, if appropriate.

Abstract for Systematic Review Articles should be no more than 300 words giving details of what was done using the following structure where applicable:

• **Background**: Provide a brief introduction of the subject and why it is important.
• **Aim**: Give a clear statement of the main aim of the study and the main hypothesis tested, if any.
• **Data sources**: Describe the databases searched.
• **Study eligibility criteria, participants, and interventions**: Briefly describe the methods adopted including exclusion/inclusion criteria.
• **Study appraisal and synthesis methods**: Describe bias, study type and quality
• **Results**: Give the main results of the review, including the outcome of any statistical meta-analysis.
• **Limitations**: Highlight problems with the current review end research area
• **Conclusions and implications of key findings**: State the primary conclusions of the study and their implications. Suggest areas for further research, if appropriate.

Abstract for Case Reports should be no more than 300 words using the following structure:

• **Aim**: Give a clear statement of the main aim of the report and the clinical problem which is addressed.
• **Summary**: Describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and analysis if any.
• **Key learning points**: Provide up to 5 short, bullet-pointed statements to highlight the key messages of the report. All points must be fully justified by material presented in the report.

Abstract for Clinical Articles should be no more than 300 words using the following structure:

• **Aim:** Give a clear statement of the main aim of the report and the clinical problem which is addressed.
• **Methodology:** Describe the methods adopted.
• **Results:** Give the main results of the study.
• **Conclusions:** State the primary conclusions of the study.

Main Text of Original Scientific Article should include Introduction, Materials and Methods, Results, Discussion and Conclusion

Introduction: should be focused, outlining the historical or logical origins of the study and gaps in knowledge. Exhaustive literature reviews are not appropriate. It should close with the explicit statement of the specific aims of the investigation, or hypothesis to be tested.

Material and Methods: must contain sufficient detail such that, in combination with the references cited, all clinical trials and experiments reported can be fully reproduced.

(i) Clinical Trials should be reported using the CONSORT guidelines available at www.consort-statement.org. A CONSORT checklist and flow diagram (as a Figure) should also be included in the submission material.

(ii) Experimental Subjects: experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration 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, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

(iii) Suppliers: Suppliers of materials should be named and their location (Company, town/city, state, country) included.

Results: should present the observations with minimal reference to earlier literature or to possible interpretations. Data should not be duplicated in Tables and Figures.

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.

Conclusion: should contain a summary of the findings.

Main Text of Review Articles should be divided into Introduction, Review and Conclusions. The Introduction section should be focused to place the subject matter in context and to justify the need for the review. The Review section should be divided into logical sub-sections in order to improve readability and enhance understanding. Search strategies must be described and the use of state-of-the-art evidence-based systematic approaches is expected. The use of tabulated and illustrative material is encouraged. The Conclusion section should reach clear conclusions and/or recommendations on the basis of the evidence presented.

Main Text of Clinical Reports and Clinical Articles should be divided into Introduction, Report, Discussion and Conclusion,. They should be well illustrated with clinical images, radiographs, diagrams and, where appropriate, supporting tables and graphs. However, all illustrations must be of the highest quality

Acknowledgements: *International Endodontic Journal* requires that all sources of institutional, private and corporate financial support for the work within the manuscript must be fully acknowledged, and any potential conflicts of interest noted. Grant or contribution numbers may be acknowledged, and principal grant holders should be listed. Acknowledgments should be brief and should not include thanks to anonymous referees and editors. See also above under Ethical Guidelines.

5.3. References

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

In the text: single or double authors should be acknowledged together with the year of publication, e.g. (Pitt Ford & Roberts 1990). If more than two authors the first author followed by *et al.* is sufficient, e.g. (Tobias *et al.* 1991). If more than 1 paper is cited the

references should be in year order and separated by "," e.g. (Pitt Ford & Roberts 1990, Tobias *et al*. 1991).

Reference list: All references should be brought together at the end of the paper in alphabetical order and should be in the following form.

(i) Names and initials of up to six authors. When there are seven or more, list the first three and add *et al.*

(ii)Year of publication in parentheses

(iii) Full title of paper followed by a full stop (.)

(iv) Title of journal in full (in italics)

(v) Volume number (bold) followed by a comma (,)

(vi) First and last pages

Examples of correct forms of reference follow:

Standard journal article

Bergenholtz G, Nagaoka S, Jontell M (1991) Class II antigen-expressing cells in experimentally induced pulpitis. *International Endodontic Journal* **24**, 8-14.

Corporate author

British Endodontic Society (1983) Guidelines for root canal treatment. *International Endodontic Journal***16**, 192-5.

Journal supplement

Frumin AM, Nussbaum J, Esposito M (1979) Functional asplenia: demonstration of splenic activity by bone marrow scan (Abstract). *Blood* **54** (Suppl. 1), 26a.

Books and other monographs

Personal author(s)

Gutmann J, Harrison JW (1991) *Surgical Endodontics*, 1st edn Boston, MA, USA: Blackwell Scientific Publications.

Chapter in a book

Wesselink P (1990) Conventional root-canal therapy III: root filling. In: Harty FJ, ed. *Endodontics in Clinical Practice*, 3rd edn; pp. 186-223. London, UK: Butterworth.

Published proceedings paper

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.

Agency publication

Ranofsky AL (1978) Surgical Operations in Short-Stay Hospitals: United States-1975. DHEW

publication no. (PHS) 78-1785 (Vital and Health Statistics; Series 13; no. 34.) Hyattsville, MD, USA: National Centre for Health Statistics.8

Dissertation or thesis

Saunders EM (1988) In vitro and in vivo investigations into root-canal obturation using thermally softened gutta-percha techniques (PhD Thesis). Dundee, UK: University of Dundee.

URLs

Full reference details must be given along with the URL, i.e. authorship, year, title of document/report and URL. If this information is not available, the reference should be removed and only the web address cited in the text.

Smith A (1999) Select committee report into social care in the community [WWW document]. URL http://www.dhss.gov.uk/reports/report015285.html
[accessed on 7 November 2003]

5.4. Tables, Figures and Figure Legends

Tables: Tables should be double-spaced with no vertical rulings, with a single bold ruling beneath the column titles. Units of measurements must be included in the column title.

Figures: All figures should be planned to fit within either 1 column width (8.0 cm), 1.5 column widths (13.0 cm) or 2 column widths (17.0 cm), and must be suitable for photocopy reproduction from the printed version of the manuscript. Lettering on figures should be in a clear, sans serif typeface (e.g. Helvetica); if possible, the same typeface should be used for all figures in a paper. After reduction for publication, upper-case text and numbers should be at least 1.5-2.0 mm high (10 point Helvetica). After reduction, symbols should be at least 2.0-3.0 mm high (10 point). All half-tone photographs should be submitted at final reproduction size. In general, multi-part figures should be arranged as they would appear in the final version. Reduction to the scale that will be used on the page is not necessary, but any special requirements (such as the separation distance of stereo pairs) should be clearly specified.

Unnecessary figures and parts (panels) of figures should be avoided: data presented in small tables or histograms, for instance, can generally be stated briefly in the text instead. Figures should not contain more than one panel unless the parts are logically connected; each panel of a multipart figure should be sized so that the whole figure can be reduced by the same amount and reproduced on the printed page at the smallest size at which essential details are visible.

Figures should be on a white background, and should avoid excessive boxing, unnecessary colour, shading and/or decorative effects (e.g. 3-dimensional skyscraper histograms) and highly pixelated computer drawings. The vertical axis of histograms should

not be truncated to exaggerate small differences. The line spacing should be wide enough to remain clear on reduction to the minimum acceptable printed size.

Figures divided into parts should be labelled with a lower-case, boldface, roman letter, a, b, and so on, in the same typesize as used elsewhere in the figure. Lettering in figures should be in lower-case type, with the first letter capitalized. Units should have a single space between the number and the unit, and follow SI nomenclature or the nomenclature common to a particular field. Thousands should be separated by a thin space (1 000). Unusual units or abbreviations should be spelled out in full or defined in the legend. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. In general, visual cues (on the figures themselves) are preferred to verbal explanations in the legend (e.g. broken line, open red triangles etc.)

Figure legends: Figure legends should begin with a brief title for the whole figure and continue with a short description of each panel and the symbols used; they should not contain any details of methods.

Permissions: If all or part of previously published illustrations are to be used, permission must be obtained from the copyright holder concerned. This is the responsibilty of the authors before submission.

Preparation of Electronic Figures for Publication: Although low quality images are adequate for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit EPS (lineart) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented programmes. Scans (TIFF only) should have a resolution of 300 dpi (halftone) or 600 to 1200 dpi (line drawings) in relation to the reproduction size (see below). EPS files should be saved with fonts embedded (and with a TIFF preview if possible). For scanned images, the scanning resolution (at final image size) should be as follows to ensure good reproduction: lineart: >600 dpi; half-tones (including gel photographs): >300 dpi; figures containing both halftone and line images: >600 dpi.

Further information can be obtained at Wiley Blackwell's guidelines for figures: http:/authorservices.wiley.com/bauthor/illustration.asp.

Check your electronic artwork before submitting it: http://authorservices.wiley.com/bauthor/eachecklist.asp.

5.5. Supporting Information

Publication in electronic formats has created opportunities for adding details or whole sections in the electronic version only. Authors need to work closely with the editors in developing or using such new publication formats.

Supporting information, such as data sets or additional figures or tables, that will not be published in the print edition of the journal, but which will be viewable via the online edition, can be submitted. It should be clearly stated at the time of submission that the supporting information is intended to be made available through the online edition. If the size or format of the supporting information is such that it cannot be accommodated on the journal's website, the author agrees to make the supporting information available free of charge on a permanent Web site, to which links will be set up from the journal's website. The author must advise Wiley Blackwell if the URL of the website where the supporting information is located changes. The content of the supporting information must not be altered after the paper has been accepted for publication.

The availability of supporting information should be indicated in the main manuscript by a paragraph, to appear after the References, headed 'Supporting Information' and providing titles of figures, tables, etc. In order to protect reviewer anonymity, material posted on the authors Web site cannot be reviewed. The supporting information is an integral part of the article and will be reviewed accordingly.

Preparation of Supporting Information: Although provision of content through the web in any format is straightforward, supporting information is best provided either in web-ready form or in a form that can be conveniently converted into one of the standard web publishing formats:

• Simple word-processing files (.doc or .rtf) for text.
• PDF for more complex, layout-dependent text or page-based material. Acrobat files can be distilled from Postscript by the Publisher, if necessary.
• GIF or JPEG for still graphics. Graphics supplied as EPS or TIFF are also acceptable.
• MPEG or AVI for moving graphics.

Subsequent requests for changes are generally unacceptable, as for printed papers. A charge may be levied for this service.

Video Imaging: For the on-line version of the Journal the submission of illustrative video is encouraged. Authors proposing the use such media should consult with the Editor during manuscript preparation.

6. AFTER ACCEPTANCE

Upon acceptance of a paper for publication, the manuscript will be forwarded to the Production Editor who is responsible for the production of the journal.

6.1. Figures

Hard copies of all figures and tables are required when the manuscript is ready for publication. These will be requested by the Editor when required. Each Figure copy should be marked on the reverse with the figure number and the corresponding author's name.

6.2 Proof Corrections

The corresponding author will receive an email alert containing a link to a web site. A working email address must therefore be provided for the corresponding author. The proof can be downloaded as a PDF (portable document format) file from this site. Acrobat Reader will be required in order to read this file. This software can be downloaded (free of charge) from the following Web site: www.adobe.com/products/acrobat/readstep2.html. This will enable the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof. Hard copy proofs will be posted if no e-mail address is available; in your absence, please arrange for a colleague to access your e-mail to retrieve the proofs. Proofs must be returned to the Production Editor within three days of receipt. As changes to proofs are costly, we ask that you only correct typesetting errors. Excessive changes made by the author in the proofs, excluding typesetting errors, will be charged separately. Other than in exceptional circumstances, all illustrations are retained by the publisher. Please note that the author is responsible for all statements made in his work, including changes made by the copy editor.

6.3 Early Online Publication Prior to Print

International Endodontic Journal is covered by Wiley Blackwell's Early View service. Early View articles are complete full-text articles published online in advance of their publication in a printed issue. Early View articles are complete and final. They have been fully reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in final form, no changes can be made after online publication. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the traditional way. They are therefore given a Digital Object Identifier (DOI), which allows the article to be cited and tracked before it is allocated to an issue. After print publication, the DOI remains valid and can continue to be used to cite and access the article.

6.4 Online Production Tracking

Online production tracking is available for your article through Blackwell's Author Services. Author Services enables authors to track their article - once it has been accepted - through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated e-mails at key stages of production. The author will receive an e-mail with a unique link that enables them to register and have their article automatically added to the system. Please ensure that a complete e-mail address is provided when submitting the manuscript. Visit http://authorservices.wiley.com/bauthor/ for more details on online production tracking and for a wealth of resources including FAQs and tips on article preparation, submission and more.

6.5 Author Material Archive Policy

Please note that unless specifically requested, Wiley Blackwell will dispose of all hardcopy or electronic material submitted two months after publication. If you require the return of any material submitted, please inform the editorial office or production editor as soon as possible.

6.6 Offprints

Free access to the final PDF offprint of your article will be available via Author Services only. Please therefore sign up for Author Services if you would like to access your article PDF offprint and enjoy the many other benefits the service offers.

Additional paper offprints may be ordered online. Please click on the following link, fill in the necessary details and ensure that you type information in all of the required fields: Offprint Cosprinters. If you have queries about offprints please email offprint@cosprinters.com

The corresponding author will be sent complimentary copies of the issue in which the paper is published (one copy per author).

6.7 Author Services

For more substantial information on the services provided for authors, please see Wiley Blackwell Author Services

6.8 Note to NIH Grantees: Pursuant to NIH mandate, Wiley Blackwell will post the accepted version of contributions authored by NIH grant-holders to PubMed Central upon acceptance. This accepted version will be made publicly available 12 months after publication. For further information, see www.wiley.com/go/nihmandate

7. Guidelines for reporting of DNA microarray data

The *International Endodontic Journal* gives authors notice that, with effect from 1st January 2011, submission to the *International Endodontic Journal* requires the reporting of microarray data to conform to the MIAME guidelines. After this date, submissions will be assessed according to MIAME standards. The complete current guidelines are available at http://www.mged.org/Workgroups/MIAME/miame_2.0.html. Also, manuscripts will be published only after the complete data has been submitted into the public repositories, such as GEO (http://www.ncbi.nlm.nih.gov/geo/) or ArrayExpress (http://www.ebi.ac.uk/microarray/submissions_overview.html), in MIAME compliant format, with the data accession number (the identification number of the data set in the database) quoted in the manuscript. Both databases are committed to keeping the data private until the associated manuscript is published, if requested.

Prospective authors are also encouraged to search for previously published microarray data with relevance to their own data, and to report whether such data exists. Furthermore, they are encouraged to use the previously published data for qualitative and/or quantitative comparison with their own data, whenever suitable. To fully acknowledge the original work, an appropriate reference should be given not only to the database in question, but also to the original article in which the data was first published. This open approach will increase the availability and use of these large-scale data sets and improve the reporting and interpretation of the findings, and in increasing the comprehensive understanding of the physiology and pathology of endodontically related tissues and diseases, result eventually in better patient care.