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THAYANA KARLA GUERRA LIRA DOS SANTOS

INFLUÊNCIA DE DIFERENTES SISTEMAS MECANIZADOS PARA PREPAROS ENDODÔNTICOS NA FORMAÇÃO DE TRINCAS RADICULARES E NA RESISTÊNCIA À FRATURA EM DENTES HUMANOS

Recife
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Tese apresentada à banca da Pós-Graduação em Clínica Integrada do Centro de Ciências da Saúde da Universidade Federal de Pernambuco, como requisito para obtenção do grau de doutor em Clínica Odontológica Integrada.

Orientador: Prof. Dr. Anderson Stevens Leônidas Gomes.
Coorientadora: Profa. Dra. Cláudia Cristina Brainer de Oliveira
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Orientador: Prof. Dr. Anderson Stevens Leônidas Gomes

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RESUMO

O preparo químico mecânico dos canais radiculares tem como objetivo realizar uma adequada desinfecção, respeitando a anatomia. Contudo, esse processo resulta na redução da dentina radicular, além da possibilidade de gerar trincas dentinárias, que por sua vez, que podem ou não influenciar a sobrevivência do dente, e levar a fratura vertical da raiz. Assim, o objetivo desse trabalho foi avaliar os efeitos do uso de sistemas reciprocantes e rotatórios na formação de trincas dentinárias e na resistência a fratura vertical da raiz de dentes unirradiculares humanos. Trata-se de um estudo *in vitro* com incisivos inferiores, aprovado pelo comitê de ética e pesquisa do Centro Universitário UNIESP (Número de parecer: 3.719.153). As coroas foram seccionadas e as raízes avaliadas em microscópio cirúrgico, usando uma magnificação de 16x. Os sistemas endodônticos utilizados foram: Wave One Gold 25,07 / Dentsply (WOG); Reciproc Blue 25,08 / VDW (RB); Protaper Next X1 e X2 / Dentsply (PTN); Protaper Gold SX, S1, S2, F1 e F2 / Dentsply (PTG). Os dentes foram avaliados através da microtomografia computadorizada antes e após a instrumentação dos canais radiculares. As imagens obtidas foram reconstruídas em software específico e analisadas pelo software ImageJ. Para o primeiro objetivo específico, detecção das trincas, a amostra foi de 80 dentes (n=20), avaliando as imagens referente aos 4mm apicais de cada raiz. O teste de Wilcoxon verificou as diferenças entre as imagens antes e após a instrumentação, o teste de Kruskal-Wallis as diferenças entre os grupos e o teste de Mann Whitney avaliou os diferentes movimentos de instrumentação. Para o segundo objetivo específico, avaliação da resistência à fratura (RF), 30 incisivos inferiores foram selecionados e divididos em cinco grupos (n = 6), sendo um controle e os demais grupos instrumentados com os respectivos sistemas e obturados pela técnica de cone único. O teste de resistência foi realizado em máquina de ensaio universal, com carga de 5000N, velocidade de 1 mm / min até a detecção da fratura. A força máxima foi registrada em Newton. O teste ANOVA foi utilizado para comparar a variável RF em situações que envolviam mais de dois grupos de comparação, enquanto o teste t de Student foi empregado para apenas dois grupos de comparação. Observando que em todos os sistemas utilizados, diferenças significativas foram encontradas entre o número de trincas antes e após a instrumentação (p <0,05). No entanto, não houve diferença estatística entre os quatro sistemas (p = 0,182), nem quando comparados os reciprocantes e rotatórios (p = 0,1048). Como também, não houve diferença estatisticamente significante na RF entre os grupos quando comparados com o controle (p = 0,064), nem entre os sistemas endodônticos (p = 0,13). Contudo, houve diferença significativa na RF entre os sistemas reciprocantes e rotatórios (p = 0,016). Desta forma, nota-se que a maioria dos defeitos dentinários estavam presente nas imagens pré-operatórias, porém, houve um aumento significativo no número de imagens comprometidas com as trincas, independentemente dos sistemas endodônticos utilizados. Enquanto, a RF dos dentes avaliados não foi influenciada pela instrumentação mecânica, todavia a RF dos dentes tratados com sistemas reciprocantes foi significativamente maior do que os rotatórios.

Palavras-chave: endodontia; raiz dentária; preparo do canal radicular; microtomografia por raio-x; resistência à flexão.

ABSTRACT

The chemical mechanical preparation of root canals aims to carry out an adequate disinfection, respecting the anatomy. However, this process results in the reduction of root dentin, in addition to the possibility of generating dentinal cracks, which may or may not influence tooth survival, and lead to vertical root fracture. Thus, the objective of this work was to evaluate the effects of the use of reciprocating and rotary systems on the formation of dentinal cracks and resistance to vertical fracture of the root of human single-rooted teeth. This is an in vitro study with lower incisors, approved by the ethics and research committee (University Center UNIESP, opinion number: 3.719.153). The crowns were sectioned and the roots evaluated under a surgical microscope (magnification 16x). The endodontic systems used were: Wave One Gold 25.07 / Dentsply (WOG); Reciproc Blue 25.08 / VDW (RB); Protaper Next X1 and X2 / Dentsply (PTN); Protaper Gold SX, S1, S2, F1 and F2 / Dentsply (PTG). The teeth were evaluated in computerized microtomography before and after the instrumentation of the root canals. The images obtained were reconstructed in specific software and analysed by the ImageJ software. For the first specific objective, detection of cracks, the sample consisted of 80 teeth (n=20), and the images referring to the apical 4mm of each root were evaluated. The Wilcoxon test verified the differences between the images before and after instrumentation, the Kruskal-Wallis test verified the differences between groups and the Mann Whitney test evaluated the different instrumentation movements. For the second specific objective, assessment of fracture resistance (FR), 30 mandibular incisors were selected and divided into five groups ($n = 6$), with a control and the other groups instrumented with the respective systems and filled using the single cone technique. The resistance test was carried out in a universal testing machine, with a load of 5000N, speed of 1 mm/min until fracture detection. The maximum force was recorded in Newton. The ANOVA test was used to compare the RF variable in situations involving more than two comparison groups, while the Student t test was used for only two comparison groups. Noting that in all systems used, significant differences were found between the number of cracks before and after instrumentation ($p < 0.05$). However, there was no statistical difference between the four systems ($p = 0.182$), nor when comparing reciprocating and rotary systems ($p = 0.1048$). In addition, there was no statistically significant difference in RF between the groups when compared with the control ($p = 0.064$), nor between the endodontic systems ($p = 0.13$). However, there was a significant difference in RF between reciprocating and rotary systems ($p = 0.016$). Thus, it is noted that most dentinal defects were present in preoperative images, however, there was a significant increase in the number of images affected by cracks, regardless of the endodontic systems used. While, the RF of the evaluated teeth was not influenced by the mechanical instrumentation, however, the RF of treated teeth with the reciprocating systems was significantly higher than the rotary systems.

Keywords: endodontics; tooth root; root canal preparation; x-ray microtomography; flexural strength.

LISTA DE ABREVIATURAS E SIGLAS

CNS	Conselho Nacional de Saúde
CRD	comprimento real do dente
CRT	comprimento real de trabalho
EDTA	ácido etilenodiaminotetracético
kV	quilovolt
LABIO	Laboratório Integrado de Biomaterias
ml	mililitro
mm	milímetro
N	newton
NaOCl	hipoclorito de sódio
NiTi	níquel-titânio
PTG	Protaper Gold
PTN	Protaper Next
PTU	Protaper Universal
RB	Reciproc Blue
RF	resistência máxima à fratura
T0	número de falhas presentes nas imagens da microtomografia antes da instrumentação
T1	número de falhas presentes após a instrumentação
UFPB	Universidade Federal da Paraíba
UFPE	Universidade Federal de Pernambuco
WO	Wave One
WOG	Wave One Gold
µA	microampere
µCT	microtomografia computadorizada
µm	micrômetro
Δ	delta (resultante das falhas causadas e/ou aumentadas pela instrumentação)
#10K	Lima manual tipo Kerr, número 10
#15K	Lima manual tipo Kerr, número 15

SUMÁRIO

1 INTRODUÇÃO

Avanços nas técnicas de preparação do canal radicular foram desenvolvidas para superar problemas como o transporte de canal, centralização do preparo e quantidade de remoção da dentina radicular (MESGARANI *et al*., 2018). A endodontia atual visa melhorar a qualidade do tratamento do canal radicular e as taxas de sucesso clínico, com instrumentos de níquel-titânio (NiTi) rotatórios e reciprocantes, que possibilitam melhor modelagem, especialmente em canais radiculares curvos e estreitos, por causa de seus designs e flexibilidade (GU *et al*., 2017), melhorando a adaptação das limas endodônticas a anatomia do canal radicular (CAVIEDES BUCHELI *et al.*, 2021).

No entanto, além da alteração na anatomia dos canais radiculares, os procedimentos de modelagem e ampliação, tem sido sugerido como um fator contribuinte na formação de trincas dentinárias, que pode evoluir para fraturas verticais da raiz (BIER *et al.*, 2009; GERGI, OSTA, NAAMAN, 2015; OLIVEIRA *et al.*, 2017).

A trinca dentinária é uma condição específica que pode influenciar a longo prazo a sobrevivência do dente afetado (SIM *et al*., 2016). Além da instrumentação e obturação, a formação de trincas tem se mostrado proveniente de outros fatores, como a presença de hábitos parafuncionais (BIER *et al*., 2009; TANG, WU, SMALES, 2010; LIU *et al*., 2013), como também uma alta concentração de hipoclorito de sódio (usado para irrigação do canal radicular) e desidratação da dentina (MILANI *et al*., 2012). Além disso, microorganismos podem proliferar em linhas de fissuras, levando ao estabelecimento de biofilme na superfície da raiz (SHEN, STOJICIC, HAAPASALO, 2011).

Estudos relatam que, quando a instrumentação é realizada no forame apical, especulam um aumento do risco de produzir tais defeitos na dentina radicular apical (ADORNO, YOSHIOKA, SUDA, 2011; LIU *et al*., 2013). Assim como, o contato oclusal prematuro e uma mastigação pesada repetitiva, podem agravar essas possíveis trincas, podendo evoluir para as fraturas verticais (COHEN *et al*., 2003). Esta é uma das complicações mais desalentadoras do tratamento endodôntico, pois leva ao insucesso e perda do dente (TSESIS *et al*., 2010).

Os sistemas mecanizados de NiTi são atualmente a tecnologia líder em instrumentação de canal radicular (KFIR *et al*., 2017). Esses instrumentos têm sido utilizados com uma maior frequência na prática endodôntica, apresentando diferentes designs, oferecendo muitas vantagens em relação a outros tradicionais, como maior confiabilidade, maior flexibilidade e diminuição do tempo de trabalho (BANE *et al.*, 2015; YUSUFOGLU *et al.* 2019). No entanto, instrumentos em NiTi ainda estão associados a um risco inerente a fratura do instrumento e incidência de trincas dentinárias (PEDULLA *et al.*, 2017).

Dentre os sistemas endodônticos, os reciprocantes são bastante utilizados, dentre eles temos o Reciproc (VDW, Munique, Alemanha) e WaveOne (WO) (Dentsply Maillefer, Ballaigues, Suíça), em que é possível moldar canais com apenas um instrumento, exigindo assim menos tempo do que a sequência completa dos sistemas rotatórios (GERGI, OSTA, NAAMAN, 2015). Seus movimentos recíprocos com velocidade predeterminada e ângulos de rotação, ajuda a aliviar o estresse sobre a lima, diminuindo o risco de fadiga cíclica de compressão e tensão (PLOTINO et al., 2012).

Esses instrumentos foram produzidos com uma liga de NiTi especial, a liga de M-Wire, criada através de um processo de tratamento térmico inovador, para aumentar a flexibilidade do instrumento (GUTMANN, GAO, 2012; GERGI, OSTA, NAAMAN, 2015). Além de ser uma liga mais resistente à fadiga e mais seguro em canais curvos e atrésicos. E, recentemente, avançados procedimentos térmicos têm sido aplicados às ligas de NiTi, a fim de desenvolver e aprimorar as características mecânicas dos instrumentos (DE DEUS *et al.*, 2017).

Em 2015, a Dentsply Maillefer (Ballaigues, Suíça) lançou o Wave One Gold (WOG) no mercado, fabricado com um novo tratamento térmico, o tratamento de ouro. O WOG usa a mesma cinemática recíproca do WO (PEDULLA *et al.*, 2017). É um instrumento produzido no NiTi CM, em que os instrumentos são repetidamente tratados termicamente e então resfriado, o que resulta em uma cor de superfície correspondente à espessura da camada de óxido de titânio, atribuindo uma cor dourada ao instrumento (SHEN *et al.*, 2016; DELAI *et al.*, 2018). Este processo de aquecimento, junto com a mais recente tecnologia recíproca, aumenta sua resistência à fadiga cíclica e dá uma maior flexibilidade (ÖZYÜREK *et al.*, 2016).

Além disso, o design do WaveOne Gold tem quatro arestas de corte com ângulo de ataque de 85°, mas apenas duas bordas estão em contato permanente com as paredes do canal, o que mantém o instrumento centrado no eixo longitudinal do canal radicular (GAVANI *et al.*, 2018). A instrumentação pode ser realizada com um único instrumento, com secção transversal em forma de paralelogramo descentralizado (KARATAS *et al.*, 2016a). Assim, apenas uma aresta de corte está em contato com a parede do canal, o que diminui a área de contato entre o instrumento e a parede do canal. Juntos, esse design resulta em um movimento recíproco suave, eliminando a necessidade para colocar qualquer pressão sobre a lima, aumentando a segurança e capacidade de corte (GAVANI *et al*., 2018).

Em 2016, o mesmo fabricante do Reciproc, lançou o Reciproc Blue (RB) (VDW, Munique, Alemanha), que tem o mesmo design, mas passa por um novo tratamento térmico de superfície, o que torna a lima mais flexível, com menor risco de fratura e maior potencial de pré-flexão, considerado uma evolução do sistema Reciproc (DE DEUS *et al*., 2017). O sistema Blue foi desenvolvido usando o processo de controle termomecânico (aquecimento e resfriamento) para modificar sua estrutura de moléculas, resultando em uma maior resistência a fadiga cíclica, mais flexibilidade e uma redução na memória de forma, possibilitando a pré-curvatura dos instrumentos antes do uso (DE DEUS *et al*., 2017; TOPÇUOGLU, TOPÇUOGLU, 2017).

A alta eficiência e desempenho de corte são fornecidos com a combinação de seção transversal em forma de S, conicidade, ângulos de corte e material melhorado termicamente. O instrumento tem uma ponta não cortante, associada a resistência à fadiga cíclica e a alta flexibilidade, permite uma melhor centralização entre as paredes do canal (ÖZYÜREK *et al*., 2018).

Estudos especulam que, ao usar apenas um instrumento, mais estresse será gerado durante a instrumentação, aumentando a frequência de defeitos (LIU *et al*., 2013; GERGI, OSTA, NAAMAN, 2015; LI *et al*., 2015). Segundo Bürklein *et al.* (2013) e Gergi, Osta, Naaman (2015), a instrumentação do canal radicular com sistemas reciprocantes produziram, significativamente, mais fissuras dentinárias do que os sistemas rotatórios, e observaram que a incidência de defeitos dentinários foi maior com uso de uma única lima.

Outros sistemas endodônticos também são utilizados na prática clínica, com uma cinemática já conhecida e mais antiga, os instrumentos com movimentos rotatórios. Dentre os sistemas rotatórios, o Protaper next (PTN) (Dentsply Maillefer, Ballaigues, Suíça), foi lançado no mercado odontológico, desenvolvido com a liga de M-Wire e suas características possuem um design inovador, com conicidade variável, secção transversal retangular (PEREZ-HIGUERAS *et al*, 2014). Esse formato retangular descentralizado permite apenas duas bordas de cortes tocando nas paredes do canal radicular, simultaneamente, enquanto as outras giram livremente, resultando em um movimento giratório externo de seu centro de massa (SHEN *et al*., 2013). Esta cinemática de movimento gera menos estresse nos instrumentos, proporcionando um espaço, através do qual os resíduos produzidos na instrumentação possam circular, sendo facilmente removidos (WU *et al*., 2015).

Além disso, a rotação assimétrica presente nesses instrumentos, é uma proposta para aumentar ainda mais a eficiência no preparo e moldagem do canal, garantindo uma liga de M-Wire com uma maior flexibilidade e resistência à fadiga cíclica (PEREZ-HIGUERAS *et al*, 2014). E o PTN é composto por 5 instrumentos disponíveis: X1 (17.04), X2 (25.06), X3 (30.07), X4 (40.06) e X5 (50.06) (VAN DER VYVER *et al*., 2019).

Relacionando tal sistema a produção de trincas, evidências sugerem que o sistema ProTaper Universal (PTU) (Dentsply Maillefer, Ballaigues, Suíça), desenvolvido anteriormente ao PTN, produz, significativamente, mais trincas dentinárias, principalmente, em canais curvos e atrésicos (CAPAR *et al*., 2014; KARATAS *et al*., 2016a).

Visto também que a conicidade dos instrumentos pode contribuir para a formação de trincas dentinárias, quanto maior a conicidade, mais a dentina radicular é removida e provável risco de trincas (BIER *et al*., 2009). Entretanto, o design de secção transversal retangular descentralizado e a conicidade do PTN, minimiza o contato entre a lima e dentina, reduzindo os danos dentinários (CAPAR *et al*., 2014).

No estudo de Capar *et al*. (2014), observaram que PTN causou menos trincas em comparação com o sistema PTU, contudo utilizou uma metodologia destrutiva, podendo os defeitos terem surgido no corte dos espécimes. Li *et al.* (2015), afirmam que o sistema PTN induziu menos microtrincas dentinárias durante a instrumentação em canais radiculares em comparação com o PTU e WaveOne. Como também, De-Deus *et al*. (2015), observaram através de microtomografia computadorizada, que o sistema PTN não induziu a formação de novas trincas dentinárias.

O sistema ProTaper Gold (PTG) (Dentsply Maillefer, Ballaigues, Suíça) é outro exemplo de sistema rotatório fabricado com tratamento térmico inovador. O tratamento *Gold* visa melhorar as propriedades mecânicas, especialmente, a resistência à fadiga cíclica da liga NiTi convencional, dando assim uma maior flexibilidade, ajudando a garantir uma preparação mais centrada (GAGLIARDI *et al*., 2015; ELNAGHY, ELSAKA, 2016) e gerando um fio de memória controlada (HIEAWY *et al*., 2015). O sistema tem exatamente a mesma sequência de 6 instrumentos, o mesmo design e características geométricas do sistema PTU (ELNAGHY, ELSAKA, 2016; PINHEIRO *et al.*, 2018), onde incluem três instrumentos modeladores (SX, S1 e S2) e cinco de finalização (F1, F2, F3, F4 e F5) (KARATAS *et al*., 2016).

Karatas *et al.* (2016b) compararam a presença de trincas utilizando o PTG, o ProFile Vortex, F360, Protaper Universal e Reciproc, observando que todos os sistemas que foram testados produziram trincas dentinárias, mas o PTU foi associado a uma maior formação de trinca que os demais ao nível de 3 mm.

Nishad *et al*. (2018), avaliaram a incidência de propagação de trincas apicais após o preparo de canais radiculares em diferentes comprimentos de instrumentação utilizando limas rotatórias do PTU, PTN e PTG e observou que o PTG mostrou menor dano dentário seguido por PTN e PTU devido às suas vantagens de fabricação e seu tratamento termomecânico.

Com a presença das possíveis trincas pode-se observar como consequência, o aparecimento de fratura vertical de raiz, que não é um fenômeno instantâneo, mas sim um resultado da propagação das trincas dentinárias (BÜRKLEIN *et al*., 2013; KFIR et al., 2017). E é um importante problema clínico, pois compromete o resultado do tratamento endodôntico (KHOSHBIN *et al.*, 2018).

Dentes tratados endodonticamente são considerados como tendo uma taxa de sobrevivência mais baixa em comparação aos dentes vitais, porém quase da metade das falhas dos dentes tratados são devido à fratura da coroa (59,4%), como a falha protética (ACHARYA *et al*., 2020). Assim, essa resistência à fratura vertical da raiz é diretamente proporcional à quantidade de estrutura do remanescente dentário presente (GANESH et al, 2014; YUSUFOGLU et al. 2019).

Embora as razões para a fratura vertical da raiz sejam multifatoriais, a perda de volume da estrutura do dente durante o tratamento endodôntico desempenha um papel importante na sobrevivência do dente a longo prazo. Assim, a preservação de uma maior quantidade de estrutura dentária não só aumenta a resistência à fratura, mas também mantém a integridade estrutural da restauração pós endodontia (ACHARYA *et al*., 2020).

Por isso, o diagnóstico precoce das trincas dentinárias e dos demais defeitos dentinários, é fundamental para evitar outras complicações (OLIVEIRA *et al*., 2017). No entanto, os dentes trincados são considerados um desafio diagnóstico para os clínicos (SIM et al., 2016).

Nos últimos anos, imagens de microtomografia computadorizada (µCT) surgiram como um método altamente preciso e amplamente utilizado nas pesquisas endodônticas laboratoriais (SIQUEIRA *et al*., 2013), considerada um padrão ouro para esses resultados, uma vez que permite análises morfológicas quantitativas e qualitativas dos canais radiculares, e uma de suas principais aplicações é a avaliação dos efeitos da instrumentação na anatomia do canal e dentina radicular (SIQUEIRA *et al*., 2013; BRASIL *et al*.., 2017; SILVA *et al.*, 2020).

Com esta tecnologia, é possível obter uma imagem tridimensional de alta resolução dos dentes antes e depois da preparação do canal, com alto nível de precisão, sem seccionar as amostras, pois é um método não destrutivo. Este método permite não apenas a visualização dos defeitos dentinários preexistentes, mas também sua localização precisa ao longo da raiz, aumentando a validade interna de experimentos *in vitro*, onde cada espécime age como seu próprio controle (DE DEUS et al., 2015).

Desse modo, com os diversos sistemas endodônticos presentes no mercado e a importância de avaliar a qualidade e possíveis defeitos causados pela instrumentação dos canais radiculares tratados com tais sistemas, trazendo assim benefícios para a prática clínica na endodontia, o objetivo desse trabalho foi avaliar a influência do uso de sistemas reciprocantes (Reciproc Blue e Wave One Gold) e rotatórios (Protaper Next e Protaper Gold) na formação de trincas na dentina radicular e na resistência a fratura vertical da raiz de dentes unirradiculares humanos, após instrumentação dos canais radiculares.

Sendo assim, a hipótese afirmativa desse trabalho é que os sistemas rotatórios e reciprocantes podem gerar trincas na dentina radicular e assim, diminuir a resistência à fratura vertical da raiz. E a hipótese nula é que os sistemas mecanizados (rotatórios e reciprocantes) não formam trincas dentinárias e nem afetam a resistência vertical das raízes após instrumentação e obturação dos canais.

2 METODOLOGIA

Esta tese está apresentada na forma de artigos (para submissão). Descreveremos em seguida a metodologia do preparo das amostras, que foram semelhantes para ambos.

2.1 LOCAIS DO ESTUDO

Esse estudo foi desenvolvido em algumas etapas. A obtenção dos dentes foi através do Banco de dentes da Universidade Federal de Pernambuco (Recife, Pernambuco), a seleção da amostra foi feita no Centro Universitário UNIESP (João Pessoa, Paraíba). O preparo da amostra foi feita no Laboratório de Biofotônica do Departamento de Física da Universidade Federal de Pernambuco.

A análise em microtomografia computadorizada foi feita no Laboratório de Tomografia Computadorizada de Raio X, do Departamento de Energia Nuclear da Universidade Federal de Pernambuco. E o teste de resistência a fratura foi realizado no Laboratório de Biomateriais do Programa de Pós Graduação em Odontologia da Universidade Federal da Paraíba.

2.2 CÁLCULOS AMOSTRAIS

Em razão dos diferentes desfechos do estudo, foram realizados dois cálculos amostrais; um para o desfecho “trincas”; e outro para o desfecho “resistência à fratura”. Para ambos os cálculos se adotou um erro tipo I (α) de 5%, um erro tipo II (β) de 20%, um poder de 80% e os efeitos (effect sizes = g de Hedge) verificados nos estudos de Uğur Aydin, Kerkin e Ozyurek (2019), e de Abdulrahman et al. (2018), para os desfechos “trincas” e “resistência à fratura”, respectivamente.

Para o cálculo amostral relacionado ao desfecho “trincas” o *effect size* calculado (g de Hedge) foi de 0,94, sendo necessária uma amostra mínima de 19 unidades amostrais por grupo experimental para se ter um estudo com poder de 80%.

Já para o desfecho “resistência à fratura”, o *effect size* calculado comparando resultados de dentes tratados endodonticamente e cimentados com dois tipos de pino diferentes foi de 1,82, sendo necessária uma amostra mínima de 6 unidades amostrais por grupo experimental para se ter um estudo com um poder de 80%.

2.3 SELEÇÃO DAS AMOSTRAS

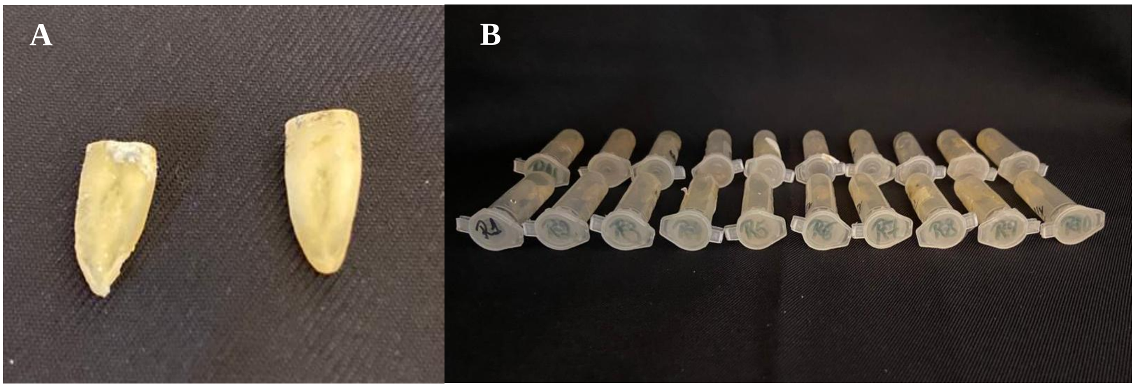
Este estudo seguiu os preceitos estabelecidos da Resolução do Conselho Nacional de Saúde (CNS/MS) 466/12 que trata da Pesquisa envolvendo Seres Humanos, foi devidamente registrado na Base de Registros de Pesquisa envolvendo seres humanos (Plataforma Brasil) e submetido à apreciação e aprovação do Comitê de Ética em Pesquisa do Centro Universitário UNIESP (Número de parecer: 3.719.153) (Anexo 3).

Os dentes utilizados foram obtidos através do banco de dentes da Universidade Federal de Pernambuco. Com um total de 100 dentes incisivos inferiores permanentes selecionados, com raízes e canais retos, avaliados previamente.

Inicialmente, os dentes foram limpos e armazenadas em solução de Timol a 0,1% por 24 horas, para desinfecção. Logo após, esses dentes foram radiografados e avaliados sob um microscópio de operação (Alliance, São José dos Campos, SP, Brasil) com 16x de magnificação. Foram excluídos os dentes com presença de rachaduras radiculares ou linhas de fraturas em suas superfícies externas, com reabsorção, calcificação e amostras que possuíam raízes curvas. Todos os procedimentos laboratoriais foram realizados pelo mesmo operador.

As coroas foram removidas sob refrigeração com discos diamantados (KG Sorensen Ind., Cotia, Brasil) montado em um motor de baixa velocidade (Kavo Ind., Joinville, SC, Brasil), deixando um comprimento de 13mm. As raízes foram mantidas hidratadas durante todo o experimento, armazenadas em água destilada individualmente em tubos de polipropileno tipo Eppendorf (Kasvi, Curitiba, PR, Brasil), numerados e divididos de acordo com cada grupo (Figura 1). Posteriormente, foram analisadas inicialmente através da microtomografia computadorizada (µCT), para obtenção das imagens iniciais.

Figura 1. Armazenamento dos espécimes. A: Espécimes sem a coroa; B: Raízes armazenadas nos tubos Eppendorf com água destilada, divididos em grupos experimentais.



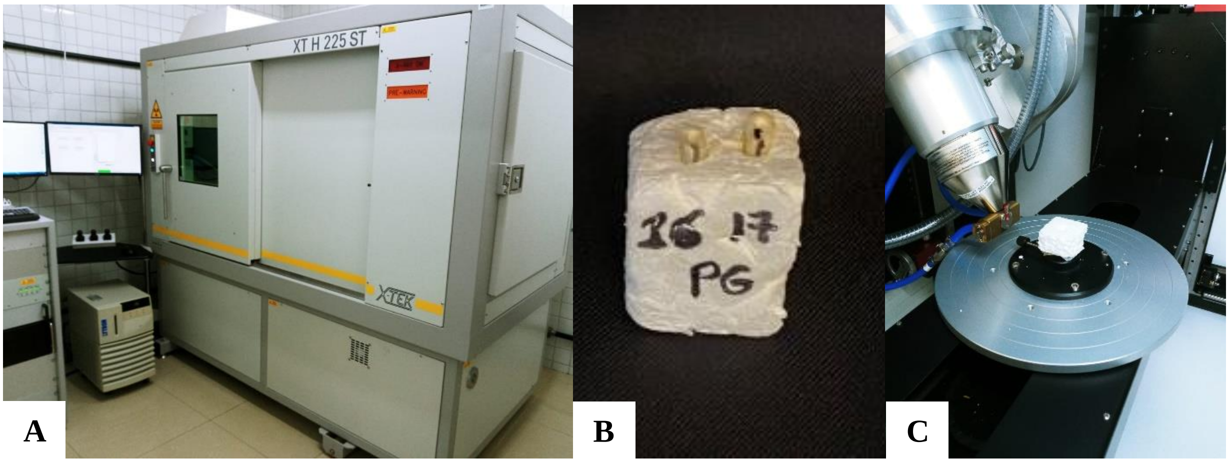
Fonte: Autoria própria (2021).

2.4 MICROTOMOGRAFIA COMPUTADORIZADA (µCT)

Antes e após preparo químico mecânico, os espécimes foram submetidos à análise de microtromografia computadorizada. O tomógrafo de µCT utilizado para os escaneamentos foi o de modelo XTH225ST (Nikon Metrology, Inc, Tokyo, Japão), pertencente ao Laboratório de Tomografia Computadorizada de Raio X, do Departamento de Energia Nuclear da UFPE (Figura 2A).

Os parâmetros foram ajustados a uma resolução isotrópica de 10 µm, tensão de 100 kV, um filtro de Alumínio com 1mm espessura e corrente de 100 µA. As raízes foram inseridas aos pares em blocos de isopor, tendo em vista que sua densidade radiográfica está próxima do ar (Figura 2B). Os blocos foram então fixados a plataforma de suporte para o escaneamento (Figura 2C). A digitalização realizada com uma rotação de 360° da amostra em torno do eixo vertical com um passo angular de 0,12° por projeção. Sendo geradas 3016 projeções para cada amostra.

Figura 2. Escaneamento de µCT. A: Tomógrafo modelo XTH225ST do Laboratório de Tomografia Computadorizada de Raio X; B: Raízes inseridas no bloco de isopor; C: Bloco de isopor na plataforma do Tomógrafo, pronto para o escaneamento.



Fonte: Autoria própria (2021).

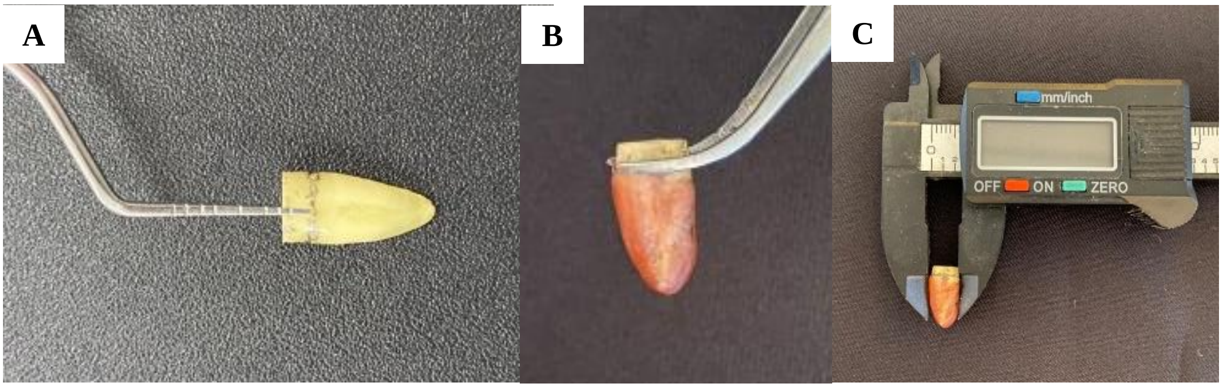
Para reduzir artefatos de anel e minimizar o efeito de endurecimento do feixe, uma calibração de ar do detector foi realizada antes das varreduras. Imagens de cada espécime foram reconstruídas com o software CT Pro 3D v.XT3.1.3 (Nikon Metrology NV, Tring, United Kingdom). Então, o software VGStudio MAX v.2.2 (Volume Graphics, Heidelberg, Germany) foi usado para aplicação de filtragem Gaussiana nas imagens e para fornecer secções transversais da estrutura interna das raízes. Em seguida, as imagens foram transferidas para o ImageJ (National Institutes of Health, Bethesda, United States), onde foram selecionados os cortes de interesse para análise.

2.5 PREPARO DO CANAL RADICULAR

Em seguida, o comprimento de trabalho foi determinado pela introdução de instrumentos #10K e #15K (Dentsply Maillefer, Ballaigues, Suíça) no canal até atingir o forame apical e então medido na régua endodôntica (Maquira Industry Dental Products, Maringá, Brasil), referindo-se ao comprimento real do dente (CRD), garantindo que todos foram instrumentados no comprimento correto e sem ultrapassar a saída foraminal. O comprimento real de trabalho (CRT) estabelecido é igual ao comprimento real do dente, com limite apical 0mm, e o comprimento de obturação com 1mm aquém do forame.

Para simulação do "ligamento periodontal", foi adotada a metodologia adaptada de Clavijo *et al.* (2009), em que todas as raízes foram imersas em cera 7 (Duradent, Odonto Ltda, São Paulo, Brasil) derretida para obter um espaço de 0,2 ± 0,3mm em torno de toda a raiz, que forneceu o espaço a ser preenchido por silicone de adição, simulando o ligamento, deixando 3mm demarcado com lápis na cervical da raiz, espaço esse para simular as distâncias biológicas (Figura 3). Os dentes foram mantidos em temperatura ambiente e, dessa forma, a cera solidificou imediatamente, evitando o escoamento. Em seguida, os mesmos foram imersos em água fria para que a cera solidificasse completamente e não sofresse deformações.

Figura 3. Raízes cobertas com cera 7. A: Demarcação dos 3mm em lápis; B: Recobrimento da raiz com cera aquecida; C: Aferição para simulação do espaço do ligamento periodontal artificial.



Fonte: Autoria própria (2021).

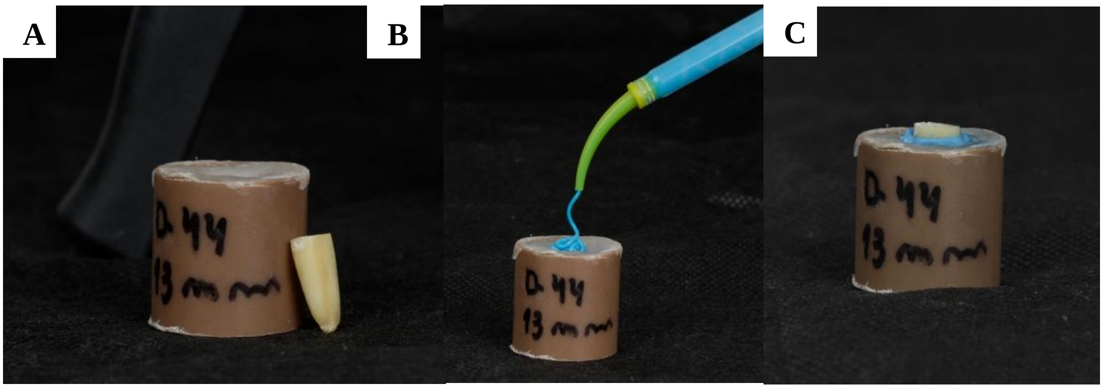
Então, em lâminas de cera utilidade, foram demarcadas uma circunferência referente ao diâmetro do tubo de PVC (Tigre do Brasil, Osasco, Brasil) e a raiz centralizada na circunferência, inserindo-se os 3mm demarcados referente às distâncias biológicas. Foi colocado o tubo de PVC sobre a demarcação na cera, vedando a parte externa do tubo para fixá-lo e não deixar a resina extravasar. A resina acrílica (Vipi Flash, Pirassununga, Brasil) foi manipulada e inserida no interior dos tubos de PVC (Figura 4).

Figura 4. Inserção das raízes nos tubos de PVC. A: Raíz centralizada na circunferência referente ao tubo de PVC; B: Preenchimento do tubo com resina acrílica; C: Após presa da resina acrílica.

Fonte: Autoria própria (2021).

Em seguida, após a presa da resina, a cera em torno das raízes e do interior do alvéolo artificial foi removida com a ajuda de uma cureta longa (Golgran, São Caetano do Sul, Brasil) e essas superfícies cobertas com um material de impressão de silicona de adição (Silicona de adição pasta fluída, Express XT 3M, Sumaré, Brasil), inseridas no interior do alvéolo artificial com a ponta de auto mistura, simulando o ligamento periodontal artificial (Figura 5). Após a presa do material, foi removido todo o excesso do mesmo com uma lâmina de bisturi. Terminado a inserção das raízes nos tubos de PVC, os mesmos foram mantidos em condições úmidas no interior de recipientes, hermeticamente fechados.

Figura 5. Raízes inseridas no alvéolo artificial com silicona de adição (simulando o ligamento periodontal). A: Espaço deixado no alvéolo artificial; B: Silicona de adição fluída Express XT 3M inserida no alvéolo artificial; C: Inserção da raiz após preenchimento com a silicona de adição.



Fonte: Autoria própria (2021).

A instrumentação foi feita de acordo com a cinemática indicada para cada instrumento com um motor endodôntico (X-Smart Plus, Dentsply Maillefer, Ballaigues, Suíça) através da técnica coroa-ápice e seguindo a programação já presente no motor. Os canais foram irrigados com Hipoclorito de sódio (NaOCl) a 2,5% usando uma seringa descartável de 5ml e agulha Endo-Eze (Ultradent Products, South Jordan, United States) e explorados com uma lima #10K e #15K entre os instrumentos, até que o comprimento de trabalho fosse alcançado, repetindo até três vezes esse movimento em cada dente. Cada instrumento foi usado para preparar até 3 dentes e trocados por um novo (AKSOY *et al.*, 2019), como também realizado por um único operador, seguindo as instruções dos fabricantes, descritos no Quadro 1.

Quadro 1. Informações dos instrumentos utilizados para cada grupo.

Grupo	Sistema endodôntico	Instrumentos	Fabricantes	Movimento	Protocolo
WOG	Wave One Gold	Primary (25 / 0.07)	Dentsply-Maillefer, Ballaigues, Suíça	Reciprocante	Foram realizados três movimentos de bicada e saída, até atingir o CRT.
RB	Reciproc Blue	RB25 (25/ 0.08)	VDW GmbH, Munich, Germany	Reciprocante	O instrumento foi usado em até três movimentos de bicada. Este procedimento foi realizado até atingir o CRT.
PTN	Protaper Next	X1 (17 / 0.04)	Dentsply-	Rotatório	Os instrumentos foram

		X2 (25 / 0.06)	Maillefer, Ballaigues, Suíça		utilizados em sequência contínua (X1 e X2), com três movimentos de bicada e saída cada, até atingir o CRT.
PTG	Protaper Gold	Sx, S1, S2, F1 (20/ 0.07) e F2 (25/ 0.08)	Dentsply-Maillefer, Ballaigues, Suíça	Rotatório	Os instrumentos seguiram a sequência: SX (1/3 CRT), S1 e S2 (2/3 CRT). E três movimentos com F1 e F2 até alcançar o CRT.

Fonte: Autoria própria (2021).

Ao final do procedimento foi feita uma irrigação final com 2 ml de EDTA (Biodinâmica, Ibiporã, Brasil) e 2 ml de NaOCl 2,5%, com agitação manual (com lima #15K). O volume total de NaOCl usado durante todos os procedimentos foi de 10 ml. Em seguida, as amostras foram irrigadas com 5 ml de água destilada e secadas no canal radicular com pontas de papel absorvente (Dentsply, Petrópolis, Rio de Janeiro, Brasil) e posteriormente avaliadas com µCT.

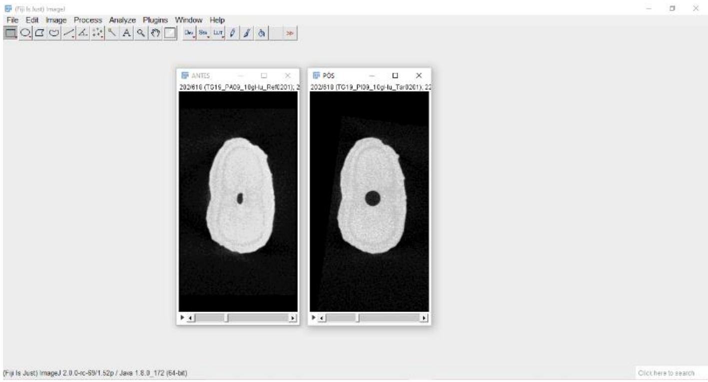
A partir deste ponto, e para facilitar o entendimento, descreveremos a continuação da metodologia específica para cada trabalho, e os principais resultados e discussão no corpo dos artigos.

Artigo 1: Cracks Incidence in Unirradicular Human Teeth Related to Different Root Instrumentation Techniques

2.6 AVALIAÇÃO DAS TRINCAS DENTINÁRIAS

As imagens obtidas a partir do escaneamento foram avaliadas por dois observadores calibrados, um especialista em Endodontia e com experiência na área e outro com habilidade em análise µCT. A avaliação de defeitos dentinários foi realizada com base nas divergências de resultados encontrados em estudos (DE-DEUS et al., 2014; DE-DEUS et al., 2015), assim, utilizou-se um primeiro método de avaliação completa das imagens transversais realizadas antes e após a preparação do canal radicular, para encontrar os possíveis defeitos dentinários presente na raiz do dente. Em uma segunda análise, as imagens foram divididas em intervalos de 1 mm (representado por 100 imagens), partindo do ápice, analisando e emparelhando os 4 mm finais com o software ImageJ (Figura 6). Nos dois métodos, as imagens foram avaliadas duas vezes com intervalo de 2 semanas e casos de divergência, foram reavaliadas (DE-DEUS et al., 2014; CASSIMIRO et al., 2017). A quantidade de imagens com presença de trincas foram contabilizadas manualmente, considerando as trincas que iniciavam a partir da parte interna do canal radicular, totalizando 64.000 cortes (imagens).

Figura 6. Imagens avaliadas no software ImageJ.



Fonte: Autoria própria (2021).

Em seguida, o valor Delta (Δ) foi calculado da seguinte forma:

$$\Delta = (T1 - T0)$$

Onde,

Δ: será o valor resultante das falhas causadas e / ou aumentadas pela instrumentação;

T0: número de falhas presentes nas imagens µCT antes da instrumentação;

T1: número de falhas presentes após a instrumentação.

2.6.1 Análise Estatística das trincas dentinárias

A análise estatística foi realizada utilizando o software GraphPad Prism 7 (GraphPad Software, Inc.). A distribuição não normal foi determinada pelo teste de Kolmogorov-Smirnov. Para verificar se há diferenças entre o T0 (antes) e T1 (depois) da instrumentação, foi utilizado o teste de Wilcoxon. Para conferir se há diferenças estatísticas significativas entre os valores de Delta dos sistemas endodônticos utilizados foi realizado o teste de Kruskal-Wallis com o teste de comparação múltiplas de Dunn. Além disso, o teste de Mann Whitney foi aplicado para avaliar se há diferença estatística entre os movimentos de instrumentação (reciprocante e rotatório), através da soma do delta dos grupos de instrumentação. A significância estatística de todos os testes foi considerada como $p<0,05$.

Artigo 2: Vertical Fracture Resistance of Teeth After Instrumentation With Rotary and Reciprocal Endodontic Systems

2.7 OBTURAÇÃO DO CANAL RADICULAR

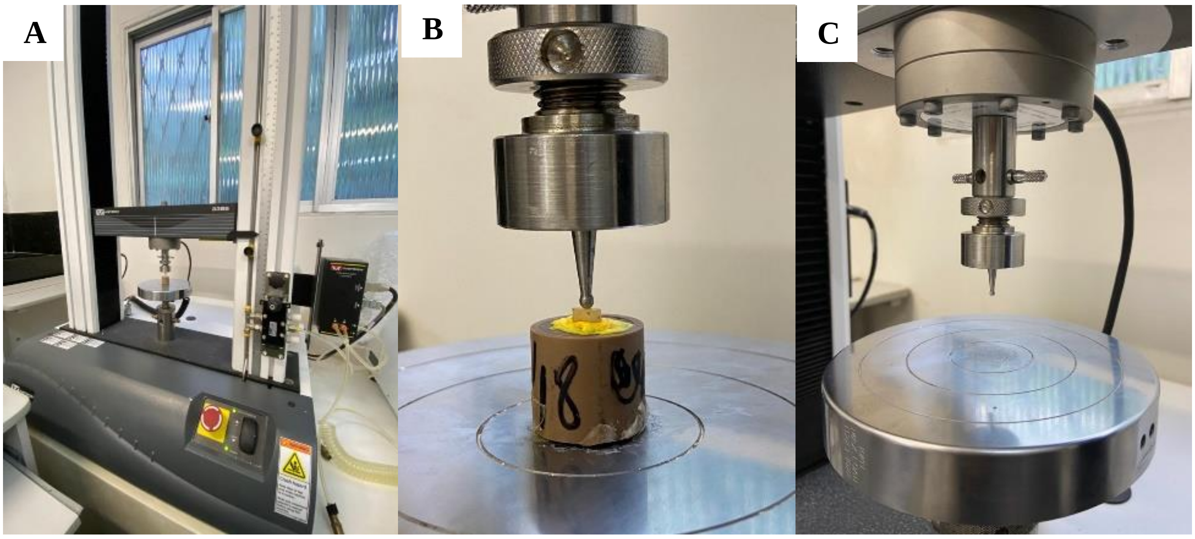
Os dentes foram selecionados para análise após avaliação na µCT, para avaliação de possíveis defeitos, examinando integralmente as imagens transversais de toda a extensão radicular. Excluindo do teste qualquer dente que apresentasse qualquer trinca ou fratura intracanal nas imagens iniciais.

Após o preparo dos canais radiculares, os dentes foram todos obturados pela técnica de cone único. Os canais foram secos e o cone de guta percha 35.04 foi adaptado (MK Life, Porto Alegre, Brazil), diminuindo 1 mm do CRT. Após a prova do cone, foi realizado o preparo do cimento endodôntico AH plus (Dentsply Maillefer, Ballaigues, Suíça) e o cone inserido no canal embebido em cimento. Em seguida, a condensação vertical foi realizada com condensadores duplos (Odous de Deus, Belo Horizonte, Brazil) aquecido e, posteriormente, frio para condensar a guta, até o limite adequado. Após obturação, os dentes foram preparados para análise da resistência a fratura vertical das raízes.

2.8 TESTE DE RESISTÊNCIA A FRATURA VERTICAL DA RAIZ

As raízes embutidas nos tubos de PVC with light body PVS foram fixadas no dispositivo da máquina de ensaio universal (Instron 3365, Instron Brasil Equipamentos Científicos Ltda, São José dos Pinhais, Paraná, Brasil) do Laboratório Integrado de Biomaterias (LABIO) do Programa de Pós-Graduação em Odontologia da UFPB (Figura 7A). E então, submetidas a um carregamento de compressão axial com a uma velocidade de cruzeta de 1 mm/min até a fratura ser detectada, com uma célula de carga de 5000 N (Figura 7B). Isso foi feito usando uma esfera metálica de 2,5 mm de diâmetro, posicionado na entrada do canal, de modo que contate a face plana da raiz em ambos os lados mesial e distal para espalhar a carga uniformemente na superfície da raiz (Figura 7C).

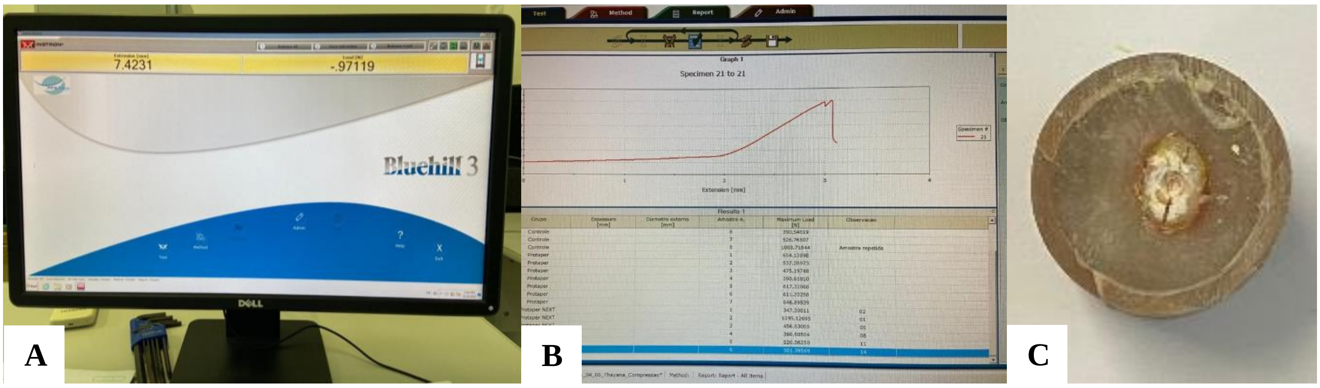
Figura 7. Amostras posicionadas na máquina de Ensaio para teste de resistência a fratura. A: Máquina de ensaio universal (Instron 3365); B: Raízes embutidas no tubo de PVC posicionada na plataforma da máquina; C: Esfera metálica que foi posicionada na entrada do canal radicular.



Fonte: Autoria própria (2021).

À medida que a carga foi aplicada o computador registrou uma curva ascendente que foi abruptamente interrompida no momento que ocorreu a fratura do espécime, e simultaneamente emitido o som do crack da amostra. O limiar de fratura foi definido como a carga que revelou a primeira fratura (a queda repentina de carga durante a compressão). O ensaio foi terminado neste ponto e a força medida em Newton (N) referente a da resistência máxima à fratura (RF) (Figura 8).

Figura 8. Software utilizado para registrar a força da resistência máxima de fratura. A: Software Bluehill; B: Gráfico mostrando a queda após fratura do espécime; C: Dente fraturado.



Fonte: Autoria própria (2021).

2.8.1 Análise Estatística da resistência a fratura vertical da raiz

Os dados foram analisados a partir de estatística descritiva e inferencial. A variável resistência à fratura foi analisada descritivamente a partir medidas de tendência central e dispersão. A normalidade da variável resistência à fratura foi verificada a partir do teste de Shapiro-Wilk, bem como através da análise visual de histograma. Como a variável apresentou distribuição normal, foram empregados testes paramétricos. O teste de Análise de Variância a um fator (ANOVA) foi utilizado para comparar a variável resistência à fratura em situações que envolviam mais de dois grupos de comparação, enquanto o teste t de Student foi empregado quando havia apenas dois grupos de comparação. Todas as análises foram realizadas utilizando o software *Statistical Package for the Social Sciences* (SPSS para Windows, versão 25.0, IBM Corp., Armonk, NY, EUA) com um nível de significância de 5%.

3 ARTIGO 1

CRACKS INCIDENCE IN UNIRRADICULAR HUMAN TEETH RELATED TO DIFFERENT ROOT INSTRUMENTATION TECHNIQUES

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CRACKS INCIDENCE IN UNIRRADICULAR HUMAN TEETH RELATED TO DIFFERENT ROOT INSTRUMENTATION TECHNIQUES

Introduction

The main objectives of the chemical-mechanical preparation of the root canals are to preserve the original anatomy and to promote a disinfection of the root canals system (Arslan *et al.* 2014). However, modelling procedures and expansion of the root canal has been a contributing factor in the formation of dentinal cracks, which can evolve to vertical fracture of the root (Bier *et al.* 2009; Gergi *et al.* 2015) due to the possibility of the propagation of these cracks (Oliveira *et al.* 2017).

Nickel-titanium (NiTi) based endodontic files are currently among the leading technology in instrumentation of the root canals (Kfir *et al.* 2017). There are several commercially available instruments with different designs, which offer many advantages over other traditional instruments, such as increased flexibility and reduction of working time (Bier *et al.* 2009, Bane *et al.* 2015).

In reciprocal movement, the instrument rotates clockwise and anticlockwise, with less stress applied to the instrument, increasing the resistance to cyclic fatigue (Pedullà *et al.* 2017). The Wave One Gold uses this mutual kinematic (Pedullà *et al.* 2017) and the instrumentation is accomplished with a single instrument, with a cross section in the form of decentralized parallelogram (Karataş *et al.* 2016a). The Reciproc Blue is also a reciprocating system with an innovative heating process that modifies their molecular structure and improves the flexibility and the resistance to cyclic fatigue (Aksoy *et al.*, 2019). With high efficiency and cutting performance, better heat treatment, besides allowing a better centralization between the canal walls (Özyürek *et al.*, 2018).

However, it can be speculated that when using only one instrument more stress will be generated during the instrumentation, by increasing the frequency of dentinal defects (Liu *et al.* 2013, Li *et al.* 2015, Gergi *et al.* 2015). Therefore, there is the possibility of using systems with rotational movements, such as Protaper Next, developed with the M-Wire alloy (Pérez-Higueras *et al.* 2014). In addition, it incorporates a decentralized rectangular cross section, giving it exclusive movement and flexibility (Nishad & Shivamurthy, 2018). Then, Protaper Gold instruments were introduced, with a transformation in the metallurgical characteristics of the instruments, with controlled memory and high austenite finishing temperature, responsible for increasing their flexibility (Hieawy *et al.* 2015, Nishad & Shivamurthy, 2018).

The early diagnosis of dentin cracks is essential to avoid further complications, which can lead to endodontic treatment failure. However, cracked teeth are considered a diagnostic challenge for clinicians (Oliveira *et al.* 2017). In recent years, microcomputed tomography (μCT) images have emerged as a generalized method applied to endodontic research, being an imaging technique recommended as the gold standard for analyzing dentin and its changes, such as dental cracks, without damaging the tooth structure (De-Deus *et al.* 2015, Ceyhanli *et al.* 2016). It is frequently used due to its high accuracy (PradeepKumar *et al.* 2017, Cassimiro *et al.* 2017, Stringheta *et al.* 2017, Zuolo *et al.* 2017, Oliveira *et al.* 2017, Aksoy *et al.* 2019, Shemesh *et al.* 2018, Van Der Vyver *et al.* 2019).

There seems to be no clear consensus in the literature regarding the dentin cracks propagation related to the use of these instruments. Additionally, it is necessary to evaluate the commercially available endodontic systems. In this way, the present study aimed to evaluate the effects of reciprocating (Reciproc Blue and Wave One Gold) and rotatory systems (Protaper Next and Protaper Gold) in the formation of cracks after root canals instrumentation.

Material and Methods

Sample Selection

The sample size calculation was determined considering a type I error (α) of 5%, a type II error (β) of 20%, a power of 80% and the effects (effect sizes = g of Hedge) verified in the study (Uğur Aydın *et al*. 2019). The calculated effect size was 0.94, requiring a minimum of 19 sample units per group. This study was approved by Research Ethics Committee of the UNIESP University Center (Number: 3.719.153).

One hundred permanent lower incisors were obtained from the tooth bank of the Universidade Federal of Pernambuco. Freshly extracted teeth were cleaned and then stored in 0.1% thymol solution during 24h for necessary disinfection. The teeth were radiographed and evaluated under a surgical microscope (Alliance, São José dos Campos, São Paulo, Brazil) with a 16x magnification. The teeth in which it was possible to detect the presence of root fissures or fracture lines on their external surfaces, resorption, calcification, and curved channels were excluded. The same operator performed all laboratory procedures.

The final sample was composed 80 teeth who attended the above cited selection criteria. Then their crowns were removed under refrigeration using diamond discs (KG Sorensen Ind., Cotia, São Paulo, Brazil) mounted on a low-speed motor (Kavo Ind., Joinville, Santa Catarina, Brazil), leaving approximately 13 mm of length. The teeth were kept hydrated throughout the experiment, stored in distilled water. Subsequently, these teeth were analyzed by µCT, to obtain the initial images.

Root Canal Preparation

The working length was determined by introducing instruments #10K and #15K (Dentsply Maillefer, Ballaigues, Switzerland) in the canal up to the apical foramen. The length was then measured with an endodontic ruler (Maquira Industry Dental Products, Maringá, Paraná, Brazil) and referred to as the actual tooth length (ATL), ensuring that all specimens were instrumented at the correct length and without exceeding the apical foramen. The actual working length (AWL) established is equal to the ATL.

The following steps were adapted from the methodology of a previous study (Clavijo et al., 2009). A space around all roots was created to simulate the periodontal ligament with polyvinyl siloxane (PVS). Initially, all roots were immersed in baseplate wax (Duradent, Odonto Ltda, São Paulo, São Paulo, Brazil) to obtain a space of 0.2 ± 0.3 mm around the entire root, a 3 mm distance was demarcated at the cervical third of the root to simulate the biological distance. Over a sheet of utility wax, the diameter of the PVC tube (Tigre do Brasil, Osasco, São Paulo, Brazil) was demarcated. The roots were inserted in the cervical third (3 mm) at the center of the demarcated circumference. The PVC tube was then positioned and fixed at the demarcation in the wax, and the self-curing acrylic resin (Vipi Flash, Pirassununga, São Paulo, Brazil) was poured into the tubes.

After polymerization of the acrylic resin, the roots were removed, and the wax around the roots was removed with the aid of a long curette 23-24 (Golgran, São Caetano do Sul, São Paulo, Brazil). The artificial alveoli were filled with light body PVS (Addition Silicone Express XT Paste Fluid 3M, Sumaré, São Paulo, Brazil), and the root was then inserted inside the cavity. The samples were kept under moist conditions in hermetically sealed containers.

Root instrumentation was performed according to the kinematics indicated for each instrument with an endodontic motor (X-Smart Plus, Dentsply Maillefer, Ballaigues, Suíça) using the crown-apex technique and following the programming already present in the motor. The root canals were irrigated with 1mL of 2.5% sodium hypochlorite (NaOCl) using a disposable syringe and Endo-Eze needle (Ultradent Products, South Jordan, Utah, United States) after each instrument. Each instrument was used to prepare up to three teeth and exchanged for a new one (Aksoy *et al.* 2019). A single operator prepared all root canals. The root canals preparation followed the manufacturer’s instructions, as described in Table 1.

Table 1 Information of the instruments used for each group.

Group	Endodontic system	Instruments	Manufacturers	Samples (n)	Movements	Protocol
WOG	Wave One Gold	Primary (25 / 0.07)	Dentsply-Maillefer, Ballaigues, Suíça	20	Reciprocating	The three movements of peck and exit were necessary, until reaching the AWL.
RB	Reciproc Blue	RB25 (25/ 0.08)	VDW GmbH, Munich, Germany	20	Reciprocating	Instrument was used in up to three pecking movements. This procedure was performed 3 times, until reaching the AWL.
PTN	Protaper Next	X1 (17 / 0.04) X2 (25 / 0.06)	Dentsply-Maillefer, Ballaigues, Suíça	20	Rotary	Instruments were used in a continuous sequence, with three spout and outlet movements, until reaching the AWL.
PTG	Protaper Gold	Sx, S1, S2, F1 (20/ 0,07) e F2 (25/ 0,08)	Dentsply-Maillefer, Ballaigues, Suíça	20	Rotary	Instruments following sequence: Sx (1/3 AWL), S1 and S2 (2/3 AWL), F1 and F2 in AWL.

At the end of the instrumentation, a final irrigation with was done with 2 ml EDTA (Biodinâmica, Ibiporã, Paraná, Brazil) and 2 ml of NaOCl 2.5%. The total volume of NaOCl used during all procedures was 10 ml for each tooth. Then the samples were irrigated to with 5 ml of distilled water and the root canals were dried with absorbent paper points (Dentsply, Petrópolis, Rio de Janeiro, Brazil) and then underwent to the second μCT scanning.

Microcomputed Tomography

Specimens underwent to µCT analysis (XT H 225 ST; Nikon Metrology, Inc., Tokyo, Japan) in two moments, before and after the biomechanical preparation. The parameters were adjusted to an isotropic resolution of 10 µm, 100kV voltage, an aluminum filter with 1 mm thickness, and a current of 100 µA. The roots were inserted in pairs in polystyrene blocks, considering that their density radiographic is close to the air. The blocks were then attached to the support for scanning.

An air calibration of the detector was performed before the scans to reduce ring artifacts and minimize the beam hardening effect. Images of each specimen were reconstructed with the CT Pro 3D v.XT3.1.3 software (Nikon Metrology NV, Tring, United Kingdom). The software VGStudio MAX v.2.2 (Volume Graphics, Heidelberg, Baden-Württemberg, Germany) applied Gaussian filtering to images and provided cross-sections of the internal structure of the roots. Then, the images were transferred to ImageJ (v.1.48; National Institutes of Health, Bethesda, Maryland, United States), and the cuts of interest were selected for analysis.

Dentinal Defects

Two calibrated observers evaluated the images, a specialist in Endodontics and another with experience and skill in µCT analysis. The evaluation of dentinal defects was performed based on the divergence of results found in studies (De-Deus *et al.* 2014, De-Deus *et al.* 2015). Thus, a first method of complete evaluation of the images used cross-sectional images performed before and after the root canal preparation, aiming to find possible dentinal defects present at the root of the tooth. The images were divided into 1 mm intervals (represented by 100 images) in second analysis, starting from the apex, analyzing and matching the final 4 mm in ImageJ. In both methods, the images were evaluated twice with an interval of 2 weeks, and cases of divergence were reassessed (De-Deus *et al.* 2014, Cassimiro *et al.* 2017).

The number of images with cracks was counted in each millimeter, considering the cracks that started from the inner part of the root canal. The images of the last 4 mm apical were selected 64,000 slices. Then, the Delta value (Δ) was calculated as follows: $\Delta = (T1 - T0)$, where Δ: will is the value resulting from the failures caused and/or increased by the instrumentation; T0: number of failures present in µCT images before instrumentation; T1: number of failures present after instrumentation.

Statistical Analysis

Statistical analysis was performed using the GraphPad Prism 7 software (GraphPad Software, San Diego, Califórnia, United States). The Kolmogorov-Smirnov test determined the non-normal distribution. The Wilcoxon test was used to check for differences between T0 and T1. The Kruskal-Wallis test was performed with Dunn's multiple comparison test to check whether there were statistical differences between the Delta values of the file systems used. In addition, the Mann-Whitney test assessed statistical differences between instrumentation movements (reciprocating and rotary). The statistical significance of all tests was considered to be $p < 0.05$.

Results

Figures 1 and 2 shows μCT images obtained from samples of the studied groups by comparing the images before and after the root instrumentation. Observe the presence of cracks only after instrumentation for samples shown in Figure 1 (indicated by arrows). Figure 2 shows crack points in initial images, which increased after the instrumentation (indicated by arrows).

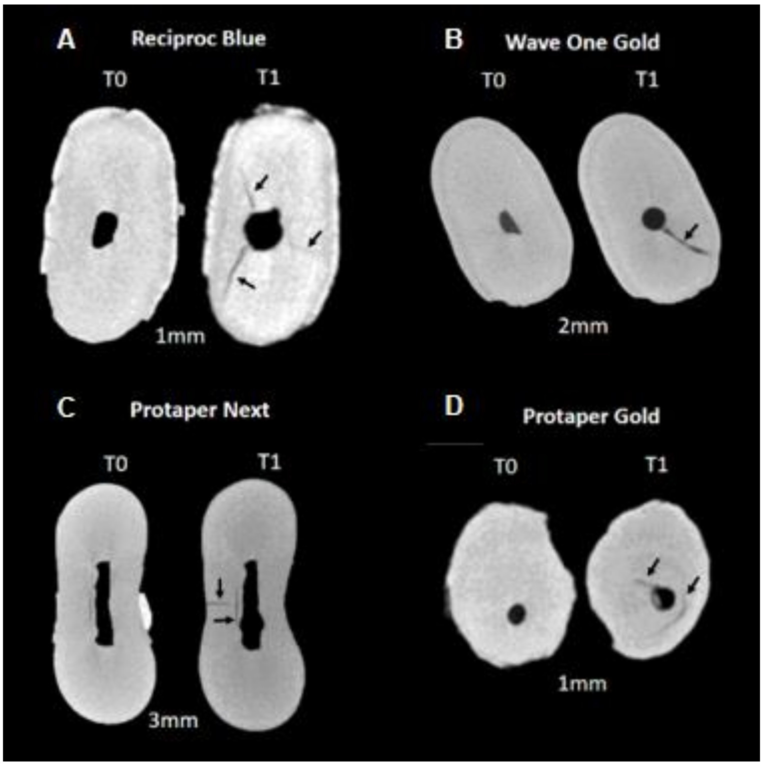

Figure 1. Representative cross-sectional microcomputed tomography images obtained with Reciproc Blue (A), Wave one Gold (B), Protaper Next (C), and Protaper Gold (D) systems, showing cracks formed (indicated by arrows) after (T1) the instrumentation.

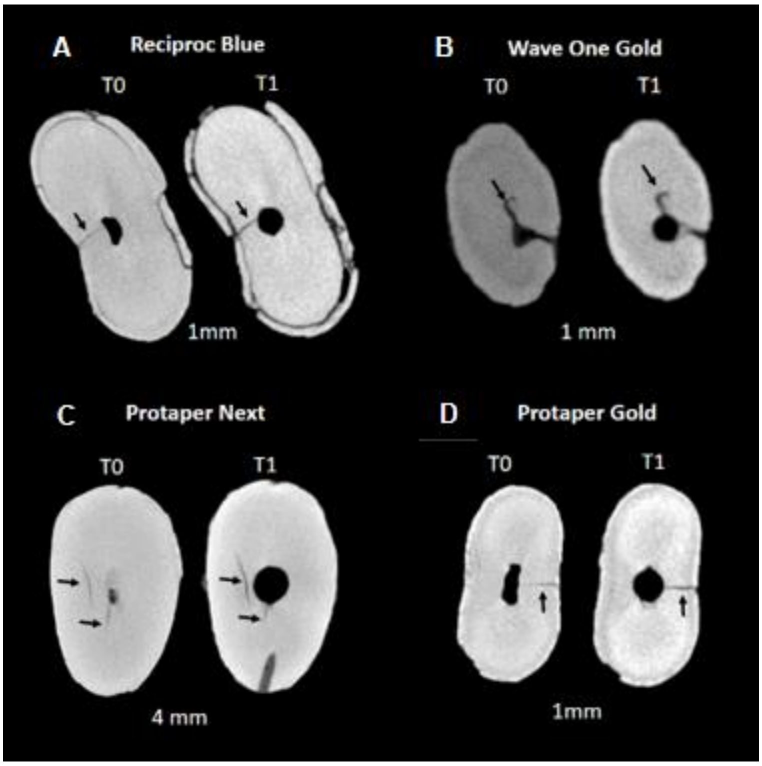

Figure 2. Representative cross-sectional microcomputed tomography images obtained with Reciproc Blue (A), Wave one Gold (B), Protaper Next (C) and Protaper Gold (D) systems root cracks (indicated by arrows), which continued or increased after (T1) instrumentation.

Statistically significant differences were found between the number of cracks before and after instrumentation (Table 2) in all systems used.

Table 2. Frequency of cracks T0 (before) and T1 (after) instrumentation.

System	Time	Number of images	Number of cracks found	p-value[1]
WOG	T0	8000	193	<0.0001*
	T1	8000	511	
RB	T0	8000	576	0.001*
	T1	8000	1237	
PTN	T0	8000	111	<0.0001*
	T1	8000	210	
PTG	T0	8000	75	0.004*

T1	8000	309

[1] Wilcoxon Test (EN: Wilcoxon matched pairs)

* Significant statistical differences.

There was an increase in the number of cracks for all groups after root instrumentation, confirmed by the Δ values showed in Table 3, which consists of the difference between the number of failures observed after and previous the root canals preparation. However, The Kruskal-Wallis test showed that there was not statistically significant difference between the groups (p=0.182).

Table 3. Comparison between the Δ values of the instrumentation systems used in the study.

System	Number of cracks found			p-value[1] Δ
	T0	T1	Δ	
WOG	193	511	318	0.182
RB	576	1237	661	
PTN	111	210	99	
PTG	75	309	234	

[1] Kruskal-Wallis test

When comparing the groups regarding instrumentation movement (reciprocating and rotary), no statistically significant differences were found in the number of cracks between instrumentation T0 and T1 (Table 4).

Table 4. Comparison between instrumentation movements (reciprocating and rotary).

System	Number of cracks found			p-value[1] Δ
	T0	T1	Δ	
Reciprocating	769	1.748	979	0.1048
Rotatory	186	519	333	

[1] Mann-Whitney test

Discussion

Four mechanized systems were chosen for this study, with different instrumentation movements and manufactured with alloys that have different heat treatments, in order to evaluate the formation of tooth cracks after instrumentation. So far, there are no studies in the literature that compare these instruments in a single study.

Several studies have been published trying to understand if endodontic mechanized instrumentation systems can result in a higher incidence of cracks. Some of these authors reported that instrumentation systems are not related to the formation of dentin cracks (Cassimiro *et al.* 2017, PradeepKumar *et al.* 2019, Valle *et al.* 2020), whilst others, similar to the present study, noted the formation of new cracks after instrumentation (Pedullà *et al.* 2017, Shantiaee *et al.* 2019).

With new systems appearing on the market, there is a need to continue this research, in which data extrapolate to clinical practice. Well, the presence of previous cracks suggest the possibility of risks for the root canal instrumentation, since it may progress to vertical root fracture (PradeepKumar *et al.* 2017, Bahrami *et al.* 2017).

The present study investigated the presence of dentin cracks in all µCT images obtained previous and after the instrumentation. Statistical difference in all groups evaluated in T0 and T1instrumentation were obtained ($p < 0.05$). Other studies published in the literature (Gergi *et al.* 2015, Pedullà *et al.* 2017, Nishad & Shivamurthy 2018, Karataş *et al.* 2016b, Ceyhanli *et al.* 2016, Kfir *et al.* 2017, Shantiaee *et al.* 2019), also showed a statistically significant difference the frequency of crack formation in the studied groups. On the other hand, other authors did not observe new dentinal defects, reporting that the post-instrumentation dentinal cracks were the same as those seen in the initial images (De-Deus *et al.* 2014, De-Deus *et al.* 2015, Bahrami *et al.* 2017, Cassimiro *et al.* 2017, Oliveira *et al.* 2017, Zuolo *et al.* 2017, Stringheta *et al.* 2017, Aksoy *et al.* 2019, PradeepKumar *et al.* 2019, Vieira *et al.* 2020, Uğur Aydın *et al*. 2019).

The preexisting cracks may be due to several causes, such as age or the presence of parafunctional habits (PradeepKumar *et al.* 2017, Aksoy *et al.* 2019). Moreover, any excessive force on the teeth during extraction (PradeepKumar *et al.* 2017, Bahrami *et al.* 2017) or environmental changes in the dentin, such as dehydration (Shemesh *et al.* 2018), can result in cracks dentin.

In one study were carried out atraumatic extractions; even keeping the samples hydrated, and observed a percentage of cracks of 7.1% (PradeepKumar et al. 2017). In another, showed cracks were frequently found in intact extracted teeth, present in 5.8% of the initial sample of extracted healthy maxillary premolars, observed under a microscope (Kfir *et al.* 2017). In this study, pre-existing cracks were present in 11.92% of the initial images, probably due to the same reasons previously mentioned.

For this reason, the teeth selected for the present study had their external root surfaces carefully examined under an operating microscope, and were kept hydrated throughout the experiment, similar to another study (Cassimiro *et al*. 2017) – aiming to avoid any structural changes in the roots before and during the experiment.

Another important fact is that some of the studies present in the literature (Bahrami *et al.* 2017, Karatas *et al.* 2016b, Pedullà *et al.* 2017) evaluated the defects after chemical-mechanical preparation through cutting and root analysis in a stereomicroscope, others using µCT (Cassimiro *et al.* 2017, PradeepKumar *et al.* 2017, Aksoy *et al.* 2019, De-Deus *et al.* 2015, Zuolo *et al.* 2017, Uğur Aydın *et al*. 2019). While other authors report that, the occurrence of these dentinal damages observed in some studies can also be related to the sectioning of the samples. It is known that pre-existing internal defects are impossible to view externally under a stereomicroscope (De-Deus *et al*. 2015).

The methodology chosen in the present study to assess cracks was the µCT analysis, considered the gold standard (Cassimiro *et al.* 2017, De-Deus *et al.* 2014, Aksoy *et al.* 2019), observing the last apical 4 mm , in agreement with other studies described in the literature (Cassimiro *et al.* 2017, Oliveira *et al.* 2017).

The superiority of µCT compared to other imaging technique methods, is due to its accurate and non-destructive character, which allows the evaluation of samples before and after root canal preparation (De-Deus *et al.* 2014, Aksoy *et al.* 2019). With this method, dentin cracks can be examined in their exact location and each specimen functions as its own control group, through pre- and postoperative assessments (De-Deus *et al.* 2015, Ceyhanli *et al.* 2016), good resolution, avoiding generating misinterpretation (Aksoy *et al.*, 2019). Moreover, other data should be evaluated, since it could be confounding variables, which limits the interpretation and the statistical power of the sample, such as the standardization of the shape of teeth canals in studied groups.

In this study, the selected specimens were lower incisors with unique and straight canals, in agreement with other authors (Karatas *et al.* 2016b, Zuolo *et al.* 2017, Cassimiro *et al.* 2017, Oliveira *et al.* 2017), others used lower premolars for their experiments (Aksoy *et al.* 2019, PradeepKumar *et al.*, 2019), whilst others used lower molars (Ceyhanli *et al.* 2016, De-Deus *et al.* 2015, Uğur Aydın *et al.* 2019). The results found were similar to the findings of studies that used other teeth, having no influence from the dental group.

Analyzing the morphology of the lower incisor, the literature reports a deep flattening in the mesiodistal direction of long cross sections and a larger diameter in the buccolingual direction (Karatas *et al.* 2016b). Therefore, instrumentation and choice of instruments have been standardized, due to the small apical diameters. So, in this study, all of the root canals were prepared with a size of up to 0.25 initial diameter, similar to other studies (Karatas *et al.* 2016b).

One possibility of the possible divergences found in this study compared to others in the literature, in which new crack formation was not observed, is regarding the working length that was used in the preparation of the samples. Using this methodology, the root preparation in the foramen, measuring the AWL through the placement of a manual file in the foraminal exit. However, other studies performed the instrumentation by subtracting 1mm from the foraminal outlet (De Deus *et al.* 2014, De Deus *et al*. 2015, Cassimiro *et al.* 2017, Aksoy *et al*. 2019), which did not observe differences in the detection of cracks. On the other hand, in studies that compared different instrumentation sizes, they observed that regardless of the working length used to prepare the root canals, no new apical crack was generated (Oliveira *et al.* 2017b, Vieira *et al*. 2020).

In the present study, four different endodontic systems were evaluated, all groups presented new dentin cracks after instrumentation and/or increasing of those already existents, with a statistically significant difference between the in T0 e T1 instrumentation. However, no significant differences were observed between the four groups.

There was no data in the literature comparing the same systems, however, several studies cite data referring to each of them, some agree with the results of this study, in other studies, no differences were seen when comparing RB (Aksoy *et al.* 2019), WOG (Cassimiro *et al*. 2017; Bahrami *et al*. 2017), RB with WOG (Uğur Aydın *et al*. 2019) and PTN with other systems (De-Deus *et al.* 2015, Cassimiro *et al.* 2017). Although many of the aforementioned studies have observed the formation of cracks, they were not significant. In contrast, observing a statistically significant difference between the pre and post instrumentation when comparing WOG with other instruments (Pedullà *et al.* 2017), as well as between PTN and PTG when compared to with PTU and group control (Nishad & Shivamurthy, 2018, Karatas *et al.* 2016b).

In the present study, the comparison between the systems showed reciprocating systems promoted more cracks than the rotatory systems; however, they did not present statistical differences. Agreeing with other studies compared the formation of cracks when using reciprocating and rotary systems (De-Deus *et al.* 2014, Coelho *et al*. 2016).

The cross section of the instrument influences the contact with the root dentin during the preparation of the canal, promoting different degrees of tension. The WOG with cross section in the form of a decentralized parallelogram (Karatas *et al.* 2016a), in which only one cutting edge is in contact with the canal wall, decreases the contact area between the file and the canal wall, thus reducing taper lock (Gavani *et al.* 2018). The system RB has cross section in "S" shape, with a blunt tip and is a developed version of the instrument Reciproc (Aksoy *et al.* 2019). The PTN system has a decentralized rectangular cross section with eccentric movement, minimizing contact with the dentin and two cutting blades (De Deus *et al.* 2015; Stringheta *et al.* 2017). In addition, according to the manufacturer, PTG instruments have greater flexibility (Karatas *et al.* 2016b), with a convex triangular cross section, which increases its cutting action, while decreasing the rotational friction between the file blade and the dentin. However, such differences did not influence the results obtained.

Moreover, the manufacturing processes in recent years allow the development of new instruments types of NiTi, with the best heat treatments of the alloy and, consequently, better mechanical characteristics. Still, studies stated that there was no difference between instruments with different heat treatments. In agreement with the data obtained in this study the different heat treatments of the NiTi alloys that compose the systems evaluated did not produce different results regarding (De Deus *et al.* 2015, Karatas *et al*. 2016b).

Based on the results of the present study it is possible to affirm that all mechanical systems caused cracks, but with no statistically significant difference between the tested groups. So, the choice of the endodontic instrument system other factors should be considered, such as the professional preference, the particularities of each clinical case, or even the number of used files/steps, the cost-benefit ratio, the technique, the root canal anatomy itself, as well as the professional's learning curve.

Regarding the cracks observed in the present research, only the cracks formed from the intracanal region (internal) were considered, excluding the external ones, which may have been generated by other factors already discussed. In contrast, in other study (Aksoy *et al*. 2019), the cracks that developed after instrumentation looked away from the canal and start on the external surface. As well as observed that many of the defects had no connection with the pulp space, were located in areas far from the intracanal region (Shemesh *et al*. 2018).

However, the stresses generated by the instruments happens from the root canal and are transmitted by the root dentin, reaching the outer surface. Moreover, the extension of the cracks were not evaluated in this study, so it is not possible to affirm if the instruments tested affect these measures neither if they are related to changes in the morphology of cracks.

Conclusion

From this study, it was observed that there was a statistically significant increase in the number of images compromised with cracks. Nonetheless, no differences were observed between the studied endodontic systems, neither in terms of movement (reciprocal or rotational) nor in the heat treatment of the alloys between them.

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4 ARTIGO 2

VERTICAL FRACTURE RESISTANCE OF TEETH AFTER INSTRUMENTATION WITH ROTARY AND RECIPROCAL ENDODONTIC SYSTEMS

- ✓ Artigo para submissão na **Clinical Oral Investigations** – A1 – Impact factor: 2.812, após avaliação da banca

Vertical Fracture Resistance of teeth after instrumentation with Rotary and Reciprocal Endodontic Systems

Introduction

One of the main steps in root canal treatment is mechanical instrumentation [1]. In preparing the root canal, the anatomical path of the canal must be preserved, while endodontic files remove dentin and irrigation facilitate disinfection [2]. During instrumentation, it can produce greater friction and tension in the canal wall, which can generate dentin defects in the root structure [3, 4].

Technological innovations in nickel titanium (NiTi) instruments have led to new instrumentation concepts, including an increase in the preparation diameter. Greater mechanical preparation taper provides sufficient widening in the root canal for better debris and smear layer removal, improved irrigant flow, and better stress distribution during lateral and vertical gutta-percha compaction [5]. However, excessive dentin removal has raised concerns about the possible susceptibility of roots to fractures [5, 6].

Because dentinal cracks can occur during mechanical instrumentation of root canals [1], [7], such defects can result in the weakening of dentin integrity, causing a reduction in the fracture strength of the treated tooth [3, 8]. It may also lead to a vertical root fracture (VRF), with extraction being the last treatment option [9]. Such fractures occur when the tensile stress on the root canal wall exceeds the remaining tensile strength of the dentin [4, 10].

Although VRFs are mainly associated with endodontically treated teeth, due to dentin wear generated during instrumentation, it is also a complication observed in vital teeth without endodontic treatment [4, 11]. Besides, the predisposing factors for root fractures are widely discussed in the literature [5, 12] and its etiology is multifactorial, including anatomical and iatrogenic factors that may contribute [13], in addition to traumatic physical injuries, occlusion problems, parafunctional habits [4] and root resorption [13].

Furthermore, professionals often face challenges in diagnosis, because signs and symptoms are not completely pathognomonic [14]. Also, the prognosis of vertically fractured teeth is very poor [8]. Therefore, professionals have sought to strengthen the remaining tooth structure so that the fracture resistance of the endodontically treated tooth is increased [13].

There are several NiTi systems available on the market, with different production phases (M, R, austenite and martensite wire), alloys, heat treatment and work movement. The design characteristics of NiTi systems have been described in detail in previous articles, however, with regard to these different designs, there is no direct association between which of them cause more or less dentin defects, being more susceptible or not to fractures [1, 3, 10, 15, 16].

Many studies have confirmed the association between NiTi systems and dentinal cracks, which can result in VRFs [1, 15, 17], but there is not enough information in the literature about the strength of teeth instrumented with current NiTi systems.

With new instruments on the market, with characteristics that increasingly seek to reduce the stresses generated in dentin and preserve the maximum dentin structure, the objective of this research is to evaluate the VFR of endodontically treated teeth, instrumented with different endodontic systems: reciprocating (Reciproc Blue and Wave One Gold) and rotary (Protaper Next and Protaper Gold).

Materials and Methods

Sample selection

This study followed the precepts established by the Resolution of the National Health Council (CNS / MS) the Research Ethics Committee, duly registered 466/12, in the Base of Research Records involving human beings and approval (Number: 3.719.153). In addition, the sample size was determined considering a type I error (α) of 5%, a type II error (β) of 20%, a power of 80% and the effects (effect sizes = g of Hedge) verified in the study [18]. The calculated effect size was 1.82, requiring a minimum of six sample units per experimental group.

Permanent lower incisors with straight roots were obtained, previously donated to the teeth bank, cleaned, and then stored in 0.1% Thymol solution during 24h, for necessary disinfection. These teeth were x-rayed and evaluated under a surgical microscope (Alliance, São José dos Campos, Brazil) with a 16x magnification. The teeth in which presence of root fissures or fracture lines on their external surfaces, resorption, calcification, and curved channels were excluded. The same operator performed all laboratory procedures.

Thirty teeth who attended the selection criteria composed the final sample (n=6). Then their crowns were removed under refrigeration using diamond discs (KG Sorensen Ind., Cotia, Brazil) mounted on a low-speed motor (Kavo Ind. Ltda., Joinville, Brazil), with approximately 13 mm of length. The teeth were kept hydrated throughout the experiment, stored in distilled water. Subsequently, these teeth were submitted for an initial evaluation of the teeth, through the µCT, to rule out the presence of any intracanal fracture.

Microcomputed tomography (µCT)

Specimens underwent µCT analysis (XT H 225 ST; Nikon Metrology, Inc., Tokyo, Japan) before the biomechanical preparation. Following the parameters with voltage of 100 kV, an isotropic resolution of 10 µm, an aluminum filter with 1 mm thickness and a current of 100 µA. The roots were inserted in pairs in polystyrene blocks, considering that their radiographic density is close to the air, and attached to the support for scanning. The scan was performed with a 360° rotation of the sample around the vertical axis with an angular pitch of 0.12° per projection 3016 projections were generated for each sample.

Images of each specimen were reconstructed with the CT Pro 3D v.XT3.1.3 software (Nikon Metrology NV, Tring, United Kingdom). The software VGStudio MAX v.2.2 (Volume Graphics, Heidelberg, Germany) was used to apply Gaussian filtering to the images and to provide cross sections of the internal structure of the roots. Then, the images were transferred to ImageJ (v.1.48; National Institutes of Health, Bethesda, Maryland, United States) for analysis.

Two calibrated observers evaluated the images, a specialist in Endodontics and with practical in area, and another with experience and skill in µCT analysis. An assessment of possible defects was carried out, fully examining the transverse images taken of the entire root extension. Excluding from the test any tooth that had any intracanal crack or fracture.

Root canal preparation

Files #10K and #15K (Dentsply Maillefer, Ballaigues, Suíça) were introducing in the canal up to the apical foramen and then measured in endodontic ruler (Maquira Industry Dental Products SA, Maringá, Brazil), to get the actual tooth length (ATL), being equal to actual working length (AWL), ensuring that all teeth were instrumented at the correct length.

The periodontal ligament simulation was adapted from the methodology of a previous study [19]. The roots were immersed in modelling wax n°7 (Duradent, Odonto Ltda, São Paulo, Brazil) to obtain a space of 0.2 ± 0.3 mm around the entire root, which provided the space to be filled with polyvinyl siloxane (PVS) and 3mm demarcated with pencil at the cervical, to simulate biological distances. The teeth were kept at room temperature and, afterwards, they were immersed in cold water so that the wax completely solidified and without suffer deformations.

A circumference was demarcated on the utility wax strips referring to the diameter of the PVC tube (Tigre do Brasil, Osasco, Brazil). Therefore, the root was centered and the cervical 3.0 mm demarcated for biological distances was inserted in the wax. The PVC tube was placed over the circumferential demarcation in the wax, sealing the outside of the tube to fix it and not let the resin overflow. The self-curing acrylic resin (Vipi Flash, Pirassununga, Brazil) was inserted into the tubes.

After polymerization of the resin, the wax around the roots and inside the artificial socket was removed with the aid of a long curette (Golgran, São Caetano do Sul, Brazil) and these surfaces were covered with light body PVS (Express Addition Silicone Express XT Paste Fluid - 3M, Sumaré, Brazil) and inserted inside the artificial socket. The samples kept under moist conditions in containers, hermetically sealed.

Root instrumentation was performed according to the kinematics indicated for each instrument with an endodontic motor (X-Smart Plus Bivolt, Dentsply-Maillefer, Ballaigues, Suíça) using the crown-apex technique. A single operator prepared all root canals. The root canals were irrigated with 1mL of 2.5% sodium hypochlorite (NaOCl) using a disposable syringe and Endo-Eze needle (Ultradent Products Inc., South Jordan, United States) after each instrument and explored with a #15K file between the instruments, until the work length has been achieved. Each instrument was used to prepare up to three teeth and exchanged for a new one (20). The root canals preparation followed the manufacturer's instructions for all systems:

Group CTR - Control: Healthy root, without instrumentation.

Group WOG - Wave One Gold (Dentsply-Maillefer, Ballaigues, Suíça): The Primary instrument (25 / 0.07) was used, applying three spout and exit movements, until reaching the ATL, with torque and speed already programmed in the motor.

Group RB - Reciproc Blue (VDW GmbH, Munich, Germany): The RB 25 instrument (25/ 0.08) was used in up to three pecking movements. This procedure was performed 3 times, until reaching the ATL.

Group PTN - Protaper Next (Dentsply-Maillefer, Ballaigues, Suíça): The X1 (17 / 0.04) and X2 (25 / 0.06) instruments were used in a continuous sequence, with three spout and outlet movements, until reaching the ATL, with the torque and speed recommended in the engine.

Group PTG - Protaper Gold (Dentsply-Maillefer, Ballaigues, Suíça): Instruments were used with a programmed endodontic motor controlled by torque and speed. The following sequence was used: Sx (1/2 AWL), S1 and S2 (2/3 AWL), F1 (20 / 0.07) and F2 (25 / 0.08) in ATL.

After instrumentation, a final irrigation with was done with 2 ml EDTA (Biodinâmica LTDA, Ibiporã, Brazil) and 2 ml of NaOCl 2.5%. The total volume of NaOCl used during all procedures was 10 ml for each tooth. Then the root canals were irrigated to with 5 ml of distilled water and dried with absorbent paper points (Dentsply, Petrópolis, Brazil).

Then, the teeth were all obturated using the single cone technique. The 35.04 gutta-percha cone was adapted (MK Life, Porto Alegre, Brazil), decreasing 1mm from the ATL. After proof of the cone, the endodontic cement AH plus (Dentsply Maillefer, Ballaigues, Switzerland) was applied into the canal and the cone and then inserted. Behind, vertical condensation was carried out with double condensers (Odous de Deus, Belo Horizonte, Brazil) warmed and, later, cold to condense the gutta. The teeth were prepared for analysis of the vertical fracture strength of the roots.

Fracture resistance test

The roots embedded in the PVC tubes were fixed in the device of the universal testing machine (Instron 3365, Instron Brasil Equipamentos Científicos Ltda, São José dos Pinhais, Brazil). Specimens were subjected to axial compression loading using a 2.5 mm diameter metallic attached to a 5000 N load cell at 1 mm/min crosshead speed until fracture was detected. The actuator was positioned at the access opening of the root canal so that it contacts the flat surface of the root on both the mesial and distal sides, evenly applying the load over the root surface.

As the load was applied, the computer recorded an ascending curve that was abruptly interrupted when the specimen fractur, and simultaneously emitted the crack sound of the sample. The fracture threshold was defined as the load that revealed the first fracture (the sudden drop in load during compression). The test was finished at this point and the force measured in Newton (N) referring to the maximum fracture resistance (FR).

Statistical analysis

Data were analyzed using descriptive and inferential statistics. The fracture resistance variable was analyzed descriptively from measures of central tendency and dispersion. The normality of the fracture resistance variable was verified through the Shapiro-Wilk test, as well as through the histogram visual analysis. As the variable had a normal distribution, parametric tests were used. The one-way Analysis of Variance (ANOVA) test was used to compare the fracture resistance variable in situations involving more than two comparison groups, while the Student t test was used when there were only two comparison groups. All analyzes were performed using the Statistical Package for Social Sciences software (SPSS for Windows, version 25.0, IBM Corp., Armonk, United States) with a significance level of 5%.

Results

There was no statistically significant difference in fracture resistance between the groups evaluated when compared to the control group (p=0.064) (Table 1).

Table 1. Comparison of fracture resistance (N) between studied groups (n=6).

Groups	Mean	SD	Median	Minimum	Maximum	*p* value*
RB	548.6	186.0	527.5	362.9	866.0	
WOG	527.8	140.9	563.4	322.5	671.0	
PTN	382.6	151.5	486.0	109.5	520.0	0.064
PTG	397.7	86.2	370.0	311.8	507.7	
CTR	631.1	214.0	570.5	406.1	1008.1	

SD: standard deviation. * Analysis of Variance (ANOVA).

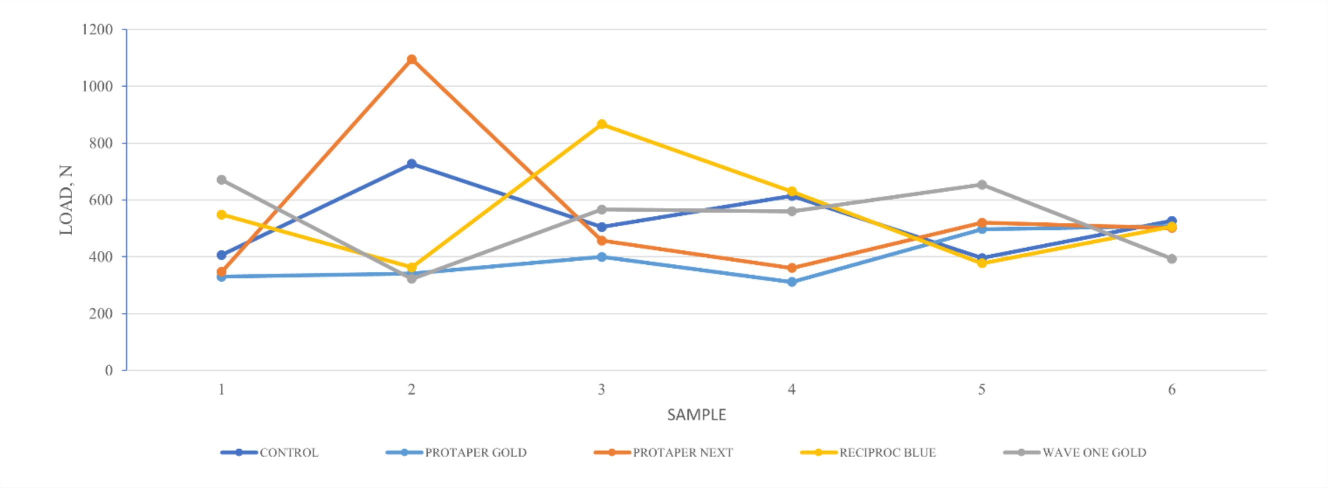
There was no statistically significant difference in fracture resistance between the endodontic systems evaluated (p=0.13) (Table 2). And the pattern of load distribution in all four experimental groups is shown in Figure 1.

Table 2. Comparison of fracture strength between Reciproc Blue, Waveone Gold, Protaper Next and Protaper Gold groups.

Groups	Mean	SD	Median	Minimum	Maximum	*p* value*
RB	548.6	186.0	527.5	362.9	866.0	
WOG	527.8	140.9	563.4	322.5	671.0	
PTN	382.6	151.5	486.0	109.5	520.0	0.133
PTG	397.7	86.2	370.0	311.8	507.7	

SD: standard deviation. * Analysis of Variance (ANOVA).

Figure 1. Graphical representation of the pattern of load distribution in different groups



There was a statistically significant difference when compared to the mechanized systems, presenting a greater resistance to vertical fracture or the instrumented group with reciprocating system (Table 3).

Table 3. Comparison of fracture strength (N) between the reciprocating and the rotating group.

Groups	Mean	SD	Median	Minimum	Maximum	*p* value*
Reciprocating	538.2	157.7	554.4	322.5	866.0	
Rotary	390.2	117.8	380.0	109.5	520.0	**0.016**

SD: standard deviation. * Student's t test.

Discussion

At least three studies claim that the removal of tooth structure and wear on the root dentin decrease the fracture resistance of the tooth [5, 6, 21], being directly related to the amount of remnant tooth. Different types of instruments, with different designs, movements, heat treatments, and other characteristics can influence this excessive wear. There are many systems and the combination of those studied here has not yet been found in the literature.

When realizing *in vitro* studies, which perform mechanical tests, the standardization of samples is an essential factor in assessing of fracture resistance with natural teeth [5, 8]. Teeth with similar characteristics were selected to standardize the root canal anatomy [1]. In addition, all were kept under the same storage conditions during the entire sample preparation and mechanical test.

Single mandibular incisors were used in this study, as in the work of Patil et al. [13]. However, other studies have evaluated molars [11] and premolars [1, 8, 10].

Some authors report that endodontic treatment can result in tooth weakening, especially after instrumentation (modeling and cleaning), reducing vertical fracture resistance [8, 9, 22, 23]. The possibility of fracture may be due to possible dentinal cracks formed during instrumentation [1, 3, 16, 24, 25] and may lead to fracture [26, 27]. However, the cause-effect relationship between the number of dentinal cracks and fracture strength still needs to be evaluated [28, 29].

The present study results indicated that the fracture resistance of endodontically treated teeth was reduced compared to the control group, but no statistically significant difference was observed. Demonstrating that the instrumentation had no direct relationship with fracture resistance, corroborating the data found by Capar et al. [10]. While Pawar et al. [8] and Keles et al. [11] observed a difference between the instrumented groups, concluding that the resistance of the endodontically treated tooth was reduced compared to the control group.

Systems with different instrumentation movements and NiTi alloys with different heat treatments were used, having a design that may or may not facilitate the presence of cracks. Among the studied endodontic systems, no statistical difference was observed.

On the other hand, other studies have pointed out the PTN with a greater resistance to fracture when compared to other systems [1, 8, 21]. However, the mean force (N) observed by these authors was similar to those in the present study. It is possible to suppose that such results would be related to comparison of the PTN system with other older instruments in the dental market which have alloys with inferior heat treatments, different from this study. On the other hand, in the findings of Acharya et al. [21] which compared the PTN with the Universal Protaper (PTU) and manual files, showed a statistically significant difference between the groups, in which the instrumented tooth with PTN presented greater resistance than the PTU and less than the manual files. However, no similar studies were found that compared the other systems, as in the present study.

Pawar et al. [8] evaluated the Wave One with NiTi M-wire alloy and observed that the instrumented samples exhibited the lowest fracture resistance in the tested groups. However, the system studied in this research was the Wave One Gold, which represents a better version of the old instrument, with a Gold Wire heat treatment and characteristics that ensure less dentinal wear, with a cross-section in the shape of a decentralized parallelogram [30]. This design results in a very smooth reciprocal movement, eliminating the need to place any strain on the file, enhancing safety, and significantly greatly improving cutting capacity [31].

This cross-section influences the possibility of generating different degrees of stress in the root dentin. The RB system has an "S" cross-section with a blunt tip, a version developed from Reciproc [20], while the PTN system has an offset rectangular cross-section with eccentric movement, minimizes contact with the dentin, and has two blades of cutting [10]. Moreover, PTG instruments have greater flexibility than PTN and PTU [24]. However, from our results, these characteristics do not seem to influence the vertical fracture resistance between the groups.

Manufacturing processes in recent years allow the development of new types of NiTi instruments, with the best heat treatments of the alloy and, consequently, better mechanical characteristics. Still, De Deus et al. [32] stated no difference in dentin wear between instruments with different heat treatments.

When comparing the reciprocating and rotational systems in the formation of cracks, the authors [28] did not observe differences that would not influence the possible fracture resistance. However, other authors [8, 16] reported having differences between movements, pointing out that reciprocating systems tend to generate more cracks when compared to rotational ones, which can influence the reduction of fracture resistance of treated teeth. In yet another study, the authors stated that Reciproc Blue and Wave One Gold's reciprocal movement generates a larger contact area between the instrument and the canal walls, cutting large amounts of dentin [33]. However, in the present study, a statistical difference was observed between the different instrumentation movements, where the rotary systems showed less resistance when compared to the reciprocating ones.

While the continuous rotational movements make a complete rotation (360°) within the root canal, the reciprocating movement drives the instruments in asymmetric rotation angles, in the counterclockwise and clockwise directions, facilitating the instrument's advancement with little apical pressure. Rotation angles range from approximately 120° to 270° counterclockwise (to cut the dentin) and from approximately 60° to 90° clockwise (to clean it) [31].

Thus, Hin et al. [34] confirmed that rotary files have an active movement and in the formation of cracks, which reduces fracture resistance of instrumented teeth. On the other hand, the reciprocating movement presents reduced flexion and torsion stresses acting on the dentin walls, mainly using only a single file for root canal instrumentation [10, 17, 35]. Cicek et al. [1] suggest that the instrumentation movement has more influence on cracks and possible VRF than the taper of the instruments.

Furthermore, as this is an *in vitro* study, it is not always possible to portray what happens in the clinical reality, leading to some biases in the results obtained. Thus, to validate techniques and the clinical use of instruments, a rigorous scientific method is needed. Because these studies help to support clinical models, although their results provide limited analogies to real situations, they give an idea of what can be expected from the clinical use of the instruments [36].

Therefore, simulation of the periodontal ligament was performed in this research to instrumentate the root canals and perform the mechanical testing [1, 8, 10, 11, 21]. The periodontal ligament serves to absorb the stresses of the preparation and limit the tooth's movement while avoiding external forces against the root [37]. The artificial periodontal ligament should be used for this type of mechanical test because the force applied directly to the tooth, with the absence of any material to imitate the periodontal ligament, can alter the data and clinical simulation [1, 10]. However, Yusufoglu et al. [23] did not use VPS material to simulate the periodontal ligament to counteract the vertical force.

A limitation of the present study is the lack of knowledge about the age of the tooth donor. Root dentin strength can be affected by age [38], although this degradation does not exceed the decrease in root dentin strength caused by endodontic treatment [39]. In addition, the sample was acquired from a tooth bank with no knowledge of the extraction time of the teeth used. Therefore, we believe that future investigations should be carried out using a fresh in situ human cadaver model combined with an appropriate mechanical assay method.

Considering that the influence of instrumentation on VRF is not yet fully consolidated, future studies should be designed to assess the influence of the preparation with other instruments. Different tapers and diameters, other metallic alloys, variable cross-sections, newly extracted teeth obtained from patients with known age, and an analysis method closer to clinical reality.

Conclusion

In the present study, the mechanical instrumentation tested did not influence the vertical fracture resistance of prepared teeth compared to the control group, no differences between the systems, even different design characteristics, section transverse, and heat treatment of the alloy. However, it was noticed that the roots instrumented with the instruments with reciprocating movement showed higher resistance to fracture than the rotatory ones.

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5 CONCLUSÕES GERAIS E TRABALHOS FUTUROS

A partir da presente pesquisa, pode-se observar que todos os sistemas endodônticos avaliados causaram trincas dentinárias, mesmo a maioria delas estando presente nas imagens pré-operatórias, houve um aumento estatisticamente significativo no número de imagens comprometidas com as trincas. Contudo, não foram observadas diferenças entre os sistemas, nem quanto ao movimento de instrumentação (reciprocantes e rotatórios).

Assim como, não houve influência da instrumentação mecânica na resistência à fratura vertical de dentes preparados com os sistemas endodônticos avaliados quando comparados ao grupo controle, bem como não houve diferença entre os sistemas, mesmo com características e design diferentes. Porém, observou-se que as raízes instrumentadas com os instrumentos de movimento recíproco apresentaram maior resistência à fratura do que os rotatórios, sendo estatisticamente significante.

Por fim, outras análises foram realizadas, através das imagens obtidas na microtomografia computadorizada, quanto a avaliação do transporte de canal e a capacidade de centralização dos instrumentos reciprocantes (Wave One Gold e Reciproc Blue) e os rotatórios (Protaper Next e Protaper Gold). No entanto, os dados desse trabalho futuro, estão com a análise estatística e escrita do artigo em andamento, constando um resumo do objetivo e metodologia utilizada no Apêndice 1.

Diante dos dados obtidos e analisados na presente tese, pode-se ter conhecimento quanto a influência dos sistemas endodônticos avaliados na instrumentação dos canais radiculares e assim, auxiliar o endodontista na escolha do instrumento que será utilizado na prática clínica. Associando a outros fatores, como as particularidades de cada caso clínico, ou mesmo a quantidade de limas/ etapas utilizadas, a relação custo-benefício, a técnica, a própria anatomia do canal radicular, bem como a curva de aprendizado de cada profissional.

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ANEXO A – NORMAS DE PUBLICAÇÃO DA REVISTA INTERNATIONAL ENDODONTIC JOURNAL, REFERENTE AO ARTIGO 1

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.

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.

4. MANUSCRIPT TYPES ACCEPTED

Original Scientific Articles (includes Clinical Research (randomized control trials, cohort studies, case control studies, cross sectional studies, case series), Basic Research – Biological, Basic Research - Technical and Education): 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.

5. MANUSCRIPT FORMAT AND STRUCTURE

5.1. Format Language: The language of publication is English. It is preferred that manuscript is professionally edited.

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.

Article Preparation Support: **Wiley Editing Services** offers expert help with English Language Editing, as well as translation, manuscript formatting, figure illustration, figure formatting, and graphical abstract design – so you can submit your manuscript with confidence. Also, check out our resources for **Preparing Your Article** for general guidance about writing and preparing your manuscript.

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 350 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.

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 PRIRATE 2020 guidelines. A PRIRATE 2020 checklist must be completed and included along with a flow diagram (as a Figure) in the submission material. These are available at **http://pride-endodonticguidelines.org/prirate/**.
(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.

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**

ANEXO B – NORMAS DE PUBLICAÇÃO DA REVISTA CLINICAL ORAL INVESTIGATIONS REFERENTE AO ARTIGO 2

INSTRUCTIONS FOR AUTHORS

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Online Submission

Please follow the hyperlink "Submit manuscript" on the right and upload all of your manuscript files following the instructions given on the screen.

Please ensure you provide all relevant editable source files. Failing to submit these source files might cause unnecessary delays in the review and production process.

Further Useful Information

please follow the link below

Further Useful Information

The Springer Author Academy is a set of comprehensive online training pages mainly geared towards first-time authors. At this point, more than 50 pages offer advice to authors on how to write and publish a journal article.

Title Page

The title page should include:

The name(s) of the author(s)

A concise and informative title

The affiliation(s) and address(es) of the author(s)

The e-mail address, telephone and fax numbers of the corresponding author

Abstract

Please provide a structured abstract of 150 to 250 words which should be divided into the following sections:

Objectives (stating the main purposes and research question), Materials and Methods, Results, Conclusions and Clinical Relevance.

These headings must appear in the abstract.

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Text Formatting

Manuscripts should be submitted in Word.

Use a normal, plain font (e.g., 10-point Times Roman) for text.

Use italics for emphasis.

Use the automatic page numbering function to number the pages.

Do not use field functions.

Use tab stops or other commands for indents, not the space bar.

Use the table function, not spreadsheets, to make tables.

Use the equation editor or MathType for equations.

Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX.

LaTeX macro package (Download zip, 190 kB)

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables. Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols. Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

References

Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:

1. Negotiation research spans many disciplines [3].
2. This result was later contradicted by Becker and Seligman [5].
3. This effect has been widely studied [1-3, 7].

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. The entries in the list should be numbered consecutively. If available, please always include DOIs as full DOI links in your reference list (e.g. “https://doi.org/abc”).

Journal article

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8

Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

Article by DOI

Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. https://doi.org/10.1007/s001090000086

Book

South J, Blass B (2001) The future of modern genomics. Blackwell, London

Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

Dissertation

Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see ISSN.org LTWA. If you are unsure, please use the full journal title.

Tables

All tables are to be numbered using Arabic numerals. Tables should always be cited in text in consecutive numerical order. For each table, please supply a table caption (title) explaining the components of the table. Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.

Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Artwork and Illustrations Guidelines

Electronic Figure Submission

Supply all figures electronically.

Indicate what graphics program was used to create the artwork.

For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.

Vector graphics containing fonts must have the fonts embedded in the files.

Name your figure files with "Fig" and the figure number, e.g., Fig1.eps. Line Art

Definition: Black and white graphic with no shading.

Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size. All lines should be at least 0.1 mm (0.3 pt) wide. Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi. Vector graphics containing fonts must have the fonts embedded in the files. Halftone Art

Definition: Photographs, drawings, or paintings with fine shading, etc.

If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves. Halftones should have a minimum resolution of 300 dpi. Combination Art

Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.

Combination artwork should have a minimum resolution of 600 dpi.

Color Art Color art is free of charge for online publication. If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.

If the figures will be printed in black and white, do not refer to color in the captions. Color illustrations should be submitted as RGB (8 bits per channel).

Figure Lettering

To add lettering, it is best to use Helvetica or Arial (sans serif fonts). Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt). Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.

Avoid effects such as shading, outline letters, etc.

Do not include titles or captions within your illustrations. Figure Numbering.

All figures are to be numbered using Arabic numerals. Figures should always be cited in text in consecutive numerical order. Figure parts should be denoted by lowercase letters (a, b, c, etc.). If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures,"A1, A2, A3, etc." Figures in online appendices [Supplementary Information (SI)] should, however, be numbered separately.

Figure Captions

Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.

Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type. No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption. Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs. Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

Figures should be submitted separately from the text, if possible. When preparing your figures, size figures to fit in the column width. For large-sized journals the figures should be 84 mm (for double-column text areas), or 174 mm (for single-column text areas) wide and not higher than 234 mm. For small-sized journals, the figures should be 119 mm wide and not higher than 195 mm.

Permissions

If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s) for both the print and online format. Please be aware that some publishers do not grant electronic rights for free and that Springer will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used.

Ethical Responsibilities of Authors

This journal is committed to upholding the integrity of the scientific record. As a member of the Committee on Publication Ethics (COPE) the journal will follow the COPE guidelines on how to deal with potential acts of misconduct.

Authors should refrain from misrepresenting research results which could damage the trust in the journal, the professionalism of scientific authorship, and ultimately the entire scientific endeavour. Maintaining integrity of the research and its presentation is helped by following the rules of good scientific practice, which include*:

The manuscript should not be submitted to more than one journal for simultaneous consideration.

The submitted work should be original and should not have been published elsewhere in any form or language (partially or in full), unless the new work concerns an expansion of previous work. (Please provide transparency on the re-use of material to avoid the concerns about text-recycling ('self-plagiarism').

A single study should not be split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (i.e. 'salami-slicing/publishing').

Concurrent or secondary publication is sometimes justifiable, provided certain conditions are met. Examples include: translations or a manuscript that is intended for a different group of readers.

Results should be presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation (including image based manipulation). Authors should adhere to discipline-specific rules for acquiring, selecting and processing data.

No data, text, or theories by others are presented as if they were the author's own ('plagiarism'). Proper acknowledgements to other works must be given (this includes material that is closely copied (near verbatim), summarized and/or paraphrased), quotation marks (to indicate words taken from another source) are used for verbatim copying of material, and permissions secured for material that is copyrighted.

Important note: the journal may use software to screen for plagiarism.

Authors should make sure they have permissions for the use of software, questionnaires/(web) surveys and scales in their studies (if appropriate).

Research articles and non-research articles (e.g. Opinion, Review, and Commentary articles) must cite appropriate and relevant literature in support of the claims made. Excessive and inappropriate self-citation or coordinated efforts among several authors to collectively self-cite is strongly discouraged.

Authors should avoid untrue statements about an entity (who can be an individual person or a company) or descriptions of their behavior or actions that could potentially be seen as personal attacks or allegations about that person.
Research that may be misapplied to pose a threat to public health or national security should be clearly identified in the manuscript (e.g. dual use of research). Examples include creation of harmful consequences of biological agents or toxins, disruption of immunity of vaccines, unusual hazards in the use of chemicals, weaponization of research/technology (amongst others).
Authors are strongly advised to ensure the author group, the Corresponding Author, and the order of authors are all correct at submission. Adding and/or deleting authors during the revision stages is generally not permitted, but in some cases may be warranted. Reasons for changes in authorship should be explained in detail. Please note that changes to authorship cannot be made after acceptance of a manuscript.
*All of the above are guidelines and authors need to make sure to respect third parties rights such as copyright and/or moral rights.

Upon request authors should be prepared to send relevant documentation or data in order to verify the validity of the results presented. This could be in the form of raw data, samples, records, etc. Sensitive information in the form of confidential or proprietary data is excluded.

If there is suspicion of misbehavior or alleged fraud the Journal and/or Publisher will carry out an investigation following COPE guidelines. If, after investigation, there are valid concerns, the author(s) concerned will be contacted under their given e-mail address and given an opportunity to address the issue. Depending on the situation, this may result in the Journal's and/or Publisher's implementation of the following measures, including, but not limited to:

If the manuscript is still under consideration, it may be rejected and returned to the author.
If the article has already been published online, depending on the nature and severity of the infraction:
- an erratum/correction may be placed with the article
- an expression of concern may be placed with the article
- or in severe cases retraction of the article may occur.

The reason will be given in the published erratum/correction, expression of concern or retraction note. Please note that retraction means that the article is maintained on the platform, watermarked “retracted” and the explanation for the retraction is provided in a note linked to the watermarked article.

The author’s institution may be informed

A notice of suspected transgression of ethical standards in the peer review system may be included as part of the author’s and article’s bibliographic record.

Research involving human participants, their data or biological material

Ethics approval

When reporting a study that involved human participants, their data or biological material, authors should include a statement that confirms that the study was approved (or granted exemption) by the appropriate institutional and/or national research ethics committee (including the name of the ethics committee) and certify that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. If doubt exists whether the research was conducted in accordance with the 1964 Helsinki Declaration or comparable standards, the authors must explain the reasons for their approach, and demonstrate that an independent ethics committee or institutional review board explicitly approved the doubtful aspects of the study. If a study was granted exemption from requiring ethics approval, this should also be detailed in the manuscript (including the reasons for the exemption).

Retrospective ethics approval

If a study has not been granted ethics committee approval prior to commencing, retrospective ethics approval usually cannot be obtained and it may not be possible to consider the manuscript for peer review. The decision on whether to proceed to peer review in such cases is at the Editor's discretion.

Ethics approval for retrospective studies

Although retrospective studies are conducted on already available data or biological material (for which formal consent may not be needed or is difficult to obtain) ethics approval may be required dependent on the law and the national ethical guidelines of a country. Authors should check with their institution to make sure they are complying with the specific requirements of their country.

Ethics approval for case studies

Case reports require ethics approval. Most institutions will have specific policies on this subject. Authors should check with their institution to make sure they are complying with the specific requirements of their institution and seek ethics approval where needed. Authors should be aware to secure informed consent from the individual (or parent or guardian if the participant is a minor or incapable) See also section on Informed Consent.

ANEXO C – PARECER DO COMITÊ DE ÉTICA EM PESQUISA

INSTITUTO DE EDUCAÇÃO SUPERIOR DA PARAÍBA - IESP

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: INFLUÊNCIA DE DIFERENTES TÉCNICAS DE INSTRUMENTAÇÃO, OBTURAÇÃO E CIMENTAÇÃO DE PINO NA DETECÇÃO DE TRINCAS E RESISTÊNCIA À FRATURA, EM DENTES HUMANOS UNIRRADICULARES

Pesquisador: Thayana Karla Guerra Lira dos Santos

Área Temática:

Versão: 1

CAAE: 25819019.5.0000.5184

Instituição Proponente: Instituto de Educação Superior da Paraíba - IESP

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.719.153

Apresentação do Projeto:

As trincas, microtrincas e/ou fissuras dentinárias é uma condição específica que pode influenciar a longo prazo a sobrevivência do dente afetado, em que além de diferentes técnicas instrumentação, o aparecimento de trincas tem associação também com a obturação desses canais radiculares e cimentação de pino intrarradiculares. Os dentes com trincas são considerados um desafio no diagnóstico, por causa dos variáveis sinais e sintomas apresentados, juntamente com a dificuldade de localizar as linhas de trincas. Desse modo, o objetivo desse trabalho é avaliar a presença de trincas

dentinárias em dentes humanos, após instrumentação mecânica com diferentes sistemas reciprocantes e rotatórios, obturação dos canais e cimentação de pino intrarradicular, através da Microtomografia Computadorizada e a Tomografia por Coerência Óptica e, posteriormente, avaliar a resistência à fratura desses dentes.

Objetivo da Pesquisa:

Avaliar a influência dos diferentes sistemas de instrumentação, da obturação e cimentação de pino na formação de trincas dentinárias e na resistência à fratura vertical antes e após tratamento endodôntico em dentes humanos extraídos.

Endereço: BR 230 - Estrada de Cabedelo Km14
Bairro: Cabedelo **CEP:** 58.310-000
UF: PB **Município:** CABEDELO
Telefone: (83)2106-3827 **E-mail:** comiteiesp@gmail.com

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1465892.pdf	19/11/2019 09:41:03		Aceito
Projeto Detalhado / Brochura Investigador	PROJETO_CEP.docx	19/11/2019 09:39:54	Thayana Karla Guerra Lira dos Santos	Aceito
Declaração de Instituição e Infraestrutura	MicroCT.pdf	19/11/2019 09:08:50	Thayana Karla Guerra Lira dos Santos	Aceito
Declaração de Instituição e Infraestrutura	Fisica.pdf	19/11/2019 09:08:28	Thayana Karla Guerra Lira dos Santos	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	Banco_de_dentes.pdf	19/11/2019 09:06:24	Thayana Karla Guerra Lira dos Santos	Aceito
Declaração de Instituição e Infraestrutura	iesp.pdf	19/11/2019 09:02:34	Thayana Karla Guerra Lira dos Santos	Aceito
Folha de Rosto	Folha_de_rosto.pdf	19/11/2019 09:00:57	Thayana Karla Guerra Lira dos Santos	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

CABEDELO, 22 de Novembro de 2019

Assinado por:
Karelline Izaltemberg Vasconcelos Rosenstock
(Coordenador(a))

Endereço: BR 230 - Estrada de Cabedelo Km14
Bairro: Cabedelo **CEP:** 58.310-000
UF: PB **Município:** CABEDELO
Telefone: (83)2106-3827 **E-mail:** comiteiesp@gmail.com