UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

FLAVIA DARIUS VIVACQUA

Analysis of instrumentation protocols, preparation quality and final cleaning of the root canal in mandibular molars

Análise de protocolos de instrumentação, qualidade do preparo e limpeza final do conduto radicular em molares inferiores

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Tese constituída por artigo apresentada à Faculdade de Odontologia de Bauru da Universidade de São Paulo para obtenção do título de Doutor em Ciências no Programa de Ciências Odontológicas, na área de concentração Endodontia.

Orientador: Prof. Dr. Clóvis Monteiro Bramante

Co-orientador: Prof. Dr. Marco Antônio Húngaro Duarte

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“Menor que o meu sonho não posso ser”.

Lindolf Bell

RESUMO

Este trabalho analisou por meio de micro-tomografias três protocolos de instrumentação, visando a qualidade do preparo endodôntico em raízes mesiais de molares inferiores, bem como, verificou a efetividade de protocolos finais de limpeza do canal radicular, com diferentes substâncias químicas e dispositivos de agitação nas raízes distais dos molares inferiores. Foram selecionados 100 molares inferiores, sendo 45 destes com 2 condutos mesiais distintos e todos apresentando um canal distal. Após realizado o escaneamento (SkyScan 1174v2, Bruker-MicroCT, Kontich, Belgium) e análise volumétrica inicial das raízes mesiais, estas foram divididas randomicamente em 3 grupos (n=30), conforme a ampliação do sistema Bassi-Logic final e o comprimento de trabalho adotado, sendo G25.06 /+1 mm; G35.05/ forame e G50.01/ – 1 mm. Ao final de cada instrumentação os canais mesiais foram escaneados por meio de microtomógrafo SkyScan 1174v2 e analisados quanto ao aumento de volume total e apical, centralização e desvio do preparo, e a porcentagem de paredes não tocadas total e área apical. Para comparação intra-grupo foi usado o teste de Wilcoxon e na análise entre os grupos o teste Kruskal Wallis e Dunn, ($p<0,05$). Considerando-se as raízes distais, todos os canais foram instrumentados com o instrumento R50 (Reciproc®) até o forame, e divididos em 10 grupos (n=10): G1(CHX+Escova intra-canal); G2 (CHX+EasyClean); G3(CHX+Irrisonic); G4(CHX/Escova intra-canal + EasyClean); G5(CHX/Escova intra-canal + Irrisonic); G6(NaOCL/Escova intra-canal); G7(NaOCL/EasyClean); G8(NaOCl/Irissonic); G9(NaOCl/Escova intra-canal + EasyClean) e G10 (NaOCL/Escova intra-canal + Irrisonic). Foram realizados 3 ciclos de agitação de 20 segundos, com 2 mL de cada substância química, em cada ciclo. As raízes foram clivadas e observadas no Microscópio Eletrônico de Varredura (MEV), sendo feitas 3 imagens, referentes a cada terço do canal, com 1000x de aumento. Estas, foram analisadas por 2 pesquisadores cegos e estabelecidos escores (0-3) para cada terço, referentes a limpeza e exposição de túbulos dentinários. Na análise entre os grupos, foi utilizado o teste de Kruskal-Wallis, e na análise intra-grupo, o teste de Friedman e Dunn ($p<0,05$). Na análise dos canais mesiais, houve diferença estatística no volume total entre o G25.06/+1 mm e os demais grupos ($p<0,05$). No terço apical, houve diferença estatística somente entre G25.06/+1mm e G50.01/-1mm ($p<0,05$). Não foram encontradas diferenças

estatísticas entre os grupos em relação a centralização e desvio do preparo, e também não houve diferença estatística quanto à porcentagem de paredes não tocadas do canal durante a instrumentação, total ou apical($p>0,05$). Na análise nas raízes distais, não houve diferença estatística entre os grupos e nem entre os terços ($p>0,05$). Na análise intra-grupo, foi observada diferença estatística entre os terços cervical e apical nos grupos G4, G5 e G6 ($p<0,05$); e entre os terços médio e apical somente no G2 ($p<0,05$). Assim, este trabalho observou que o preparo dos canais mesiais, com limas de maior calibre de ponta e menor conicidade, mantiveram o preparo centralizado, realizando um desgaste de dentina apical seguro, sem ocasionar em desgastes cervicais severos. Também se verificou, nos condutos distais, que nenhum dos protocolos de limpeza final, promoveram uma limpeza completa no canal radicular, e em todos os grupos, o terço apical mostrou-se mais crítico em relação à limpeza.

Palavras-chave: Endodontia. Preparo do canal radicular. Microtomografia computadorizada. Microscopia Eletrônica de varredura.

ABSTRACT

Analysis of Instrumentation Protocols, preparation quality and final cleaning of the root canal in mandibular molars

This work analyzed three instrumentation protocols using micro-tomography, aiming at the quality of endodontic preparation in mesial roots, as well as, verified the effectiveness of final root canal cleaning protocols, with different chemical substances and agitation devices in distal roots. A hundred mandibular molars were selected, 45 of which had 2 distinct mesial canals and all had a distal canal. After scanning escaneamento (SkyScan 1174v2, Bruker-MicroCT, Kontich, Belgium) and volumetric analysis of the mesial roots, they were randomly divided into 3 groups, according to the enlargement of Bassi-Logic file and the working length: G25.06 / + 1 mm; G35.05 / foramen and G50.01 / - 1 mm. The mesial canals were scanned using micro-tomography SkyScan 1174v2 and analyzed for increase in total and apical volume, centralization and deviation of the preparation, and the percentage of total and apical untouched walls. For intra-group comparison, the Wilcoxon test was used and in the analysis between groups, the Kruskal Wallis and Dunn test ($p < 0.05$). Considering the distal roots, all canals were instrumented with the R50 file and divided into 10 groups according to antimicrobial irrigant and agitation device as follows: 1) CHX + Canal brush, 2) CHX + EasyClean, 3) CHX + Irrisonic, 4) CHX + Canal 1) CHX + Canal brush, 2) CHX + EasyClean, 3) CHX + Irrisonic, 4) CHX + Canal brush + EasyClean, 5) CHX + Canal brush + Irrisonic, 6) NaOCl + Canal brush, 7) NaOCl + EasyClean, 8) NaOCl + Irissonic, 9) NaOCl + Canal brush + EasyClean, and 10) NaOCl + Canal brush + Irrisonic. EDTA 17% was applied as final chelator irrigant in all protocols. Three cycles of 20s agitation were performed, with 2 mL of each irrigant. After completing the final cleaning protocols described, the samples were dried with medium-sized paper points and the roots were longitudinally sectioned for scanning electron microscopy (SEM) analysis of dentin surface. Cleaning of surfaces were assigned to scores (0-3) for quantitative evaluation. Statistical analysis was performed by Kruskal-Wallis and Dunn'S test ($p<0.05$). In the mesial root canals analysis, there was a statistical difference in the total volume between the G25.06 / + 1 mm and the other groups ($p <0.05$). In the apical third, there was a statistical difference between G25.06 / + 1 mm

and G50.01 / -1 mm ($p < 0.05$). No statistical differences were found between the groups regarding centralization and deviation of the preparation, and no statistical difference regarding the percentage of untouched walls of the canal, total or apical ($p > 0.05$). In the distal analysis, the irrigation protocols CHX+Canal brush+EasyClean, CHX+Canal brush+Irrisonic and NaOCl+Canal brush presented statistical difference between cervical and apical third and the middle and apical thirds in CHX+EasyClean. This work observed that the preparation of the mesial canals, with files of a higher tip and less taper, kept the preparation centralized, making a safe apical dentin wear, without causing severe cervical wear. We also found that none of the final cleaning distal protocols performed, did not promoted a thorough cleaning the root canal, the apical third was more critical in relation to cleaning in all groups.

Keywords: Endodontics. Root canal preparation. Microcomputed tomography. Scanning electron microscopy.

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LISTA DE ABREVIATURA E SIGLAS

CHX	Clorexidina
EDTA	Ácido etilenodiamino tetra-acético
MEV	Microscopia Eletrônica de Varredura
Micro-CT	Microtomografia Computadorizada
NaOCl	Hipoclorito de Sódio
NiTi	Níquel-Titânio
CM	Controle de Memória
PUI	Irrigação Passiva Ultrasônica

LISTA DE SÍMBOLOS

+	mais
mm	milímetro
-	menos
<	menor
=	igual
mL	mililitros
x	vezes
>	maior
rpm	rotações por minuto
N	Newton
µm	Micrômetro

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1 INTRODUÇÃO

1 INTRODUÇÃO

A Endodontia vem se beneficiando de avanços tecnológicos importantes que ocorreram nos últimos anos, principalmente no que tange aos protocolos clínicos, favorecendo de forma importante o preparo biomecânico, aumentando assim a previsibilidade do tratamento endodôntico. Tratamentos que favoreçam uma maior limpeza, remoção de dentina infectada e redução eficaz de microrganismos, contribuem para o sucesso endodôntico e reduzem as chances de reinfecções (SIQUEIRA JR *et al*., 2018).

Dentre os novos recursos utilizados, o preparo de condutos com instrumentação rotatória, reciprocante e mista acionadas por motores elétricos, representam um significativo avanço no tratamento endodôntico. Os instrumentos endodônticas, acionados por motores elétricos, fabricadas em níquel titânio (NiTi), com tratamentos térmicos diferenciados, apresentam inúmeras vantagens em relação às anteriormente utilizadas, em aço inoxidável, contribuindo principalmente com uma maior flexibilidade, mantendo a eficiência de corte na dentina, e apresentando uma maior resistência à fratura (BAHIA *et al*., 2005; AL-HADLAQ *et al*., 2010; NINAN; BERZINS, 2013; SHEN *et al*., 2013). Devido a estas propriedades, houve também uma significativa melhora durante a instrumentação, em relação à manutenção da morfologia original do canal, com menores probabilidades de desvios e consequente preservação do forame apical (AL-HADLAQ *et al*., 2010; PEREIRA *et al*., 2012).

Para Peters (2001) um dos principais objetivos do tratamento endodôntico é manter a integridade das estruturas radiculares, não modificando o trajeto do canal original. No entanto, apesar das evoluções obtidas no preparo biomecânico, a busca pela maior redução de microrganismos ainda é um desafio, principalmente devido a complexidade anatômica dos canais radiculares. Mesmo quando considera-se o canal principal, os instrumentos têm demonstrado não conseguir instrumentar todas as regiões, deixando uma porcentagem bastante expressiva de paredes não tocadas (PETERS *et al*., 2001; PAQUÉ *et al*., 2010). Esta limpeza ineficiente pode ser adivinda, segundo Siqueira Jr (2018), da instrumentação do canal com limas de baixa conicidade e ponta, gerando preparos muito conservadores, ou muito semelhantes ao original, não ocorrendo a remoção do biofilme bacteriano das paredes do canal.

Visando buscar melhorias no preparo do canal radicular, observa-se na literatura que a ampliação da porção apical, com limas de maior calibre (ponta e/ou conicidade) possa contribuir para reduzir esta porcentagem de áreas não instrumentadas dentro do canal. Contudo, a literatura ainda não é clara em determinar qual a ampliação do canal e do terço apical seriam ideais para se alcançar tal êxito (SAINI *et al*., 2012; AMINOSHARIAE; KULILD, 2015; PERÉZ *et al*., 2018; LIMA *et al*., 2020).

Resultados bastante expressivos, foram encontrados em trabalhos que avaliaram a relação entre o diâmetro apical e a maior penetração do irrigante, a redução de microrganismos e aumento de toque às paredes, e estes verificaram existir uma relação entre a maior ampliação desta região, para a obtenção de melhorias do preparo químico-mecânico (AKHLAGHI *et al*., 2014, PERÉZ *et al*., 2018, BUTCHER *et al*., 2019). Contudo, estudos clínicos realizando a ampliação foraminal tem demonstrado a ocorrência de dor pós operatória nas primeiras horas após o tratamento (CRUZ-JÚNIOR *et al*., 2016, YAYLALI *et al.,* 2017). Devido a isto, ainda há alguma falta de consenso, considerando o limite e a extensão da ampliação apical que seriam suficientemente necessárias, para uma menor porcentagem de paredes não tocadas, maior remoção de dentina contaminada no canal como um todo, principalmente na porção apical, e que também, não ocasionasse em uma remoção excessiva de dentina no terço cervical.

É de suma importância que trabalhos que avaliem protocolos de instrumetação com limas endodônticas, sejam executados e avaliados por métodos confiáveis. Dentre as metodologias utilizadas para este fim, a Microtomografia Computadorizada (Micro-CT), tem se mostrado muito eficaz (RHODES *et al*., 2000; BERGMANS *et al*., 2003). Por se tratar de uma metodologia de análise não destrutiva, possibilitou a obtenção e a avaliação de maneira mais real e fidedigna dos protocolos testados, quantificando o volume de dentina original e após a preparação do canal radicular com diferentes ampliações e formatos (PAQUÉ *et al*., 2010, VERSIANI *et al.,* 2013), auxiliando assim no estabelecimento das melhores abordagens clínicas a serem implementadas na Endodontia

Contudo, mesmo com os recentes avanços na instrumentação, a ação dos instrumentos na dentina radicular, sejam estes, manuais ou mecanizados, durante o preparo químico-mecânico, produzem raspas e debris e estes, acabam recobrindo as paredes do canal, depositando-se ao longo do leito do canal principal e principalmente em áreas de achatamentos, istmos, irregularidades e ramificações anatômicas (PAQUÉ; BOESSLER; ZEHNDER, 2011; KAMEL; KATAIA, 2014; MENDODNÇA *et al*., 2015; SCHMIDT *et al*., 2015, PRADO *et al.,* 2016; DUQUE *et al*., 2017). Esta camada, denominada lama dentinária ou "smear layer", constitui-se de elementos orgânicos e inorgânicos, podendo também conter bactérias e seus subprodutos (Prado et al., 2016), vindo a ser um importante fator de interferência na qualidade do tratamento endodôntico ou mesmo aumentando as chances de reinfecção (PAQUÉ; BOESSLER; ZEHNDER, 2011; KAMEL; KATAIA, 2014; SCHMIDT *et al*., 2015).

Como não é possível alcançarmos todas estas regiões anatômicas dos canais radiculares, com a ação mecânicas dos instrumentos (PETERS *et al*., 2001; PERÉZ *et al.,* 2018; VERSIANI *et al.,* 2018), se faz necessário o uso de substâncias químicas que tenham ação antimicrobiana comprovadas. Porém, já é conhecido que o uso somente de seringas e agulhas, de irrigação, para levar estas substâncias ao canal, é ineficiente para a remoção desta camada (KAMEL; KATAIA, 2014; SCHMIDT *et al*., 2015), e que a ampliação do canal, tem influência direta na penetração do irrigante principalmente na porção apical (CARON *et al*., 2010; SRIKANTH *et al*., 2015). A literatura tem ressaltado ainda, que o uso de irrigantes, com propriedades antimicrobianas (JUSTO *et al*., 2014; ZARGAR *et al*., 2015) associados à dispositivos de agitação, têm potencial de aumentar o contato destas substâncias com as paredes do canal, favorecendo assim a limpeza dessa camada (VAN DER SLUIS *et al*., 2010; PRADO *et al*., 2016; DUQUE *et al*., 2017; LEONI *et al*., 2017).

Atualmente, é recomendada uma sequência de irrigação final do canal com um agente quelante, como o ácido etilenodiamino tetra-acético (EDTA) e hipoclorito de sódio (NaOCl) para a remoção da camada de *smear layer*, porém diversos protocolos fazendo uso de dispositivos de agitação estão sendo estudados, obtendo bons resultados, como o uso de pontas de ultrassom, Easy Clean, limas ajustáveis (VAN DER SLUIS *et al*., 2010; KATO *et al*., 2016; LEONI *et al*., 2017; SILVA *et al*, 2018) e escovas intra-canais (KAMEL; KATAIA, 2014). Contudo, protocolos que levem em consideração a variação da substância química, e o uso isolado ou complementar dos dispositivos de agitação, ainda apresentam resultados contraditórios na literatura recente.

A microscopia eletrônica de varredura (MEV) provou ser um método valioso de comparação dos remanescentes de tecido e da camada de smear layer na parede do canal radicular. Este método permite a avaliação dos detalhes morfológicos das superfícies do canal radicular preparados após a instrumentação e uso de protocolos finais de limpeza com diferentes instrumentos e irrigantes (TORABINEJAD *et al*., 2003; YANG *et al.,* 2008).

Em função do exposto, fica evidente a necessidade e a importância de mais estudos que avaliem protocolos de instrumentação e limpeza, buscando trazer melhorias no preparo biomecânico, e no processo de desinfecção dos canais radiculares. Frente a isso, este estudo teve como objetivo avaliar por meio de Micro-CT, a qualidade do preparo de três protocolos de instrumentação endodôntica em raízes mesiais de molares inferiores, considerando a ampliação apical e o comprimento de trabalho, bem como, observar por meio da microscopia eletrônica de varredura, a remoção da "smear layer", após a implementação de protocolos de toalete final, nos canais distais de molares inferiores considerando a variação da substância química e o uso de dispositivos de agitação de maneira isolada ou combinada.

2 ARTIGOS

2 ARTIGOS

2.1 ARTIGO 1

The article presented in this thesis was written according to **The Journal of Endodontics** instruction and guidelines for article submission

Analysis of Instrumentation Protocols Regarding the Quality of Mesial Canal Preparation in Mandibular Molars – A Micro-CT study.

Flavia Darius Vivacqua, DDS, MS, PhD Sudent,*
Marco Antonio Hungaro Duarte, DDS, MS, PhD,☨
Rodrigo Ricci Vivan, DDS, MS, PhD,☨
Murilo Priori Alcalde, DDS, MS, PhD,‡
Renan Furlan DDS, MS, PhD Sudent *
Clovis Monteiro Bramante, DDS, PhD,☨

From the * Department of Dentistry, Endodontics and Dental Materials, Bauru School of Dentistry, University of São Paulo, Bauru, São Paulo, Brazil; ☨ Department of Dentistry, Endodontics and Dental Materials, Bauru School of Dentistry, University of São Paulo, Bauru, São Paulo, Brazil; and ‡ Department of Health Science, University of the Sacred Heart, Bauru, São Paulo, Brazil

Analysis of Instrumentation Protocols Regarding the Quality of Mesial Canal Preparation in Mandibular Molars – A Micro-CT Study

ABSTRACT

Introduction: This study aimed to analyze by the micro-computed tomography, the quality of the endodontic preparation of mesial canals in mandibular molars provided by three instrumentation protocols. **Methods:** Forty-five extracted mandibular molars with 2 independent mesial canals were selected and an initial micro-computed tomography was performed. The initial volume values of the canals were submitted to statistical analysis for the paired division. The groups were determined according to the canal final enlargement and the work length adopted: G25.06 /+1 mm; G35.05/foramen and G50.01/–1 mm. At the end of each instrumentation, the root canals were scanned and analyzed regarding the increase in total and apical volume, centralization and preparation deviation, and the percentage of total and apical uninstrumented walls. **Results:** For the intragroup comparison, the Wilcoxon test was employed, and for the intergroup analysis, the Kruskal Wallis and Dunn test was used, ($p<0.05$). In the analysis of canal total volume, statistical difference was found between G25.06/+1 mm and the remaining groups ($P<0.05$). In the apical third, statistical difference was observed between G25.06/+1 mm and G50.01/-1 mm ($P<0.05$). No statistical difference was found between the groups in terms of centralization and deviation of the preparation or in terms of the percentage of non-instrumented walls, total or apical. **Conclusions:** The preparation of mesial canals, of mandibular molars up to larger tip files, but with lower taper at 1mm before the foramen resulted in greater volume of apical preparation, kept the preparation centralized, providing safe apical dentin wearing without excessive cervical wearing.

KEY WORDS

Endodontics; increased apical enlargement; micro-computed tomography; root canal preparation

INTRODUCTION

Endodontics has gone through important technological advancements, however, treatment protocols that favor better cleaning and efficient reduction of microorganisms, tend to be more successful and reduce the risk of reinfection[1].

According to some studies[2-4], the instruments used in electrical motors, with thermal treatment, present several advantagens when compared to the previous stainless steel files, contributing to the procedures for presenting greater flexibility, high cutting efficacy and improvement of fracture resistance[5,6]. Because of this, these instruments provide a better maintenance of the morphology of the canal, resulting in a more centralized preparation and consequent preservation of the apical foramen [5,7].

A more efficient microbial reduction is still a challenge, mainly due to the anatomy of the root canal. Even in the main canal, the instruments have not been able to reach all the walls[8,9]. Probably because, the preparation of the canal being carried out with final instruments smaller than the canal initial diameter, that is, very conservative preparations result in the non-removal of the bacterial biofilm and in deficient cleaning[1].

The hypotheses that the size of the apical preparation might be important to achieve such improvement has been studied, as well as the determination of the ideal apical third enlargement[10-13]. Studies evaluating the influence of the apical size with the irrigating penetration, reduction in microorganisms and wall treatment have demonstrated expressive results in relation to the enlargement of this area[12,14-16]. Some studies that had performing the enlargement of the foramen have demonstrated the occurrence of post-operative pain in the first few hours after treatment[17,18]. There still some lack of consensus regarding the limit and extension of the apical enlargement that could result in a lower percentage of uninstrumented walls and greater dentin removal in the apical third[9]

This study aimed to evaluate the quality of three distinct instrumentation protocols using micro-CT, it was taking into consideration apical diameter and the work length adopted. The null hypothesis is that the instrumentation protocols present the same canal volume increase in its total extension and in the apical third, the same centralization capacity at the cervical and apical third, as well as similar percentage of walls untouched by the instrument.

MATERIALS AND METHODS

For the sample calculation the G*Power v3.1 for Mac (Heinrich Heine, Universität Düsseldorf) was used and the Wilcoxon-Mann Whitney test of the T test family was selected. The data of a previous study that evaluated the root canal preparation with curved canal[19] was used and the effect size in the present study was established (=0.88). The alpha type error of 0.05, a beta power of 0.95, and a ratio N2/N1 of 1 were also stipulated. A total of 30 canals per group were indicated as the ideal size required for noting significant differences.

After approval by the ethics committee research, ninety human permanent mesial canals of forty-five mandibular molars were selected. The specimens had been presented full root formation and curves of up to 30° according to Schneider (1971). The forty-five mandibular molars were scanned using the micro-CT system (SkyScan 1174v2, Bruker-MicroCT, Kontich, Belgium) to confirm Vertucci's Type IV Classification.

Micro-CT analysis and sample division

The teeth were scanned as follows: 19 µm voxel size, 50 kV, 800 µA, and 0,8° step-size rotation using a 1024x1304 resolution. The images were reconstructed using a specific program (NREcon v.1.6.9; Bruker – MicroCT) and stored with the axial cut, in the BMP format. These images were used to observe the anatomical configuration, and the initial volumetric analysis was carried out using the program CTAn v1.12 (Bruker – MicroCT). The initial volumes were submitted to the Kruskal-Wallis and Dunn statistical test for the sample pairing and random division of the 3 groups (n=30), no statistical difference was found between the initial volume values of the three groups ($p>0.05$).

Sample preparation and group division

After accessing the canal using a high rotation spherical diamond tip FG 1014 (KG Sorensen, Cotia, Brazil) and the ENDO ZK bur (JET, France), the patency was performed with a C-pilot #15.02 file (Dentsply VDW, Munich, Germany), and the canal length measured with file visualization in the foramen using the surgical microscope (ALLIANCE UNIQUE, São Carlos, Brazil).

Three instrumentation protocols were employed, considering the apical enlargement file and the work length used. The files used were Bassi Logic (EASY Equipamentos Odontológicos, Belo Horizonte, Brazil) #25/.06, #35/.05 and #50/.01. The full length of the canals up to the foramen, 1 mm beyond the foramen (+ 1 mm) and 1mm before the foramen (-1mm) was considered, which determinated 3 groups: G25.06/ +1 mm; G35.05/foramen and G50.01/ - 1 mm.

Root canal preparation

The instrumentation was started with the Bassi Logic #25/.06 file, in each work length of the group, Bassi Logic #35/.05 for the groups G35.05/foramen and G50.01/-1 mm and only the G50.01/-1 mm group used the Bassi Logic #50/.01 file. At the end of each instrumentation, new scanning was carried out and reconstructed using the same parameters previously described.

The whole instrumentation was carried out by a single operator, using motor ENDO SI (EASY Equipamentos Odontológicos, Belo Horizonte, Brazil) with 600 rpm 2 N for the #25/.06 and #35/.05 files; and 350 rpm and 1.5 N for the #50/.01 file**.** The instrumentation was performed with constant irrigation/aspiration, with 2.5% sodium hypochlorite and 5 mL volume per canal.

Volume analysis

The mesial canals were analyzed regarding the percentage of total volume increase, measured from the foramen up to 1mm below the furcation area, and in the apical 5 mm, which were considered from the foramen in a coronal direction in the CTAn v1.12 software (Bruker – microCT).

Canal transportation and centering ability

The measurements of centralization and transport considered apical 4 mm, from the foramen in the coronal direction, with 1 mm intervals, and cervical 4 mm, from the furcation area in the apical direction, with the same 1mm interval. The formulas used to calculate the canal transportation and centering ability were as follows: (m1-m2)-(d1-d2) and (m1-m2)/(d1-d2) or (d1-d2)/(m1-m2), respectively[20].

Percentage of uninstrumented walls

In this analysis, the entire length of the canal and the apical 4 mm were considered, measured in the same way as previously described in the volume analysis. All analyzes were performed using the CTAn v1.12 software (Bruker - microCT).

Statistical analysis

The results obtained of volume, centralization, transport, and percentage of uninstrumented areas were evaluated regarding their normality using the Kolmogorov-Smirnov test, which revealed an abnormal distribution. For this reason, in the intragroup comparison, the Wilcoxon test was employed and in the intergroup analysis, the Kruskal Wallis and Dunn test was used. The significance level was 5% ($P < .05$), and the statistical program used was GraphPad Prism version 8.4.3.

RESULTS

Considering the initial total volume and after each preparation protocol (dentin removal) within the same group, significant statistical difference ($P < .05$) was observed in all groups. The same way, in the apical volume, in the intragroup comparison (Table 1).

In the intergroup analysis, of the total volume, statistical difference ($P <0.05$) was observed between G25.06 / + 1 mm and the other groups. There was no difference between G35.05 / foramen and G50.01 / -1 mm (Table 1). In the intergroup analysis, of the apical volume, a statistically significant difference was found in the comparison between G25.06 / + 1 mm and G50.01 / -1 mm ($P <0.05$).

No statistical difference was observed in relation to the preparation centralization or in relation to transport, between groups, in the cervical 4 mm or in the apical 4 mm (Table 2).

The percentage of uninstrumented walls during instrumentation, no significant statistical difference ($P > .05$) was observed in the canal full extension or in the apical third between the groups under analysis (Table 2). Figure 1 shows micro-CT representative images of the groups studied.

DISCUSSION

This Micro-CT study analyzed the quality of 3 different instrumentation protocols, in mesial mandibular molars. The null hypothesis was partially rejected, since there was difference in relation to the percentage of increased canal volume between the groups under analysis.

Mesial canals of mandibular molars, are the root canals most commonly observed clinically and used in studies[21,22], have a challenging anatomy, causing difficulty in choosing the final apical file[23]. Due to anatomical variation, the diameter of the canal must be measured properly and before chemical-mechanical preparation, aiming to personalize the instrumentation and avoid excessive wear[24].

Canal debridement or enlargement techniques do not guarantee the elimination of bacteria during the root canal treatment[11]. However, larger apical dilations have been suggested as a way of increasing the efficacy of the canal cleaning and disinfection[10,16]. The inefficacy of the preparation might result from conservative apical instrumentations, whose consequence is the great amount of bacteria and necrotic tissue in the canal[1].

Recent studies have pointed out the importance of larger apical preparations, aiming at greater cleaning, disinfection and penetration of cement [16,24-26], in addition to better removal of contaminated dentin, and increasing the action of irrigants in the apical region[10,27].

This study considered 3 work lengths, and 3 final apical enlargements: G25.06/+ 1mm, G35.05/foramen and 50.01/- 1 mm. In the total and apical volume analysis, within the same group, relevant statistical difference was observed in all instrumentations, indicating dentin removal and increased canal diameter in all phases.

In the total volume, the protocol 35.05 / foramen and the protocol 50.01 / - 1mm, obtained a percentage increase in volume significantly greater than that found in the protocol 25.06 / + 1mm. Previous studies already demonstrated that the apical enlargement over the diameter #35, 1 mm before the foramen favors a higher percentage of instrumented areas and greater decontamination of the root canal[5,16].

The literature already suggests that apical enlargement with diameters above # 35 may improve the cleaning and disinfection of the root canal system[14,15,24,26,28]. However, the ideal apical dilation and the instrumentation limit have still been widely discussed[18,29]. Some authors have suggested that determining the initial apical diameter and enlarging it with 3 to 4 larger instruments, would be ideal[5,10,12,19].

Other studies also observed that apical enlargement over #35, reduced the total amount of contaminated debris by around 30%[27,28]. The fact that the greater apical enlargement provided less debris may be related to better irrigation of the apical third. One study, with apical patency, with file # 10 and enlargement up to 40 / .06, the irrigant reached the apical portion even more[30].

Enlargements with files between # 40 and # 50 have also been reported, promoting better removal of contaminated material, apical cleaning[5,6,12,19,28], in addition to improvements in irrigation [16]. Statistical difference was found in the removal of the canal dentin (total volume), between G25.06/+1 mm and the other groups. No difference was found between G35.05/foramen and G50.01/-1 mm. This might be explained, because the canals in G50.01/-1 mm had been previously instrumented with the 35.05 file, which resulted in a #40 diameter at 1mm before the foramen.

In the apical third, volume difference was observed between G25.06/+1 mm and G50.01/-1 mm. Demonstrating that working with instruments with a smaller tip and greater taper, 1 mm beyond the foramen does not remove more dentin than, using the same, 1 mm below the foramen. The enlargement of the foramen does not provide more cleaning to the apical third and may increase the risk of overflow of irrigating solutions and filling material[29]. Similar results were found by other authors, using the same working length[6,11,26].

Anatomical studies have shown that root canals are, mostly, irregular and with an oval cross section, and this might result in irregular preparation, removing dentin only from one of the sides [31]. Analyzing the cervical and the apical third regarding deviation and transport of canals, no estatistical difference was found in the groups tested. Due to the heat treatment of the memory control (CM) type found in the files used in the study, these are very flexible[4,6,7], which contributes to the centralization of the preparation, since the increase in the instrument tip was compensated with taper reduction that prevented canal deviation or transport. These factors are relevant, since differentiated thermal treatments and smaller taper and tip diameter aim at allowing better adaptation of the instrument to the canal anatomy[13,32], preserving the thickness of the dentin in the cervical region[32,33]

There was no statistical difference between the 3 protocols in the percentage of non-instrumented area, in relation to the total canal or apical third. Similar results were found in other studies with the same methodology and dental group [9,22,34]. Some authors have observing a reduction of the non-instrumented areas with the increase of the apical diameter[21,35]. Although the result is not significant, the group 50.01 / - 1mm reached 24.7% more canals walls than the group 25.06 / + 1mm, and presented 34.5% more surface touched when compared to the 35.05 / foramen, which should clinically result in greater cleaning and decontamination.

Within the limitations of this study and its application in the clinical environment, the authors emphasize that the apical enlargement with thermically treated nickel-titanium files, with larger tip diameters and smaller taper, 1mm before the foramen, presents great potential to promote the removal of the contaminated dentin in the apical region.

CONCLUSIONS

The preparation of the mesial canals of the lower molars with files of larger tips and smaller taper, 1mm below the foramen, resulted in a safe apical tooth wear, keeping the preparation centralized and without excessive cervical wear.

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TABLE 1 - Median, minimum and maximum values of the initial, final volume and percentage of increase in the total and apical volume of the canal according to the instrumentation protocol.

	Total volume (mm^3)			Apical volume		
Group	**Initial**	**Final**	**%increase**	**Initial**	**Final**	**%increase**
G25.06/+1 mm	1.98 [A] (0.8 – 3.9)	3,18 [B] (1.6-4.7)	48.50[b] (1.0-190)	0.45 [A] (0.0 – 0.9)	0.78 [B] (0.3-1.2)	59.0[b] (0-591.0)
G35.05/Foramen	1.76 [A] (1.7 – 5.2)	3.50 [B] (1.7-6.3)	87.10[a] (32.9-218.0)	0.43 [A] (0.4-1.2)	0.95 [B] (0.6-1.7)	125.0[a,b] (30.30-564.9)
G50.01/-1 mm	1.97 [A] (2.1- 5.7)	4.10 [B] (2.3-5.9)	95.15[a] (0.0-296.7)	0.34 [A] (0.4-1.8)	1.24 [B] (0.7-1.9)	190.2[a] (0.1- 113.0)

Different lowercase letters indicate statistically significant differences (Kruskal Wallis test, $P < 0.05$)

TABLE 2 - Median, minimum and maximum values of 4 mm cervical and 4 mm apical centralization and the total and apical uninstrumented area (%) of each group.

	Cervical Centralization (mm from furcation)				Apical Centralization (mm from foramen)				Uninstrumented area (%)	
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**Total**	**Apical**
G25.06/+1 mm	0.48 (0.0-0.9)	0.50 (0.0-1.0)	0.36 (0.0-0.9)	0.56 (0.0-1.0)	0.32 (0.0-0.9)	0.48 (0.0-1.0)	0.45 (0.0-1.0)	0.43 (0.0-0.9)	27,86 (0.0-81.9)	43.63 (0.0-98.0)
G35.05/Foramen	0.54 (0.0-0.9)	0.41 (0.0-0.9)	0.38 (0.0-0.9)	0.56 (0.0-0.9)	0.51 (0.0-0.9)	0,35 (0.0-0.9)	0.49 (0.0-0.9)	0.45 (0.0-0.9)	29.46 (0.0 –94.1)	53.38 (0.0-99.8)
G50.01/-1 mm	0.37 (0.0-0.9)	0.37 (0.0-0.9)	0.51 (0.0-0.9)	0.63 (0.1-0.9)	0.27 (0.0-0.9)	0.51 (0.0-0.9)	0.45 (0.1-0.8)	0.55 (0.0-1.0)	26.21 (0.0- 90.1)	18.88 (0.0-90.3)

No significant difference was found at all tested levels ($P > .05$).

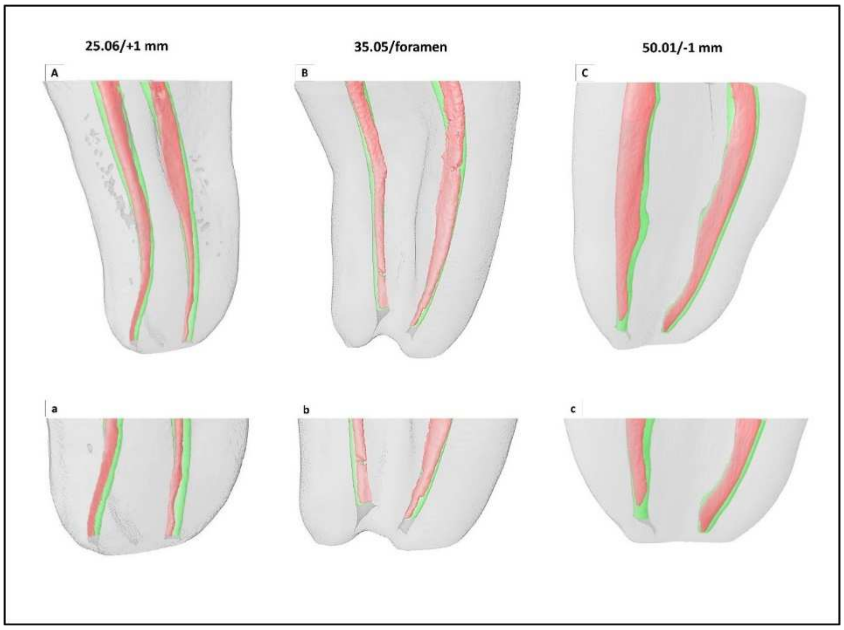

FIGURE 1 - Representative of superimposed micro-computed tomographic images of the mesial canals of mandibular molars taken before (red) and after (green) the instrumentation protocols of each group. Uppercase letters represent the mesial canals along the entire length, lowercase letters represent the apical third.

2.2 ARTIGO 2

The article presented in this tese was written according to **The Bulletin of Tokyo Dental College** instruction and guidelines for article submission

Efficacy of different protocols for root canal irrigation on ultrastructural assessment of molars' distal root canal cleaning

Effectiveness of cleaning distal root canals

Flavia Darius Vivacqua, DDS, MS[1]; Marco Antonio Hungaro Duarte, DDS, MS, PhD[1]; Rodrigo Ricci Vivan, DDS, MS, PhD[1]; Murilo Priori Alcalde, DDS, MS, PhD[2]; Tainah Oliveira Rifane DDS student [3],Victor Pinheiro Feitosa DDS, PhD[3] and Clovis Monteiro Bramante, DDS, PhD[1]

[1] Department of Dentistry, Endodontics and Dental Materials, Bauru School of Dentistry, University of São Paulo, Bauru, São Paulo, Brazil

[2] Department of Health Science, University of the Sacred Heart, Bauru, São Paulo, Brazil.

[3] Department of Dentistry, Paulo Picanço School of Dentistry, Fortaleza, CE, Brazil.

Abstract

The aim of the study was to analyze irrigation solutions, chlorhexidine gel 2% vs sodium hypochlorite 2.5% in cleaning root canals associated with different cleaning protocols. One hundred distal canals from extracted lower molars were instrumented with R50 system and divided into 10 groups according to antimicrobial irrigant and agitation device as follows: 1) CHX + Canal brush, 2) CHX + EasyClean, 3) CHX + Irrisonic, 4) CHX + Canal brush + EasyClean, 5) CHX + Canal brush + Irrisonic, 6) NaOCl + Canal brush, 7) NaOCl + EasyClean, 8) NaOCl + Irissonic, 9) NaOCl + Canal brush + EasyClean, and 10) NaOCl + Canal brush + Irrisonic. EDTA 17% was applied as final chelator irrigant in all protocols. Three cycles of 20s agitation were performed, with 2 mL of each irrigant. After completing the final cleaning protocols described, the samples were dried with medium-sized paper points and the roots were longitudinally sectioned for scanning electron microscopy (SEM) analysis of dentin surface. Cleaning of surfaces were assigned to scores for quantitative evaluation. Statistical analysis was performed by Kruskal-Wallis and Dunn'S test ($p<0.05$). The irrigation protocols CHX+Canal brush+EasyClean, CHX+Canal brush+Irrisonic and NaOCl+Canal brush presented statistical difference and between the middle and apical thirds in CHX+EasyClean. In conclusion, none of the protocols ensured effective cleaning of the root canal, and in all groups the apical portion was more critical to clean. Therefore, future studies are needed.

Keywords Chlorhexidine; endodontic; root canal; smear layer; sodium hypochlorite.

Introduction

The mechanical action of manual or mechanized instruments, along the walls of the root canal during chemical-mechanical preparation, produces scrapings and debris, and these are deposited along the main canal and in its anatomical complexities [1, 2, 3, 4, 5,6], forming a thick layer, called "smear layer" [7,8]. This layer, when not removed, can interfere in the penetration of intra-canal medication, as well as in the sealing action of the root canal sealer, or even in the perpetuation of intra-radicular infections [4], having the potential to cause endodontic failure [9,10].

As it is not possible to reach all these regions with the mechanical action of the instruments [11,12], it is necessary to use chemical substances, with disinfectant and lubricant action, and cleaning agents, helping to eliminate and neutralize bacteria and their by-products [13,14]. However, it is already known that the use of conventional irrigation is inefficient for debris removal [2,4], and that enlargement of the apical third affects directly the penetration of the irrigating agent to reach its full working length [9,15]. Therefore, the antimicrobial action of the irrigating solution [14,16] in association with devices that improve the irrigation is fundamental [5,6,7,17].

The anatomy of the distal canals, in the vast majority, presents only a root canal, wide, but quite flattened, often in the form of a tape, with mesial and distal poles of difficult access for instrumentation. Manual or mechanized action just in the canal central area, and the regions that are without instrumentation, around 35%, require solid strategies, aiming at complementing this preparation [14].

Several protocols using agitation devices are being studied, obtaining good results, and the use of ultrasound inserts, plastic devices, adjustable files [7,8,17,18] and canal brushes [2] are among them. However, there are no studies in the literature evaluating the use of these devices separately or in association with different irrigation solutions in the cleaning of root canals.

This study aimed to evaluate final root canal cleaning protocols by scanning electron microscopy, using the chemicals Sodium Hypochlorite 2.5% (NaOCl) and Chlorhexidine gel 2% (CHX) and Ethylenediaminotetraacetic Acid 17% (EDTA) associated with the irrisonic agitation devices (Helse Dental Technology, Santa Rosa de Viterbo, São Paulo, Brazil), Easy Clean (EASY Dental Equipment, Belo Horizonte, Minas Gerais, Brazil), and Intracanal Brush (MK Life, Porto Alegre, Rio Grande do Sul, Brazil), used individually or jointly for improvements in the smear layer removal process. The null hypothesis considered was that all protocols present similar cleaning ability.

Materials and Methods

This research was approved by the Human Research Ethics Committee.

A hundred human permanent lower molars were selected, extracted for reasons unrelated to this research, with complete root formation, without calcifications or resorptions, with only 1 distal root canal (Vertucci's Type I Classification), donated by the CEO-Centro, in the city of Fortaleza-CE. The

Vertucci's type I classification were verify two times, with X-ray, and after de acess with a manual hand file. After access with spherical diamond tip in high rotation FG 1014 (KG Sorensen, Cotia, Brazil) and the ENDO ZK drill (JET, France), only canals with foraminal diameter between 200 µm and 300 µm were selected. All root canals were instrumented with the Reciproc® system (VDW®, Munich, Germany) with the R50 file in the forame (0.0), with the VDW Silver engine (VDW®, Munich, Germany), in the RECIPROC ALL program, by the same operator.

The root canals were randomly divided into 2 groups (n=50), and protocols for agitation and final cleaning of the root canal were created with an antimicrobial, a chelator and an agitation device. The chemical substance used were Sodium Hypochlorite 2.5% (% (Asfer Chemical Industry, São Caetano do Sul, São Paulo, Brazil) - NaOCl + EDTA 17% and Chlorhexidine Gel 2% (Pharmacy Biophormula, Fortaleza, Ceará, Brazil - CHX+ EDTA 17% (Biodynamic Pharmacy, Santa Rosa de Viterbo, Paraná, Brazil). Were created them 10 subgroups (n=10) with the final protocols of cleaning of the root canal.

Protocols for agitation and final cleaning of the root canal:

1) CHX + Canal Brush
2) CHX + Easy Clean
3) CHX + Irrisonic
4) CHX + Canal Brush + Easy Clean
5) CHX + Canal Brush + Irrisonic
6) NaOCl + Canal Brush
7) NaOCl + Easy Clean
8) NaOCl + + Irrisonic
9) NaOCl + Canal Brush+ Easy Clean
10) NaOCl + Canal Brush+ Irrisonic

Passive ultrasonic irrigation (PUI) was performed using the Irrisonic insert (20/0.01), coupled to the Piezon Master 200 ultrasound (EMS, Vallée de Joux, Switzerland) at power 2, and 2 mm from the foramen. Easy Clean (25/0.04) was used in the Reciprocation program, in the VDW Silver electric motor, in the WaveOne function, distancing 2 mm from the foramen. The Canal Brush was used in the Rotary program, in the Dr's function in the same electric motor, with 1.5 N and 350 RPM, in the cervical and middle thirds.

Three 20-second cycles (20 s) were performed by each agitation devices, with 2 mL of each chemical substance, totaling 6 mL used (CHX/NaOCl + EDTA) and 2 minutes of agitation. Prior to the use of EDTA, all canals were irrigated with 2 mL of 0.9% saline solution. The use of disposable syringes and the use of the 24 G needle (Injex Industrias Cirurgicas, Ourinhos, São Paulo) were recommended, distancing 2 mm from the foramen (0.0), and at the end of the protocols, irrigation with 5 mL of 0.9% saline solution was carried out.

The root apices were sealed with adhesive wax (Technew, Rio de Janeiro, RJ, Brazil), before the protocols started, to prevent extrusion of irrigant agents during chemical-mechanical preparation.

Materials and methods

1.Specimen Preparation

The analysis methodology used in the present study was based on and analysis reported by Prado *et al.* [5].

After completing the final cleaning protocols described, the samples were dried with paper points (Odous de Deus, Belo Horizonte, MG, Brazil) and the distal roots were longitudinally cleaved with a hammer and chisel. The most visible root half was selected for further analysis. The halves were metallized in the Denton Desk II sputter coating system (Denton Vacuum, New Jersey, USA) and examined using scanning electron microscopy (SEM) (JOEL JSM model 5410; JEOL Ltd., Tokyo, Japan).

2. Scanning Electron Microscopy (MEV)

The total root length was divided by 3, considering the cervical third 2 mm below the entrance of the canal, middle third to half of the total length and apical third 2 mm above the apical foramen. Three representative images were made, with a 1000x increase, one of the cervical third, another of the middle third, and one apical, and these were arranged in slides in the PowerPoint program® (Windows 10), unidentified and evaluated by 2 independent blinded researchers. Scores were established according to wall cleaning, smear layer removal and dental tubules exposure, being considered score 0 – for cleaning between 100% and 75% of the walls; score 1 - between 75% and 50%; score 2 - 50% and 25% and score 3 - 25% to 0% cleaning and exposure of tubules (Fig. 1).

3.Statistical analysis

The reliability of the inter-examiner result for the SEM evaluation was assessed using the Cohen Kappa coefficient. The data were analyzed using the Kruskal-Wallis and Friedman and Dunn tests with a 5% significant index.

Results

The Kappa test showed high agreement between the observers (0.85). The data obtained were analyzed between the groups, taking into account each third, cervical, middle and apical regions. There was no statistical difference between the groups in any of the analyzed thirds, regardless of the chemical substance used or the agitating device used. Maximum and minimum values are expressed in Table 1.

In the intra-group analysis, where the thirds were compared within the same group, there was a statistically significant difference ($p<0.05$) in the CHX + Easy Clean group, between the middle and apical thirds, where the middle third was cleaner (Fig. 2), considering the score 0, there was no difference between the cervical and middle or cervical and apical thirds. There was also a statistically significant difference between the cervical and apical thirds in groups CHX + Canal Brush + Easy Clean, CHX + Canal Brush + Irrisonic and NaOCl + Canal Brush, with better results for cleaning and exposure of dentinal tubules in the cervical third (Fig. 3). There was no statistical difference between the thirds in the other groups analyzed.

Discussion

The use of different protocols and cleaning devices is essential to obtain the maximization of root canal cleaning and disinfection. Therefore, the objective of this work was to analyze final cleaning protocols of the root irrigation, using the chemicals NaOCl 2.5% or CHX 2% plus to EDTA 17% associated to the supplementary agitation devices, Irrisonic, Easy Clean and Intracanal Brush, by means of SEM.

Similar studies have been previously conducted using a similar number of samples and methodology of analysis by SEM in extracted human teeth [2,3,5,9,18]. The analysis of the images was performed independently by blind operators, and the scores were established regarding the cleaning of walls, removal of smear layer and opening of dentinal tubules, as well as in other studies already described [5,9,18,20]. The irrigation protocol used in this study aimed to ensure the standardized volume and frequency of irrigating solution to ensure a suitable root canal cleaning, and this has been reproduced in numerous studies, proving its efficacy [14,21]. This study used the R50 file during root canal preparation of distal canals of mandibular molars, and 24 G caliber hypodermic needle, ensuring that the irrigant agent reached the middle and apical third of the root canal. Previous studies suggested a minimum enlargement of the apical third, with the file #35 [8,15,17]. Our study, followed the manufacturer's instruction regarding the sample selection, making use of files smaller or equal to #30 thus the R50 file was selected.

The use of an irrigating solution with antimicrobial and bacteriostatic properties is a fundamental condition for endodontic treatment. In the present study, the chemical substances CHX and NaOCl were considered, since both have antimicrobial action proven in the literature [19,22], being NaOCl most commonly used, due to its solvent properties [20,22]. CHX, when used, is due to its substantiality and rheological capacity, due to the association of natrosol gel [9]. The most commonly used irrigation protocols for the removal of the smear layer make use of NaOCl in its various concentrations, for removal of the organic component from the layer, and complementation with chelating agents such as EDTA 17% to remove inorganic components. In our study, regardless of the chemical substance used in association with EDTA 17%, there was no statistical difference between the groups, a similar report was also found in the literature [23]. Similar results were also observed in other studies in the literature, taking into account the 2 substances, [14,20] or even, using only hypochlorite, in the form of solution and gel [19] where there was no difference between the groups in the removal of the smear layer in any of the thirds. Different results were found [20] when the substances were used independently in the groups, with EDTA 17%, getting the best results, in relation to CHX and NaOCl, demonstrating that EDTA 17% has great value in the final canal cleaning protocols. It should be emphasized that in our study, NaOCl was inactivated with 2 mL of physiological serum, before the use of EDTA 17%.

In relation to agitation devices, Irrisonic, Easy Clean and canal brush were used, which were previously described in the literature [2,8,17,18], however, these tools were never reported to be used jointly and with variation of the chemical substance. The vast majority of studies concluded that the use of ultrasound (PUI) presents the best results [5,24] in terms of final cleaning of the canal, with the exception of one study [18], where Easy Clean presented the best results in the apical third, when used in the reciprocal movement, as well as our study. In a recent study [21], Easy Clean in rotational motion obtained results similar to those by PUI and when in reciprocal movement its results were similar to those found in conventional irrigation, but with artificial and flattened canals.

In our study, in the analysis between the groups, there was no statistical difference between the groups, in any of the analyzed thirds, regardless of the agitating device and the chemical substance employed. Thus, the null hypothesis was accepted, corroborating other similar findings [8].

In the intra-group analysis, that is, comparing the thirds of the canal, within the same group, there was a statistical difference between the middle and apical thirds only in one group CHX + Easy Clean, where CHX and EDTA 17% were used in association with Easy Clean, where the middle third presented higher exposure of dentinal tubules and cleaning of the walls. However, there was no difference between the cervical and apical thirds. In groups CHX + Canal Brush + Easy Clean, CHX + Canal Brush + Irrisonic and NaOCl + Canal Brush, there was a relevant statistical difference between cervical and apical thirds, where the last third had the worst scores in the 3 groups, a similar report was also found in the literature [2]. This result can be explained by the use of the intracanal brush in the 3 groups, since it has its largest area of activity in the cervical third, performing mechanical cleaning, through its friction against the walls of the root canals. The chemical substance did not seem to be relevant, considering that CHX + Canal Brush + Easy Clean and CHX + Canal Brush + Irrisonic also used CHX and EDTA 17%, while the NaOCl + Canal Brush group used NaOCl and EDTA 17%.

There were no statistical differences in the other groups, between the thirds, however, the apical third presented the worst scores in all groups analyzed. It should be emphasized that the apical third presents many anatomical complexities, as well as a smaller diameter when compared to the other thirds, thus hindering the arrival of the irrigator, cleaning and removal of the smear layer in the region, similar results are already found in the literature [2,5,8]. However, in a study with bovine teeth and enlargement using the File K #80, the use of ultrasound showed the best results, in the apical third regardless of the chemical substance used [14].

Conclusion

Inevitably the instrumentation of the root canal promotes the production of scrapings inside it, and final irrigation protocols are necessary in order to increase the cleaning capacity of the root canal system. Through this study, it was possible to verify that none of the protocols promoted effective cleaning in all thirds, and in all groups the apical portion was more critical, regardless of the agitation technique. The chemical substances used did not interfere in the cleaning of the canals. However, more studies are still necessary to qualify and improve the final cleaning of root canals.

Disclosure statement

The authors deny any conflicts of interest related to this study.

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Ethical statement

This research was approved by the Human Research Ethics.

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Figures

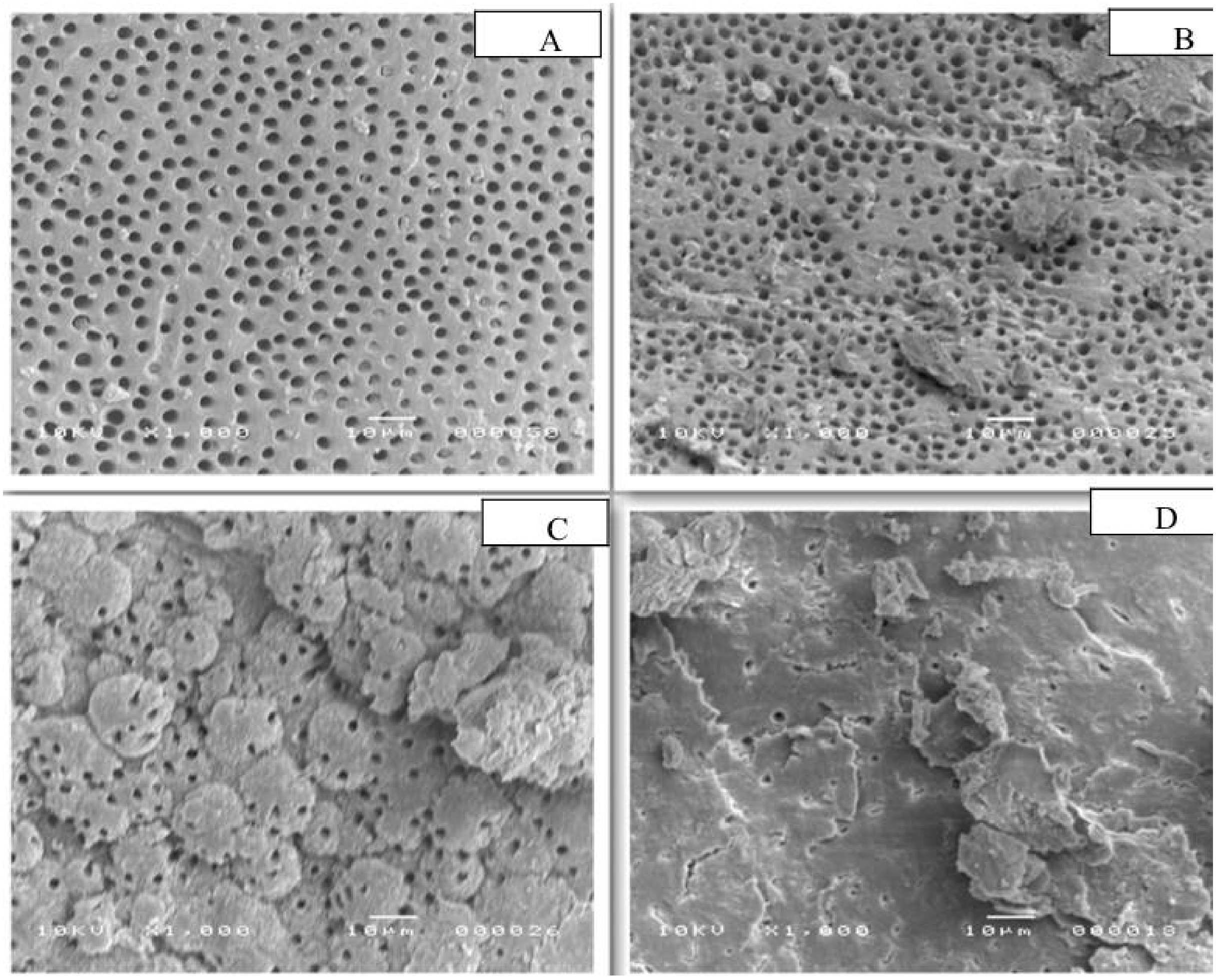

Fig. 1. Representative images of the scores established score 0 (A); 1(B); 2(C) and 3 (D) respectively.

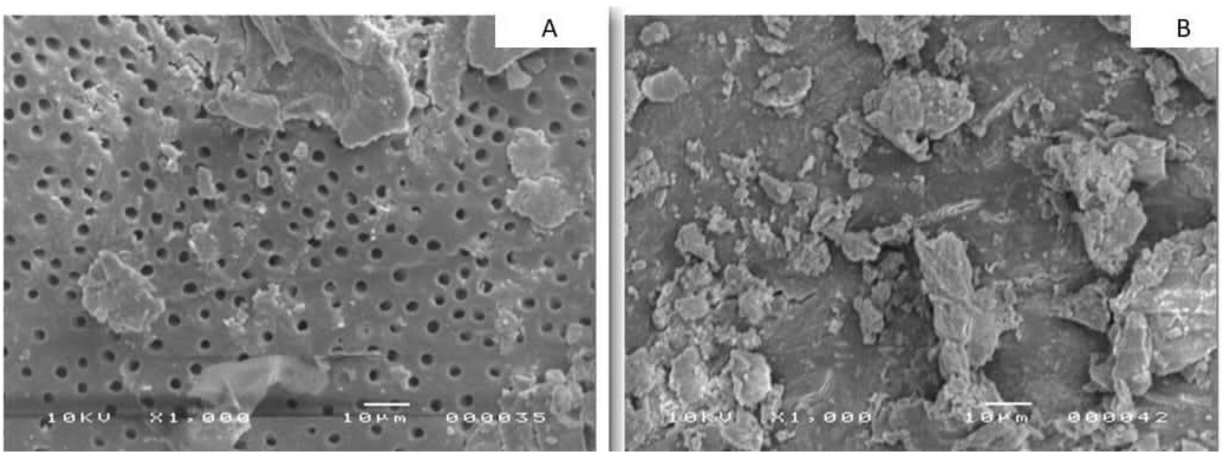

Fig. 2. Representative images of CHX + Easy Clean with statistical difference between the Middle (A) and Apical third (B)

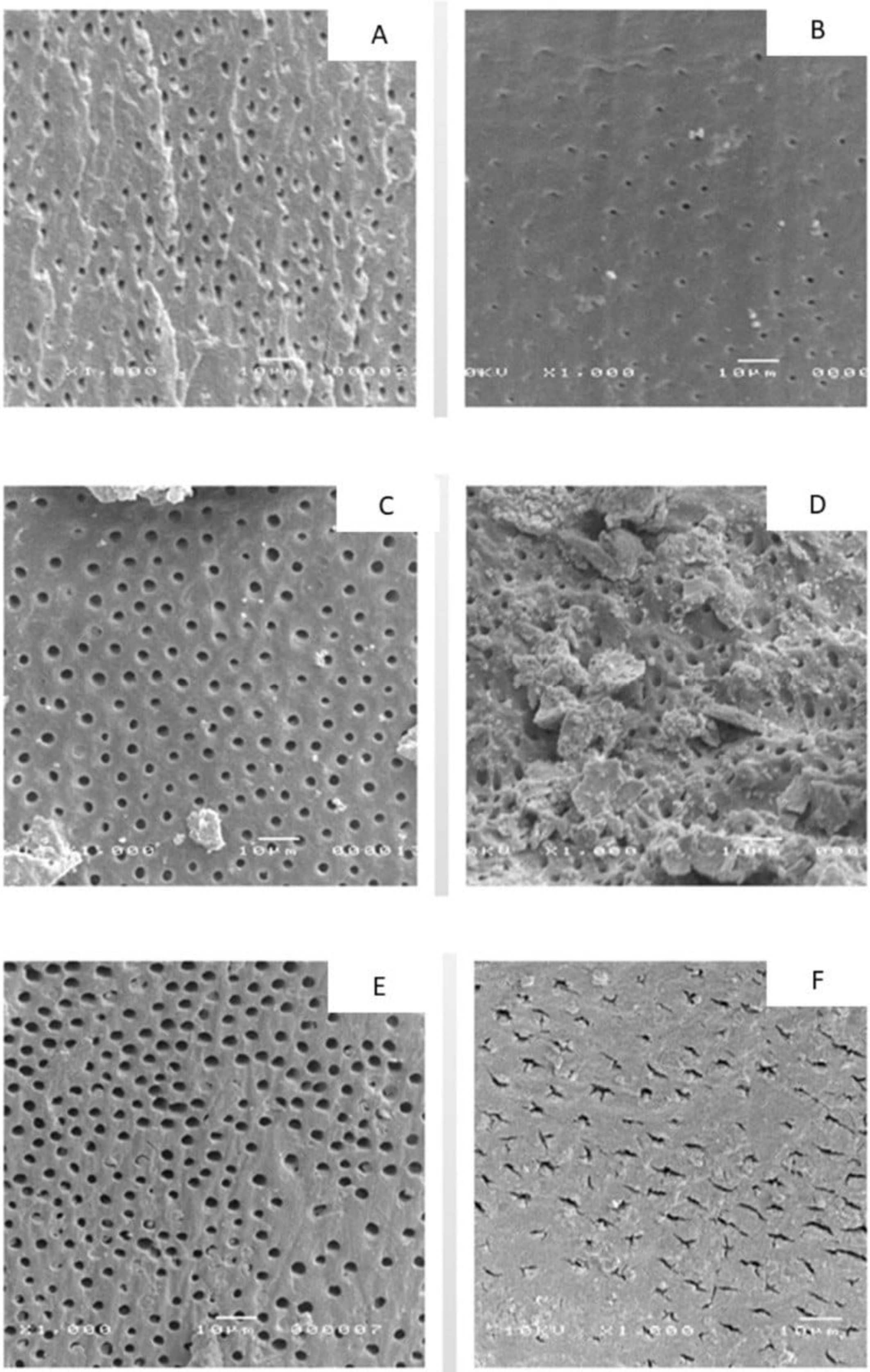

Fig. 3. Representative images of CHX + Canal Brush + Easy Clean (A,B), CHX + Canal Brush + Irrisonic (C,D) and NaOCl + Canal Brush (E,F), respectively, with statistical difference between cervical (column 1) and apical thirds (column 2).

Table 1. Median, Minimum and Maximum root canal cleansing scores, after final cleaning protocol; C (cervical third); M (middle third) and A (apical third).

	CHX + Canal Brush C	**CHX + Easy Clean C**	**CHX + Irrisonic C**	**CHX + Canal Brush + Easy Clean C**	**CHX + Canal Brush + Irrisonic C**	**NaOCl + Canal Brush C**	**NaOCl + Easy Clean C**	**NaOCl + Irrisonic C**	**NaOCl + Canal Brush+ Easy Clean C**	**NaOCl + Canal Brush+ Irrisonic C**
Median	2,0 $^{A, a}$	1,5 $^{A, ab}$	1,5 $^{A, a}$	1,0 $^{A, a}$	2,0 $^{A, a}$	1,0 $^{A, a}$	2,0 $^{A, a}$	1,0 $^{A, a}$	1,5 $^{A, a}$	1,5 $^{A, a}$
Min/Max	0/3	0/3	0/3	0/3	0/3	0/2	0/3	0/3	0/3	0/3
	CHX + Canal Brush M	**CHX + Easy Clean M**	**CHX + Irrisonic M**	**CHX + Canal Brush + Easy Clean M**	**CHX + Canal Brush + Irrisonic M**	**NaOCl + Canal Brush M**	**NaOCl + Easy Clean M**	**NaOCl + Irrisonic M**	**NaOCl + Canal Brush+ Easy Clean M**	**NaOCl + Canal Brush+ Irrisonic M**
Median	2,0 $^{A, a}$	1,5 $^{A, a}$	2,5 $^{A, a}$	1,5 $^{A, ab}$	3,0 $^{A, ab}$	2,0 $^{A, ab}$	2,0 $^{A, a}$	2,0 $^{A, a}$	2,0 $^{A, a}$	2,0 $^{A, a}$
Min/Max	0/3	0/3	1/3	0/3	1/3	0/3	1/3	0/3	0/3	0/3
	CHX + Canal Brush A	**CHX + Easy Clean A**	**CHX + Irrisonic A**	**CHX + Canal Brush + Easy Clean A**	**CHX + Canal Brush + Irrisonic A**	**NaOCl + Canal Brush A**	**NaOCl + Easy Clean A**	**NaOCl + Irrisonic A**	**NaOCl + Canal Brush+ Easy Clean A**	**NaOCl + Canal Brush+ Irrisonic A**
Median	1,0 $^{A, a}$	2,5 $^{A, b}$	3,00 $^{A, a}$	2,0 $^{A, b}$	3,0 $^{A, b}$	2,5 $^{A, b}$	3,0 $^{A, a}$	2,0 $^{A, a}$	2,0 $^{A, a}$	2,0 $^{A, a}$
Min/Max	0/3	1/3	1/3	0/3	2/3	1/2	1/3	0/3	0/3	1/3

Legend: Uppercase letters represent intergroup differences, and in each third; lowercase letters depict intragroup analysis, between thirds. Different letters represent statistical difference ($p>0.05$).

3 DISCUSSÃO

3 DISCUSSÃO

Este estudo analisou a qualidade do preparo e limpeza final do conduto radicular em molares inferiores. Foram analisados diferentes protocolos de instrumentação em função do limite de instrumentação e diâmetro de ampliação, nos canais mesiais, bem como, protocolos de limpeza final do conduto radicular, em canais distais, variando o uso das substâncias químicas, associadas a dispositivos de agitação.

Em relação aos canais mesiais analisados, foi observado o aumento de volume do canal, a centralização e o transporte do preparo, bem como a porcentagem de áreas não instrumentadas dos canais por meio de Microtomografias. A análise por Micro-CT é considerada a metodologia mais amplamente utilizada para investigar os efeitos dos instrumentos e de técnicas de instrumentação no interior do canal, devido a sua natureza não destrutiva do espécime, permite a comparação morfológica do canal radicular em dentes extraídos antes e depois do preparo realizado (SIQUEIRA JR *et al*., 2018; LIMA *et al*., 2020). Neste estudo foram considerados 3 protocolos de instrumentação, que consideraram 3 comprimentos de trabalho, e 3 ampliações apicais finais, sendo: G25.06/+ 1mm, G35.05/forame e 50.01/- 1 mm.

Em relação aos condutos distais estudados, na análise considerou-se as substâncias químicas Hipoclorito de Sódio 2.5% e Clorexidina gel 2%, juntamente com os dispositivos de agitação Irrisonic, Easy Clean, e Escova intracanal, por meio de MEV. Outros estudos semelhantes também foram realizados, utilizando-se da mesma metodologia de análise e dentes humanos extraídos (CARON *et al*., 2010; AKHLAGHI *et al*., 2014; KAMEL; KATAIA, 2014; MENDODNÇA *et al*., 2015; KATO *et al*., 2016; PRADO *et al.,* 2016). As análises das imagens foram realizadas por 2 operadores cegos independes, experientes, e estabelecidos scores quanto a limpeza de paredes, remoção de smear layer e abertura dos túbulos dentinários, semelhantemente a trabalhos já descritos anteriormente (CARON *et al*., 2010; KATO *et al*., 2016; CHARLIE *et al*., 2018; ZANDI *et al*., 2019).

No presente trabalho, foram analisados canais mesiais de molares inferiores, do tipo IV de Vertucci, e tipo I de Vertucci na raiz distal, configurando o tipo de canal radicular mais comumente observado clinicamente e utilizado em outros estudos semelhantes (PETERS *et al*., 2015; BRASIL *et al*., 2017). Estes canais apresentam uma anatomia extremamente desafiadora, gerando dificuldade na escolha da lima apical final, que possa garantir uma segurança e uma ampliação adequadas, visando a descontaminação e limpeza satisfatórias (AYDIN *et al*., 2019).

Segundo Campello *et al* (2019) o diâmetro do canal deve ser aferido adequadamente, e devido à grande variabilidade anatômica dos canais radiculares, é importante que isso seja observado antes do preparo químico-mecânico, visando personalizar a instrumentação e evitar desgastes em demasia. Para Aminoshariae e Kulild (2015), técnicas de desbridamento ou de alargamento do canal não garantem a eliminação das bactérias durante o tratamento do mesmo, independe da ampliação do canal. Contudo, dilatações apicais maiores estão sendo sugeridas, como forma de aumentar a eficácia da limpeza e desinfecção dos canais (SAINI *et al*., 2012; BUTCHER *et al*., 2019). Siqueira Jr *et al* (2018) são enfáticos ao analisar que a ineficiência do preparo pode ser resultado de um tratamento inadequado, principalmente com instrumentações apicais muito conservadoras, resultando em grandes quantidades de bactérias e tecido necrótico deixadas no canal.

Estudos recentes apontam a importância do alargamento do canal radicular para atingir a limpeza, desinfecção, penetração do irrigante e cimento obturador, considerando ser uma condição já estabelecida (LASLAMI *et al*., 2017; RODRIGUES *et al*., 2017; BUTCHER *et al*., 2019; CAMPELLO *et al*., 2019). Preparos apicais maiores permitem uma melhor remoção de dentina contaminada, aumentam a ação dos irrigantes na região apical e reduzem significativamente a carga bacteriana no sistema de canais radiculares (SAINI *et al*., 2012; DE-DEUS *et al.*, 2015).

Na análise de volume total e apical, dos canais mesiais, dentro do mesmo grupo, foi observada diferença estatística relevante, em todas as instrumentações realizadas, indicando a remoção de dentina e aumento do diâmetro do canal em todas as fases. Em relação ao volume total quando se empregou o protocolo 35/.05 no comprimento real do dente ou o protocolo 50/.01 a 1mm aquém do comprimento real do dente a porcentagem de aumento de volume foi significantemente maior do que quando se trabalhou com a 25/.06 1 mm além do forame. Estudos anteriores demonstraram que ampliação apical acima do diâmetro #35 a 1 mm aquém do forame favorecem uma maior porcentagem de áreas instrumentadas e maior descontaminação do canal radicular (FORNARI *et al*., 2010, BUTCHER *et al*., 2019).

Assim, está sendo sugerido na literatura que a ampliação da porção apical com diâmetros acima de #35 do canal podem melhorar a eficiência de limpeza do canal radicular e maximizar sua desinfecção do sistema de canais radiculares (XU *et al.*, 2001; AKHLAGHI *et al*., 2014; LASLAMI *et al*., 2017; CAMPELLO *et al*., 2019), contudo a dilatação apical ideal ainda é bastante discutida, assim como o limite de instrumentação empregado (YAYLALI *et al.,* 2017; ALBUQUERQUE *et al*., 2020). Alguns autores têm sugerido que, um critério para determinar o tamanho da preparação apical é considerar cada canal individualmente, determinando o diâmetro apical inicial e ampliando com 3 a 4 instrumentos maiores (FORNARI *et al*., 2010; ELAYOUYI *et al*., 2011; SAINI *et al*., 2012; RODRIGUES *et al*., 2017; PERÉZ *et al*., 2018).

Outros estudos também observaram que ampliação apical acima de #35, reduziu significativamente a quantidade total de detritos contaminados em mais de 30% (XU *et al*., 2011, DE-DEUS *et al.*, 2015); este resultado já havia sido encontrado em outro estudo, com preparos de canais mesiais de molares superiores e inferiores e verificaram que quanto mais dentina era cortada, menos detritos residuais permaneciam nos canais. O fato de a maior ampliação apical proporcionar menor ocorrência de detritos pode estar relacionado ao favorecimento de uma melhor irrigação no terço apical, como observado por Vera et al (2012) quando realizaram a patência apical com lima #10 e ampliação até o 40/.06, obtendo-se o maior alcance do irrigante na porção apical.

Considerando-se os condutos distais, nosso estudo, seguiu a instrução do fabricante (VDW, Munique, Alemanha) na seleção da amostra, fazendo o uso limas de diâmetro #30 para assim selecionar a lima R50, para que o irrigante alcançasse o terço médio e apical do canal radicular, corroborando com estudos que sugerem uma ampliação mínima com a lima #35 (VAN DER SLUIS *et al*., 2010; SRIKANTH *et al.*, 2015; SILVA *et al*, 2018), para tal feito.

Trabalhos descritos na literatura atual, contemplam que ampliações com limas de calibre entre #40 e #50, são capazes de promover uma maior remoção de material contaminado, melhorando a limpeza apical, resultando em uma desinfecção do canal radicular significativamente melhorada (XU *et al*., 2011; FORNARI *et al*., 2010; ELAYOUTI *et al*., 2011; RODRIGUES *et al*., 2016, PERÉZ *et al*., 2018), além de melhorar a ação do irrigante (BUTCHER *et al*., 2019) e a penetração do cimento obturador (LASLAMI *et al*., 2017).

Em nosso estudo, realizado nos canais mesiais, houve diferença estatística na remoção de dentina do canal, considerando o volume total, entre os grupos G25.06/+1 mm e os demais, porém não houve diferença entre G35.05/forame e G50.01/-1 mm, considerando o volume total. Isto pode ser explicado, devido ao fato dos canais do grupo G50.01/-1 mm terem sido instrumentados com a lima 35.05 anteriormente, e a finalização da ampliação do G35.05/forame, ter sido com esta mesma lima, no comprimento real do dente, estabelecendo um diâmetro #40 à 1mm aquém neste grupo, ficando ambos muito semelhantes em relação ao diâmetro do canal, tanto em ponta quanto em conicidade. Quando foi considerado a alteração de volume do terço apical somente, houve diferença somente entre G25.06/+1 mm e G50.01/-1 mm. Isso demonstra que trabalhar com instrumentos de menor diâmetro e maior conicidade a 1mm além não vai remover mais dentina que empregar instrumentos de maior diâmetro e menor conicidade a 1mm aquém, portanto ampliar o forame não irá limpar mais o terço apical. Além disso a ampliação foraminal pode aumentar o risco de extravasamento de soluções irrigantes e material obturador (ALBUQUERQUE *et al*., 2020). Resultados semelhantes, na limpeza apical, foram encontrados por outros autores que empregaram ampliação apical com limas de maior calibre e menor diâmetro 1mm aquém do forame (ELAYOUTI *et al*., 2011; RODRIGUES *et al*., 2016; LASLAMI *et al*., 2017). Além disso, empregar maior diâmetro de ponta e menor conicidade tende a preservar uma maior espessura de dentina na região cervical (YUAN *et al*., 2016; PLOTINO *et al*., 2019).

Entretanto, pesquisas têm mostrado, que os canais radiculares são, na sua grande maioria, irregulares e com secção transversal oval, e essa discrepância pode ocasionar preparo irregulares, removendo dentina apenas de um dos lados do canal (MARTINS *et al*., 2019), e, que a maior ampliação do canal principalmente na porção apical, pode contribuir com a extrusão de debris além do forame apical (Albuquerque et al., 2020), podendo causar inflamação pós-operatória, ou mesmo falha no tratamento, como desvios, transportes e perfurações (YL et al., 2011; ELAYOUTI *et al*., 2011).

No presente trabalho, foram analisados, nos canais mesiais os 4 mm cervicais a partir da furca e 4 mm apicais a partir do forame, com relação ao desvio e tranporte dos canais após as limas apicais, não foram encontradas diferenças estatísticas em relação aos grupos testados, considerando a maior ampliação cervical com limas de conicidade 25/0.06, 35/0.05 e ampliação a 1mm aquém com a lima 50/.01 no G50.01/-1mm. Os autores acreditam que este resultado foi encontrado, devido as limas utilizadas no estudo, serem fabricadas por meio de um processo termomecânico especial de controle da memória (CM), tornando-as extremamente flexíveis e reduzindo erros de procedimento (SHEN *et al*., 2013; PEREIRA *et al.,* 2015; RODRIGUES *et al*.,2016). Estas características podem ter contribuído para a centralização do preparo, onde o aumento da ponta do instrumento foi compensado com a diminuição da conicidade, não ocasionando desvios ou transporte dos canais. Esses fatores são importantes na determinação da quantidade total de dentina removida, pois instrumentos com diferentes desenhos, com tratamentos térmicos diferenciados e menores conicidades e diâmetros de ponta, visam permitir uma melhor adaptação do instrumento a anatomia do canal durante a instrumentação (PLOTINO *et al*., 2019; LIMA *et al*., 2020).

Considerando-se a porcentagem de paredes não tocadas pelos instrumentos nos protocolos testados, este trabalho não observou diferença estatística entre os grupos testados, considerando toda a extensão do canal ou os 4 mm apicais analisados. Trabalhos que utilizaram a mesma metodologia de análise, o mesmo grupo dental e consideraram a extensão total e/ou do terço apical do canal, também encontram resultados semelhantes (PAQUÉ *et al*., 2010; SIQUEIRA JR *et al*., 2013; BRASIL *et al*., 2017). Porém outros autores tiveram resultados distintos, com diminuição das áreas não tocadas relacionadas ao aumento do diâmetro apical (PETERS *et al*., 2015; DUQUE *et al*., 2017). Embora não se tenha evidenciado diferença significante o grupo preparado até o instrumento 50/.01 a 1mm aquém tocou 24.7% a mais as paredes do canal em relação ao grupo 25.06/+1mm e 34.5% a mais de superfície tocada em relação ao grupo 35.05/forame, o que clinicamente proporcionaria maior limpeza e descontaminação.

Contudo, devido as dificuldades anatômicas presentes no interior do canal radicular, como istmos, achatamentos, canais acessórios e os próprios túbulos dentinários, mesmo com a ampliação do canal, o uso de substância química, com ação antibacteriana é fundamental (GUTARTS *et al*., 2005). Os protocolos de irrigação mais comumente utilizados, para a remoção da camada de smaer layer, fazem uso do NaOCl em várias concentrações para remoção do componente orgânico da camada e a complementação com agentes quelantes, como o ácido etilenodiaminotetracético a 17% (EDTA) para remover os componentes inorgânicos.

No presente trabalho, nos canais distais, foram utilizadas as substâncias químicas CHX e NaOCl, com comprovada ação antimicrobiana (GOMES *et al*., 2013; ZANDI *et al*., 2019) juntamente com o EDTA. O NaOCl é considerado padrão ouro na literatura, sendo o irrigante mais comumente utilizado, principalmente devido à suas propriedades solventes (MOHAMMADI *et al.,* 2009; CARPIO-PEROCHENA *et al.*, 2011), e a CHX pela sua substantividade e capacidade reológica (MOHAMMADI *et al.,* 2009; SIQUEIRA JR *et al*., 2013; GOMES *et al*., 2013).

O protocolo utilizado no presente estudo, para averiguar a limpeza dos canais distais foi realizado após a ampliação com a lima R50, compreendendo 3 ciclos de 20s de 2 mL, com cada uma das substâncias testadas, tendo como objetivo garantir o volume e a frequência do irrigante, visando aumentar a limpeza no interior do canal. Este protocolo já foi reproduzido em outros trabalhos, com comprovada eficiência (YANG *et al.,* 2008; JUSTO *et al*., 2014; CESÁRIO *et al.,* 2018). Em relação as substancias químicas testadas, neste estudo, não se observou diferença estatística entre os grupos, considerando a remoção da smear layer, em nenhum dos terços analisados; resultados semelhantes também foram vistos, em outros trabalhos na literatura (JUSTO *et al*., 2014; JIMNA *et al.,* 2017; CHARLIE *et al*., 2018; ZANDI *et al*., 2019). Porém, resultados diferentes foram encontrados quando as substâncias foram utilizadas de maneira independente nos grupos, tendo o EDTA, os melhores resultados, em relação a CHX e o NaOCl (CHARLIE *et al*., 2018). Devemos ressaltar que em nosso estudo, foi realizada a inativação do NaOCl com 2 mL de soro fisiológico, antes do uso do EDTA, pois o mesmo parece reduzir o cloro livre da primeira substância (DOGÄN *et al*., 2001).

Porém, utilizar estas substancias químicas, e assegurar o seu alcance até as porções mais apicais, somente com o uso de seringas e agulhas não é suficiente para garantir o processo de limpeza e descontaminação (KAMEL; KATAIA, 2014; SCHMIDT *et al*., 2015). Para que as mesmas consigam alcançar estas áreas, é necessário, além da ampliação do canal radicular, por meio das limas endodônticas, (JIMNA *et al*., 2017), o uso de agulhas de pequeno calibre (GUTARTS *et al*., 2005) e dispositivos de agitação. Estes dispositivos, visam potencializar a ação dos irrigantes, para que estes entrem em contato com as paredes do canal radicular, principalmente nas áreas onde não ocorrem a ação dos instrumentos (PETERS *et al*., 2001; GUTARTS *et al*., 2005; PERÉZ *et al*., 2018; VERSIANI *et al.,* 2018), auxiliando nesta etapa de descontaminação do sistema de canais radiculares (MENDODNÇA *et al*., 2015; PRADO *et al.,* 2016).

No presente estudo, considerou-se a ampliação do canal distal com a lima R50, bem como o uso da agulha hipodérmica de 24 G, e, em relação aos dispositivos de agitação, foram testados a Irrisonic, Easy Clean e a Escova Intracanal, sendo estes já descritos anteriormente na literatura (GARIP *et a*l., 2010; VAN DER SLUIS *et al*., 2010; KATO *et al*., 2016; SILVA *et al*, 2018). Na literatura atual, no entanto, ainda são encontrados resultados contraditórios em relação ao dispositivo de agitação mais eficientes (MENDODNÇA *et al*., 2015; PRADO *et al.,* 2016). Gutarts et al (2005), observaram melhorias na limpeza de istmos, com agitação ultrassônica, por 1 minuto, após preparo manual ou rotatório. Já Yang et al (2008), relataram eficiência na limpeza final, no terço cervical e médio do canal, sem nenhum tipo de dispositivo de agitação, somente o uso de agulha convencional de 27G. Outros estudos, já contemplam que o uso de irrigação passiva ultrassônica (PUI), apresentam os melhores resultados (PARAGLIOLA *et al*., 2010; PRADO *et al.,* 2016**)**. Plotino et al (2019), só obtiveram os melhores resultados de limpeza, nos terços cervical e médio, com o uso de um instrumento sônico. Tanto Yang et al (2008) quanto Plotino et al (2019) não observaram eficiência no terço apical, em canais mesiais de molares inferiores, diferentemente de Gutarts et al (2005), que observaram melhorias nos 10 níveis apicais analisados. Já Kato et al (2016), verificaram os melhores resultados, no terço apical com o uso da Easy Clean, quando utilizada no movimento reciprocante. Porém, em um estudo recente, a Easy Clean em movimento rotatório apresentou resultados semelhante a PUI, e quando utilizada no movimento reciprocante alcançou resultados parecidos com a irrigação convencional (CESÁRIO *et al.,* 2018).

Em nosso trabalho, comparando-se os três terços (cervical, médio e apical) dos canais distais, dentro do mesmo grupo, houve diferença estatística entre os terços médio e apical apenas no G2(CHX+EasyClean), onde o terço médio apresentou-se com maior exposição de túbulos dentinários, caracterizando uma maior limpeza das paredes, em relação ao terço apical. Já nos grupos G4(CHX/Escova intra-canal+ EasyClean), G5(CHX/Escova intra-canal+Irrisonic) e G6 (NaOCL/Escova intra-canal), houve diferença estatística relevante entre os terços cervical e apical, onde o último terço apresentou os piores scores nos 3 grupos. Este resultado, segundo os autores, pode ser explicado devido ao uso da escova intra-canal, nos 3 grupos, onde a mesma tem sua maior área de atuação no terço cervical, realizando uma limpeza mecânica, através da fricção desta contra as paredes do canal. Resultados semelhantes foram encontrados no trabalho de Gorduysus et al (2012) quando observaram melhores resultados no terço médio e cervical, no uso da escova, para remoção de hidróxido de cálcio do canal, e Kamel &Kataia et al (2014) associando o uso da escova intra-canal, com os melhores resultados no terço cervical. Porém Garip et al (2010) não verificaram resultados significantes nesta associação, quando comparado ao uso exclusivo de agulha e seringa. Em nosso estudo, nos 6 grupos onde a escova intra-canal foi utilizada, em 3 foram observados, diferenças estatísticas no terço cervical, em relação ao apical. A variação da substância química, assim como relatado anteriormente, não foi relevante, tendo em vista que o G4 e G5 fizeram uso da CHX e o G6 do NaOCl, ambos com EDTA. O uso da Easy Clean, no movimento reciprocante como preconizado no estudo de Kato et al (2016) não foi associado melhorias ao terço apical do canal.

Não houve diferenças estatísticas nos demais grupos, entre os terços analisados, contudo o terço apical apresentou os piores escores em todos os grupos analisados. Deve-se ressaltar que o terço apical, apresenta muitas complexidades anatômicas, bem como um menor diâmetro em relação aos demais terços, dificultando dessa forma, a chegada do irrigante, a limpeza e remoção da smear layer na região; resultados semelhantes já foram vistos na literatura (YANG *et al.,* 2008; KAMEL; KATAIA, 2014; SCHMIDT *et al*., 2015; PRADO *et al.,* 2016; SILVA *et al*, 2018; PLOTINO *et al*., 2019). Contudo, em um estudo com dentes bovinos e ampliação com a lima K #80, o uso do Ultrassom apresentou os melhores resultados, no terço apical independente da substância química utilizada (JUSTO *et al*., 2014), assim como no estudo de Kato et al (2016) com a Easy Clean. Em outro estudo o uso da lima SAF (Self Adjusted File) em combinação com a irrigação de NaOCl e EDTA resultou em superfície apical limpa na maioria dos canais radiculares utilizados no experimento (JIMNA *et al.,* 2017).

Dentro das limitações do estudo e sua extrapolação para o meio clínico, os autores salientam que a ampliação apical com instrumentos de Níquel-titânio, tratadas termicamente, com diâmetros maiores de ponta, porém com menores conicidades, a 1mm aquém do forame, têm potencial de promover um desgaste de dentina contaminada na região apical bastante importante, mantendo ainda o preparo centralizado, não promovendo transporte ou desvios, e com a uma porcentagem de paredes tocadas dentro do mesmo perímetro muito semelhante, não ocasionando desgastes cervicais desnecessários, contribuindo assim para maior descontaminação da zona crítica do canal. Inevitavelmente, a instrumentação do canal radicular promoverá a produção de raspas de dentina no seu interior e protocolos finais de irrigação, visando aumentar a capacidade de limpeza do sistema de canais radiculares são fundamentais. Por meio deste estudo, foi possível verificar que nenhum dos protocolos testados, associando uma substância química, um quelante e dispositivos de agitação, promoveram uma limpeza efetiva em todos os terços do canal, e, em todos os grupos a porção apical foi considerada a mais crítica, independente da técnica de agitação e da ampliação com a lima R50. Observou-se uma tendência de melhoraria na limpeza do terço cervical com o uso de escovas intra-canais, porém, mais estudos ainda são necessários para qualificar e aprimorar a limpeza final dos canais radiculares.

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