UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

PABLO ANDRÉS AMOROSO SILVA

Evaluation of the internal anatomy and instrumentation techniques with new mechanized instrumentation systems in second mandibular molars with fused roots

Avaliação da anatomia interna, instrumentação e técnicas de retratamento com novos sistemas mecanizados de instrumentação em segundos molares inferiores com raízes fusionadas

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DADOS CURRICULARES

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RESUMO

Avaliação da anatomia interna e técnicas de instrumentação com novos sistemas mecanizados em segundos molares inferiores com raízes fusionadas

O objetivo deste estudo foi analisar por meio da microtomografia computadorizada os aspetos morfológicos e morfométricos da anatomia interna do sistema de canais radiculares com formato em C, e, avaliar a área de superfície, volume e áreas do canal não instrumentadas antes e após a instrumentação dos canais por meio de sistemas alternativos de instrumentação mecanizada, assim como, analisar o efeito de instrumentos manuais com movimento de 90º-oscilatório como instrumentação final. Cinquenta e dois segundos molares inferiores extraídos com canais em forma de C foram escaneados usando micro tomografia computadorizada. As secções transversais do canal radicular foram registradas de acordo com a classificação modificada de Melton. Os parâmetros morfométricos e a configuração tridimensional, foram avaliadas. Depois, 20 dentes com anatomias convergentes e configuração interna C1 foram divididos em dois grupos (n = 10) e instrumentados com Reciproc e SAF respectivamente. Em seguida, uma lima niti #30 do tipo K foi usado em movimento de 90º oscilatório como uma instrumentação final. Os espécimes foram escaneados usando Micro-CT após todos os procedimentos. Os parâmetros morfométricos foram analisados utilizando o programa CTAN. Além disso, a superfície do canal radicular não instrumentada foi calculada por terços. Os resultados indicaram uma distribuição uniforme dentro da amostra. Além disso, a análise da secção transversal revelou predominância das configurações de C4 e C3 a 1 mm a partir do ápice e as configurações C1 e C2 no terço cervical. De acordo com os parâmetros morfométricos, o tipo C1 e o canal distal do C2 apresentaram os menores valores de circularidade e valores mais elevados para a área, diâmetro maior e menor no terço apical. Todos os valores relativos a analise de instrumentação foram comparados entre os grupos utilizando o teste de Mann-Whitney e a comparação intra-grupos usando o teste de Wilcoxon. A instrumentação com Reciproc aumentou significativamente o volume do canal em comparação com SAF. Além disso, os volumes dos canais foram significativamente aumentados após a instrumentação de 90º-oscilatória ($P < 0,05$). Depois de todos os protocolos de

instrumentação, o aumento de área de superfície só revelou diferenças significativas na comparação intra-grupos (P <0,05). A instrumentação com Reciproc e SAF deixou 28% e 34%, de áreas não instrumentadas respectivamente, sem diferença estatística (P> 0,05). Já a instrumentação oscilatória final reduziu as superfícies do canal radicular não instrumentadas de 28% para 9% (Reciproc) e de 34% para 15% (SAF; P <0,05). Os molares inferiores com canais radiculares em forma de C apresentaram distribuições semelhantes de canais simétricos, assimétricos e convergentes. A configuração C1 e o aspecto distal da configuração C2 apresentaram os maiores valores de área de e diâmetros apicais. Além disso, o uso final da instrumentação com 90º oscilatório usando instrumentos manuais de NiTi diminuiu significativamente as paredes do canal não instrumentadas que permaneceram após a instrumentação com Reciproc e SAF. Finalmente, a combinação de dois sistemas ou técnicas de instrumentação provaram ser eficazes na obtenção de melhores resultados na instrumentação de segundos molares inferiores em forma de C.

Palavras chave: Dente molar; Preparo de Canal Radicular; Endodontia; Microtomografia por Raio-X.

ABSTRACT

Evaluation of the internal anatomy and instrumentation techniques with new mechanized instrumentation systems in second mandibular molars with fused roots

The present study evaluated the morphometric aspects of the internal anatomy of the root canal and the effect of 90º-oscillatory instrumentation with hand files on volume, surface area and uninstrumented surface after shaping procedures with Self adjusting file and Reciproc in mandibular second molars with C-shaped canals. 52 extracted mandibular second molars with C-shaped canals were scanned with a micro-computed tomography scanner. The root canal cross-sections were recorded according to the modified Melton classification. Morphometric parameters and the tridimensional configuration, were evaluated. Afterwards, 20 teeth with merging type canals and C1 internal configuration were divided in two groups (*n* = 10) and instrumented with Reciproc and SAF instruments respectively. Then, a size 30 Niti hand K-file used in 90º-oscillatory was used as a final instrumentation. The specimens were scanned using Micro-CT after all procedures. Morphometric parameters were analyzed using CTAn software. Also, the uninstrumented root canal surface was calculated for each canal third. The results indicated an even distribution within the sample. Also, the cross-sectional configuration analysis revealed predominance of the C4 and C3 configurations at 1 mm from the apex and the C1 and C2 configurations in the cervical third. According to the morphometric parameters, the C1 and the distal aspect of the C2 exhibited the lowest roundness values and higher values for the area, major diameter and aspect ratio in the apical third. All values were compared between groups using the Mann–Whitney test and within groups using the Wilcoxon's signed-rank test. The significance level was set at 5%. Instrumentation with Reciproc significantly increased canal volume compared to SAF and the canal volumes were significantly increased after 90º-oscillatory instrumentation ($P < 0.05$). After all instrumentation protocols the surface area increase only revealed significant differences in the within groups comparison ($P < 0.05$). Reciproc and SAF instrumentation yielded a uninstrumented root canal surface of 28% and 34%, respectively, without differences ($P > 0.05$). Final oscillatory

instrumentation reduced the uninstrumented root canal surface from 28% to 9% (Reciproc) and from 34% to 15% (SAF; P < 0.05). The apical and middle thirds exhibited higher uninstrumented root canal surfaces after the first instrumentation that was significantly reduced after oscillatory instrumentation (P < 0.05). Mandibular molars with C-shaped root canals exhibited similar distributions of symmetrical, asymmetrical and merging-type canals. The C1 configuration and the distal aspect of the C2 configuration exhibited the highest area values and large apical diameters. Furthermore, the final use of 90º-oscillatory instrumentation using NiTi hand files significantly decreased the uninstrumented canal walls that remained after Reciproc and SAF instrumentation. Finally, the combination of two instrumentation systems/techniques proved to be effective in achieving better instrumentation results in C-shaped mandibular second molars.

Keywords: Molar; Root canal preparation; Endodontics; X-Ray Microtomography.

LISTA DE ABREVIATURA E SIGLAS

%	percentagem
+	mais
x	vezes
<	menor
>	maior
#	numero
=	igual
º	grau
2D	duas dimensões
3D	três dimensões
cm	centímetro
EDTA	ácido etelino diaminotetracético
h	hora
KV	quilovolts
MA	miliampère
MEV	microscopia eletrônica de varredura
mL	mililitro
mm	milímetro
mm^2	milímetro quadrado
mm^3	milímetro cubico
n	numero
NaOCL	hipoclorito de sódio
NiTi	Níquel Titânio
P	significância estatística
rpm	revoluções por minuto
SAF	Self Adjusting File
µm	micrômetro

SUMMARY

1 Introduction

1 INTRODUCTION

The molar group possesses one of the most complex anatomies among all teeth (WEINE, 1989) and understanding its internal anatomy and possible variations is of utmost importance to enable the clinician to determine an appropriate root canal treatment approach. The mandibular molar usually has two separate roots, one mesial with two or three canals and one distal with one or two canals. However, a common variation of these teeth is the presence of fused roots in their external anatomy, accompanied by an unusual root canal system (MANNING, 1990). This is more frequently observed in the mandibular second molar than in the mandibular first molar (SKIDMORE, 1979). Manning (1990) theorized that the failure of the Hertwig's epithelial root sheath to fuse on the lingual or buccal root surface was the main cause of a C-shaped root, or, that it may also be formed by coalescence because of deposition of cementum with time.

When root fusion is present, the external surface of the tooth may be conical or square shaped (MANNING, 1990), and a shallow or deep radicular groove can be present individually or on the buccal and lingual aspects of the root (FAN et al., 2004a). Such grooves may continue to the apical area or to less than half of root length (FAN et al., 2004a; KOTOKU, 1985). When a deep radicular groove is encountered (as given by a high groove-to-thickness ratio), a C-shaped canal system is frequently found and the floor of the pulp chamber is usually located more deeply than in a normal tooth (FAN et al., 2008). However, in the existence of a shallow radicular groove or no groove, this C-shaped root canal system may or may not exist (MANNING, 1990), and single wide root canals can be expected (IOANNIDIS et al., 2011).

Although rare, C-shaped root canals can also be found in other dental groups such as the first lower and upper molars (DE MOOR, 2002; NEWTON; MCDONALD, 1984; SILVA et al., 2013), lower and upper premolars (DE MOOR, 2002; NEWTON; MCDONALD, 1984; SILVA et al., 2013) and even in upper lateral incisors (BOVEDA; FAJARDO; MILLAN, 1999). Numerous studies report that the higher prevalence of C-shaped canals occurs in Asian populations, with an incidence of 31-45% (SEO; PARK, 2004; SONG et al., 2010; WANG et al., 2012; ZHENG et al., 2011). In addition, a recent study revealed a low prevalence of mandibular second molars with C-shaped molars in a Brazilian population, which was around 3.5% (SILVA et al., 2013).

Teeth with C-shaped root canals may have different internal anatomical configurations. According to (GAO et al., 2006), the internal classification of the root canal system of the molars C can be divided into (Appendix C):

- Type I (**merging type**): The root canals merged into 1 major canal before exiting at a single apical foramen. Partial dentin fusion areas may have appeared in the coronal and/or middle portions of the canal system.
- Type II (**symmetric type**): Separate mesial and distal canals were located at the mesial and distal parts of the root, respectively. From the buccolingual view, the mesial and distal canals were symmetric along the longitudinal axis of the root.
- Type III (**asymmetric type**): Separate mesial and distal canals were evident. From the buccolingual view, the distal canal may have had a large isthmus across the furcation area that commonly made the mesial and distal canal asymmetric.

Regarding the cross-sectional shape, MELTON et al., (1991) initially classified the cross-sections in several configurations. Nonetheless, (FAN et al., 2004a) pointed out that the shape of this canals can vary along the length of the root, so the initial shape appearance of the canal orifice, may not be the same at the apical level and for this reason, one cannot assume that such a shape continues throughout its length. According to this, FAN et al., (2004a) using microcomputed tomography modified and classified the internal cross-section configuration in (Apendix D):

1. C1: an uninterrupted C-shaped canal with no separation or division;
2. C2: the canal shape resembles a semicolon resulting from a discontinuation of the C-shaped canal outline;
3. C3: two or three separate canals are present in the cross-section;

4. C4: only one round or oval canal are present in the cross-section and;
5. C5: no canal lumen is observed.

In cases that root canal treatment of C-shaped canals is needed, the complexity of cleaning and shaping (SOLOMONOV et al., 2012) and obturating (ORDINOLA-ZAPATA et al., 2009) teeth with this type of anatomy present a real challenge for the clinician. A previous study (YIN et al., 2010) concluded that rotary systems maintained the canal curvature with speediness and few procedural errors during root canal preparation in C-shaped molars, however, large amounts of uninstrumented root canal surface area were observed, whereas manual instrumentation with traditional K files cleaned more canal surface. Due to these limitations and to achieve better cleaning and shaping of C-shaped root canals, some authors recommend the combination of two or more instrumentation systems/techniques (JEROME, 1994; YIN et al., 2010).

It is known that the primary goals of chemo mechanical preparation are cleaning and shaping of the root canal through the use of instruments and irrigants. However, recent studies have shown (GERGI et al., 2015; METZGER et al., 2010; PAQUE et al., 2010; SIQUEIRA et al., 2013) that almost 35 – 60% of the root canal walls are untouched by the endodontic files during mechanical instrumentation regardless of the instruments used, and that the ease of debridement decreases as the complexity of root canal anatomy increases (DE-DEUS et al., 2010). Usually, the main canal is partially cleaned and shaped and other anatomical structures such as isthmuses, fins, lateral canals and ramifications remain completely unaffected by the procedure because of inherent physical limitations of the instruments and irrigants to enter into those complexities (SIQUEIRA et al., 2013). These limitations might decrease the success rates of endodontic treatment (VERA et al., 2012).

To instrument challenging anatomies such as C-shaped root canals (SOLOMONOV et al., 2012) studied the effectiveness of the Self Adjusting File system (SAF; ReDent Nova, Ra'anana, Israel) and verified that almost 41% of the canal walls remained unaffected by the procedure performing better instrumentation than rotary files. This alternative instrumentation system has been showing favorable results in cleaning and shaping oval-shaped root canals compared to traditional NiTi rotary files (PARANJPE et al., 2012; VERSIANI; PECORA; DE SOUSA-NETO, 2011). This file is designed to adapt itself three-dimensionally to the shape of the root canal and is composed of a thin abrasive NiTi lattice that removes dentin with a back-and-forth grinding motion with vibration. Also, continuous irrigation solution is delivered thru the hollow file via a silicone tube (DE MELO RIBEIRO et al., 2013).

Recently, a new single endodontic file (RECIPROC, VDW, Munich, Germany) has been extensively used to instrument root canals in a faster manner and in a reciprocation motion. The Reciporc is made of a special NiTi-alloy called M-Wire that is created by an innovative thermal-treatment process which increases its flexibility and its resistance to cyclic fatigue (YARED; RAMLI, 2013) and the files are presented in different sizes: 25/08; 40/06 and 50/05. Moreover, the reciprocation working motion consists of a counter-clockwise (cutting direction) and a clockwise motion (release of the instrument), while the angle of the counterclockwise cutting direction is greater than the angle of the reverse direction (BURKLEIN et al., 2012). Studies have shown that the Reciproc system removes more tooth structure tan rotary instruments due to its increased cutting ability (BUSQUIM et al., 2015). Hand files are essential components of the endodontic arsenal; however, studies into the benefits of their use after the instrumentation of C-shaped root canals with either instrumentation technique are scarce.

Therefore, the purpose of this study was to evaluate the morphologies of second mandibular molars with C-shaped canals while considering the morphometric aspects of the internal anatomies identified using micro-CT technology and to assess the effect of finishing instrumentation with hand files using a 90º-oscillatory motion on final root canal instrumentation in combination with two different instrumentation techniques. Mandibular second molars with C-shaped canals were prepared with either a single-file reciprocating system (Reciproc; VDW, Munich, Germany) or a Self-Adjusting File System (SAF; ReDent Nova, Ra'anana, Israel). Changes in dentine volume, surface area increase and the percentage of the uninstrumented canal walls were compared using micro-CT technology. For this part of the study the hypotheses were that:

1. the single-file reciprocating system and SAF have similar morphological parameters (volume, surface area and uninstrumented surface) after instrumentation of C-shaped canals;
2. finalizing instrumentation with 90º-oscillatory instrumentation using hand files does not improve the abovementioned morphological parameters in C-shaped canals prepared with either the Reciproc or SAF systems.

2 Articles

2 ARTICLES

2.1 Article 1 - Micro–computed Tomographic Analysis of Mandibular Second Molars with C-shaped Root Canals

The article presented in this thesis was published in the Journal of Endodontics. Annex B contains the permission letter to include a published article from the Journal of Endodontics in this thesis.

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Basic Research—Technology

Micro–computed Tomographic Analysis of Mandibular Second Molars with C-shaped Root Canals

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Abstract

Introduction: The goal of the present study was to evaluate the morphometric aspects of the internal anatomy of the root canal system of mandibular second molars with C-shaped canals. **Methods:** Fifty-two extracted second mandibular molars with C-shaped canals, fused roots, and radicular grooves were selected from a Brazilian population. The samples were scanned with a micro–computed tomographic scanner at a voxel size of 19.6 μm. The root canal cross sections were recorded as C1, C2, C3, and C4 root canal configurations according to the modified Melton classification. Morphometric parameters, including the major and minor diameters of the root canals, the aspect ratio, the roundness, and the tridimensional configuration (merging, symmetric, and asymmetric), were evaluated. **Results:** The 3-dimensional reconstruction images of the teeth indicated an even distribution within the sample. The analysis of the prevalence of the different cross-sectional configurations of the C-shaped molars revealed that these were predominantly of the C4 and C3 configurations (1 mm from the apex) and the C1 and C2 configurations in the cervical third. According to the morphometric parameters, the C1 and the distal aspect of the C2 configurations exhibited the lowest roundness values and higher values for the area, major diameter, and aspect ratio in the apical third. **Conclusions:** Mandibular molars with C-shaped root canals exhibited similar distributions of symmetric, asymmetric, and merging type canals. The C1 configuration and the distal aspect of the C2 configuration exhibited the highest area values, low roundness values, and large apical diameters. *(J Endod 2015;41:890–895)*

Key Words

C-shaped canals, dental anatomy, fused roots, mandibular molars, micro–computed tomographic imaging

Knowledge regarding the internal anatomy of teeth and their possible anatomic variations is important for predictable shaping, cleaning, and disinfection of the root canal system and thereby to improve the likelihood of successful root canal treatments. Mandibular molars have some of the most complex anatomies, with isthmuses in both mesial and distal root canal systems and low roundness values in the transverse root canal, which make the instrumentation of these teeth challenging (1, 2).

The mandibular second molar anatomy has been extensively studied, particularly through the use of the clearing process (3). The descriptions of the anatomy of these teeth obtained using this technique include the existence of root fusions and C-shaped canal systems (4). The latter anatomic feature is characterized by the presence of isthmuses that connect the mesial and distal root canals. These teeth typically present a deep pulp chamber floor and fused roots with a longitudinal groove (4, 5). Previous studies (primarily of Asian populations) have reported that the prevalence of C-shaped canals is between 31% and 45% (6–9). Additionally, diverse classifications of the tridimensional distributions of root canal systems and transverse sections have been previously reported (5, 10, 11).

Micro–computed tomographic (micro-CT) technology is currently being used to study dental anatomy because this technology provides high-quality imaging and detailed quantitative and qualitative descriptions of the root canal anatomy without destroying the sample when compared with older techniques (12). Previous studies that have evaluated several anatomic features of C-shaped molars with micro-CT imaging have primarily been conducted in Asian populations (10-12).

The apical 3 mm of the root canal system because of its anatomic complexity is regarded as a critical area because of the difficulty associated with achieving complete cleaning of this area (13), and failure to do so may compromise the long-term prognosis of the treatment. Regarding the transverse cross sections of the root canal anatomy of mandibular molars with C-shaped canals, previous studies have reported a complex distribution of transversal anatomies that includes the presence of complex isthmuses (11). The morphometric parameters obtained from micro-CT data, such as roundness, major diameter, and aspect ratio, allow the acquisition of detailed measurements of different cross sections. Among these parameters, the roundness and aspect ratio features have gained the attention of several researchers because the presence of oval canals and isthmuses may decrease the efficacy of several clinical procedures, such as instrumentation and filling procedures (14, 15).

Despite the difficulty associated with recognizing the internal anatomies of C-shaped molars via traditional radiographs, some anatomic characteristics, such as

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convergence and wide root canal spaces, that can be observed on preoperative films have been used to classify these anatomies into 3 types: merging, asymmetric, and symmetric (12). This information can help clinicians predict the presence of fused areas and high numbers of isthmuses in the apical third (12). Because of the highly challenging anatomy of C-shaped canals, morphometric studies addressing this variation are needed to improve the mechanical and chemical strategies for root canal cleaning and shaping procedures. A detailed description of the numerous morphologic parameters of these root canal systems and their anatomic variation does not yet exist. The aim of the present study was to evaluate the morphologies of second mandibular molars with C-shaped canals while considering the morphometric aspects of the internal anatomies identified using micro-CT technology.

Materials and Methods

After the receipt of approval from the ethics committee (CEP #703.054), a micro-CT system (SkyScan 1174v2; Bruker-microCT, Kontich, Belgium) was used to scan a total of 52 mandibular second molars with fused roots that were extracted from a Brazilian population. The selected molars exhibited C-shaped roots with a radicular groove in the lingual or buccal surface. The sex and ages of the patients are unknown. The micro-CT parameters used were 50 kV, 800 mA, 360° of rotation, and an isotropic resolution of 19.6 μm. The system included a charge-coupled device camera (1304 $\times$ 1024 pixels). The images of each specimen were reconstructed with dedicated software (NRecon v.1.6.3, Bruker-microCT) that provided axial cross sections of the inner structures of the roots in the bitmap (BMP) format.

Tridimensional Classification

Three-dimensional models were reconstructed using automatic segmentation and the surface modeling CTAn v.1.12 software (Bruker-microCT). CTVol v.2.2.1 and data viewing software (Bruker-microCT) were used for visualization and qualitative evaluation of the specimens. After tridimensional reconstruction of the models, the root canal configurations were classified (12) as follows:

Type I (merging type): The root canals merged into 1 major canal before exiting at a single apical foramen. Partial dentin fusion areas may have appeared in the coronal and/or middle portions of the canal system.
Type II (symmetric type): Separate mesial and distal canals were located at the mesial and distal parts of the root, respectively. From the buccolingual view, the mesial and distal canals were symmetric along the longitudinal axis of the root.
Type III (asymmetric type): Separate mesial and distal canals were evident. From the buccolingual view, the distal canal may have had a large isthmus across the furcation area that commonly made the mesial and distal canal asymmetric.

Classification and Morphometric Analysis of the Root Canal Cross Sections

Two-dimensional (2D) cross sections taken 1, 2, and 3 mm from the apex were selected. Furthermore, the middle and the cervical thirds of the root were considered. The middle third of the root was defined as the section equidistant between the root apex and the pulp chamber floor of the root. The cervical third was defined 1 mm apical from the pulp chamber floor. The 2D sections of both groups were classified according to Melton's modified method proposed by Fan et al (11) (Figs. 1 and 2) as follows:

C1: An uninterrupted C-shaped canal with no separation or division
C2: The canal shape resembled a semicolon resulting from a discontinuation of the C-shaped canal outline
C3: 2 or 3 separate canals were present in the cross section
C4: Only 1 round or oval canal was present in the cross section
C5: No canal lumen was observed

Quantitative analyses of the area, perimeter, major diameter, minor diameter, and aspect ratio were performed based on the apical 3-mm sections using the CTAn software (Bruker-microCT). The area and perimeter were calculated using the Pratt algorithm. The cross-sectional appearance (ie, round or more ribbon shaped) was expressed as roundness (16). The roundness of a discreet 2D object was defined as $4\,A/(\pi[\text{dmax}]2)$, where A is the area and dmax is the major diameter. The roundness values ranged from 0 to 1, where 1 indicates a perfect circle (16, 17). The major diameter was defined as the distance between the 2 most distant pixels in the object. The minor diameter was defined as the longest chord through the object that could be drawn in the direction orthogonal to that of the major diameter (17). The aspect ratio is a common measure of shape that was defined by dividing the major diameter by the minor diameter (16). All of these values were calculated using the CTAn v.1.12 (Bruker-microCT) and CTVol v.2.2.1 software. The median, minimum, and maximum ranges of all of the previously mentioned values were processed using the Prism 5.0 software (GraphPad Software Inc, La Jolla, CA).

Results

Qualitative Analyses

Tridimensional Classification. The 3-dimensional reconstruction images of the C-shaped roots exhibited an even distribution in terms of Gao's classification (ie, type I [n = 19], type II [n = 16], and type III [n = 17]).

Root Canal Cross Section Classification. The cross-sectional configurations of the C-shaped roots are presented in Table 1. All of the evaluated molars exhibited at least 1 level that was classified as C1, C2, or C3. Overall, the C-shaped molars predominantly exhibited C3 and C4 configurations at a location 1 mm from the apex. At the 2- and 3-mm levels, the C1 and C3 configurations were most prevalent. In the middle third of the root, the C3 configuration was the most prevalent, and the C1 and C2 were the second and third most prevalent configurations. In the cervical third of the root, the C1 and C2 configurations were most common.

Quantitative Analyses

Detailed data regarding the morphometric analyses of the cross-sectional 2D images of all of the C-shaped configurations are presented as medians and ranges in Figures 1 to 4. The C4 configuration was only analyzed at the 1- and 2-mm apical level because of the low number of samples with this configuration at the 3-mm level.

Overall, the C1 and the distal part of the C2 configurations exhibited the lowest roundness values and higher aspect ratios, areas, and major apical diameters of the root canals compared with the C3 and C4 configurations. The highest prevalence of round-shaped sections of the root canals was found among those with the C4 configuration; in contrast, the C1 configuration exhibited lower roundness values and higher aspect ratios. The median roundness values of the

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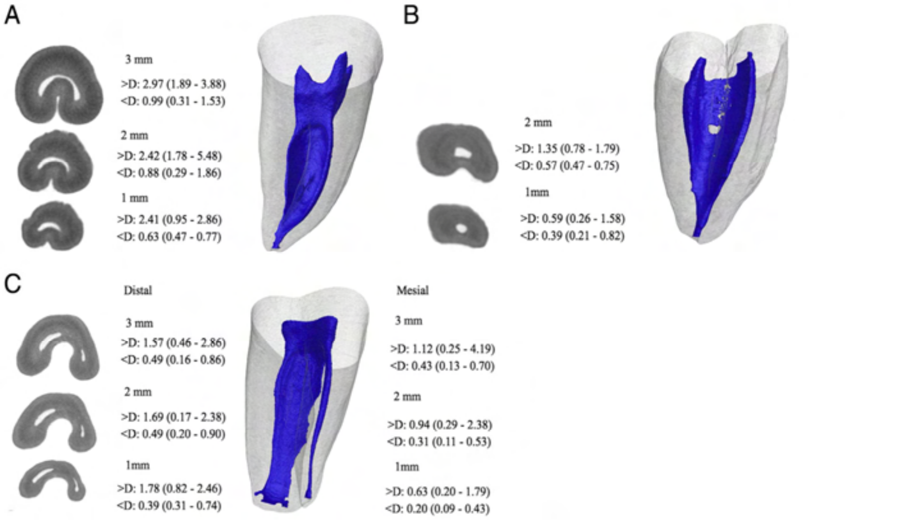

Figure 1. Medians and ranges of the major (>) and minor (<) diameters found at the apical third in the (*A*) C1, (*B*) C4, and (*C*) C2 configurations.

cross-sectional configurations at 1 mm of the foramina were as follows: 0.21 for C1, 0.21 to 0.33 for C2, 0.41 to 0.61 for C3, and 0.50 for C4.

At the 1-mm level, the C1 configuration exhibited the greatest areas (0.73 mm^2) followed by the distal aspect of the C2 (0.46 mm^2), C4 (0.23 mm^2), and distal C3 configurations (0.14 mm^2). Lower values were found in the mesial canals with the C2 (0.10 mm^2) and C3 configurations (0.03–0.09 mm^2). Other morphometric values and representative images of the studied anatomic configurations are presented in Figures 1 to 4.

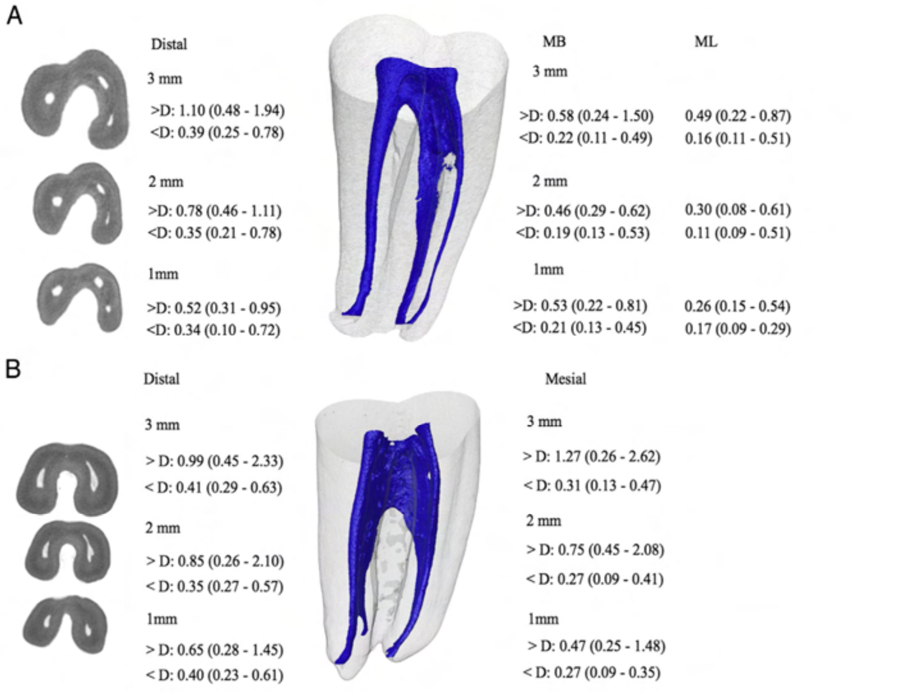

Figure 2. Medians and ranges of the major (>) and minor (<) diameters of the different cross sections evaluated at the apical third in the C3 configuration with (*A*) 2 mesial and (*B*) 1 mesial root canal system.

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TABLE 1. Distribution of Melton's Classification in Mandibular Second Molars with C-shaped Roots at the 5 Evaluated Levels

	1 mm			2 mm			3 mm			Middle third			Cervical third		
Type	S	A	M	S	A	M	S	A	M	S	A	M	S	A	M
C1	0	1	4	1	3	9	0	2	11	0	3	8	4	7	13
C2	1	8	1	1	9	2	1	5	4	2	6	7	3	8	3
C3	13	5	2	14	5	2	15	10	3	14	7	4	9	2	2
C4	2	3	12	0	0	6	0	0	1	0	1	0	0	0	1

A, asymmetric type; C1, an uninterrupted C-shaped canal with no separation or division; C2, the canal shape resembled a semicolon resulting from a discontinuation of the C-shaped canal outline; C3, 2 or 3 separate canals were present in the cross section; C4, only 1 round or oval canal was present in the cross section; C5, no canal lumen was observed; M, merging; S, symmetric type.

Discussion

The root canal anatomy of C-shaped mandibular molars is highly variable. In this group of teeth, the majority of studies that have reported the presence of C-shaped canals in mandibular second molars have been conducted in Asian populations (3, 9, 12). The prevalence of this type of anatomy has also been reported to be between 3.5% and 15% in a Brazilian population (18, 19).

The tridimensional evaluation of mandibular second molars with C-shaped root canals in the present study revealed an even distribution of the anatomic variants (ie, type I [36%], type II [30%], and type III [32%]). These findings are similar to the results reported by Gao et al (12), who found that the distributions of types I, II, and III of a sample of 98 molars were 32%, 38% and 28%, respectively, and are similar to those found in another previous study (20). C-shaped mandibular molars with merging-type canals exhibited a high prevalence of C1 and C4 configurations at the 3-mm apical location, whereas symmetric C-shaped molars exhibit a high prevalence of the C3 configuration. Additionally, the asymmetric type exhibited a high prevalence of C2 and C3 configurations at the apical third. These results are in agreement with those of a previous study conducted in a Chinese population (21).

Of all of the cross section types, the C3 and C4 configurations were the most prevalent in the last apical millimeter (11). The C4 configuration is basically the result of the convergence of the mesial and distal aspects of the C-shaped anatomy into a single canal. This anatomy was primarily found in the last apical 2-mm section and exhibited the highest roundness values (0.50–0.44), the lowest aspect ratios (1.69–2.03), and a median large apical diameter at the 1-mm level of 0.59 mm. This apical diameter is similar to the diameters of the distal canals of first and second mandibular molars (22) and the apical diameter of the distal aspect of the C3 configuration.

The presence of 2 or 3 independent root canals in the apical third has been previously reported in C-shaped molars (5). This variation is known as the C3 configuration. Examination of the morphometric data from this cross section revealed results that were similar to those that have been found in mandibular molars with separate roots (1). The median minor diameters of the mesial canals were 0.17 and 0.21 mm, and the major diameters were 0.26 and 0.53 mm. Interestingly, the presence of a single large oval canal at the mesial aspect of the C3 configuration was associated with high aspect ratios and low roundness values (Fig. 4). This finding may be expected based on the presence of an isthmus at this level. It is important to note that these apical diameters

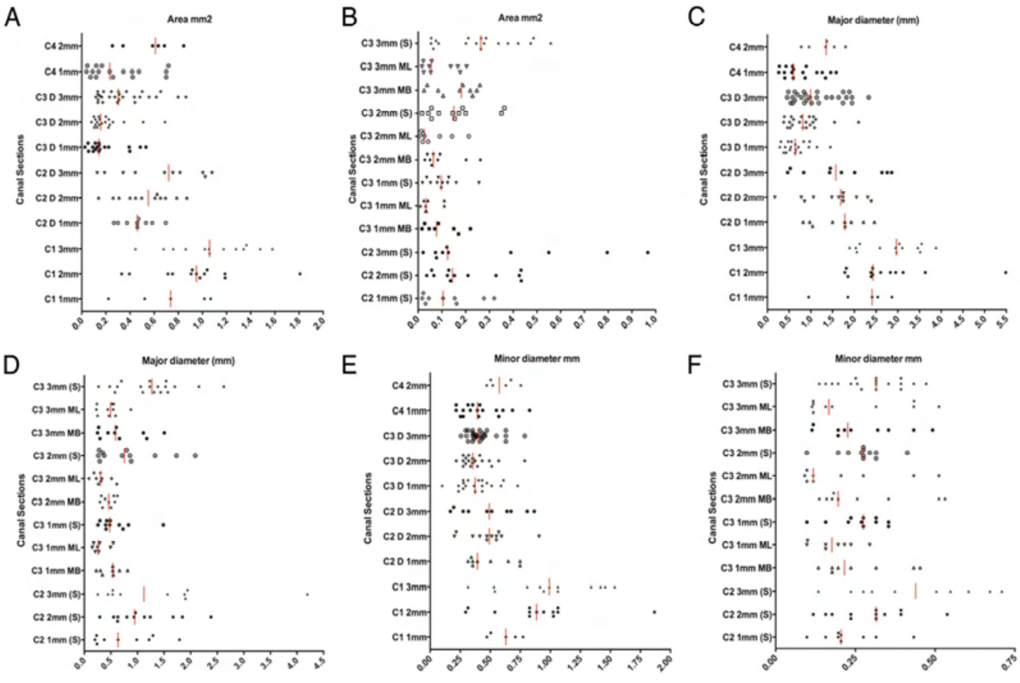

Figure 3. Distributions of the areas (mm^2) and major and minor diameters (expressed in mm) found at the apical third. The values for the C1 and C4 configurations are shown in *A*, *C*, and *E*. The distal aspects of the C2 and C3 configurations are also shown in *A*, *C*, and *E*. The mesial aspects of the C2 and C3 configurations are shown in *B*, *D*, and *F*. D, distal; MB, mesiobuccal; ML, mesiolingual; S, single mesial canal. The *red lines* indicate the medians.

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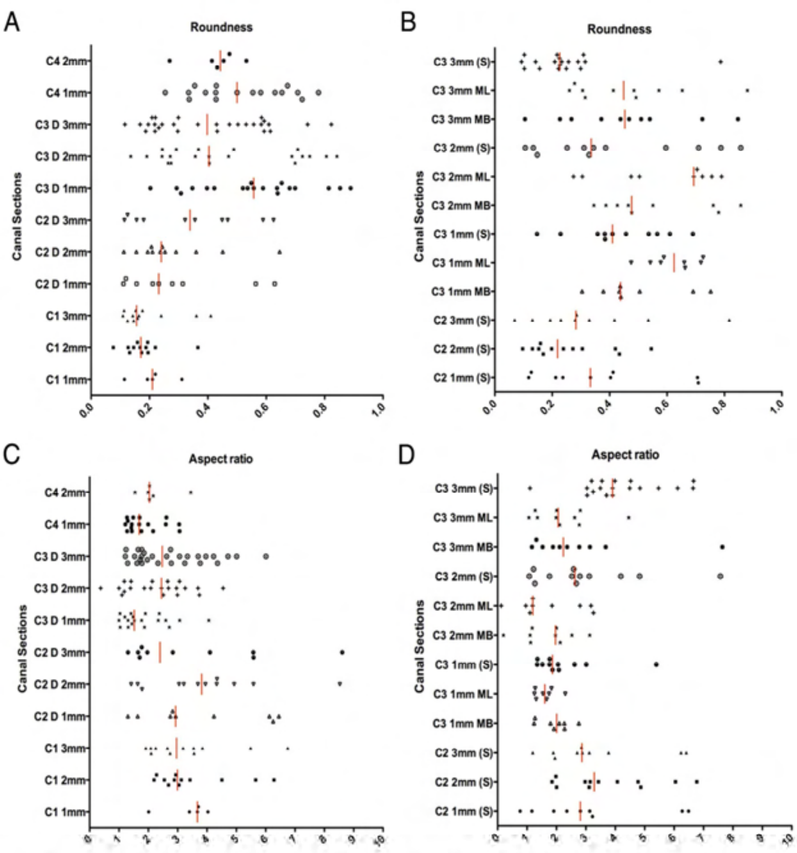

Figure 4. Roundness and aspect ratio distributions found at the apical third. The values for the C1 and C4 configurations are shown in *A* and *C*. The distal aspects of the C2 and C3 configurations are also shown in *A* and *C*. The mesial aspects of the C2 and C3 configurations are shown in *B* and *D*. D, distal; MB, mesiobuccal; ML, mesiolingual; S, single mesial canal. The *red lines* indicate the medians.

were lower than those of the C1 configuration and distal anatomies of the C2 and C3 configurations.

Flat oval canals are characterized by low roundness values or high aspect ratios. A canal can be considered oval when the aspect ratio is greater than 1 or 2 (23, 24). Based on this criterion, nearly all C-shaped canals can be considered oval canals (Fig. 4). It is accepted that large oval canals significantly affect shaping and filling procedures and decrease the quality of root canal procedures. Previous studies have shown that the percentages of uninstrumented areas of C-shaped canals can be between 59% and 66% (24, 25) when rotary instruments are used and 41% when hand instruments or self-adjusting files are used (25, 26).

The diameters and areas found in this study at the distal aspects of the C-shaped molars, including those in the C1 and C4 transverse cross sections, exhibited large apical diameters. One advantage of large apical preparations is that they may favor the irrigation process. However, clinicians should be aware of the small amount of dentin that is present in this type of anatomy. A previous study calculated the remaining wall thickness at the middle portion to be between 0.45 and 0.66 mm (12), which suggests that the ideal instrumentation technique should cut more dentin in the buccolingual direction and cut less dentin in the mesiodistal aspect of the root. In this context, the authors of previous studies do not recommend the enlargement of C-shaped canals above the 30/.06 instrument (27) because of the lack of dentin present in these roots. As previously recommended, after cleaning and shaping with rotary instruments, manual instruments should be applied conservatively with minimal pressure in the canal and directed toward the isthmus areas to enable the cleaning of the recesses and irregularities found in these anatomic types (25). Thus, the use of passive ultrasonic irrigation in combination with sodium hypochlorite is recommended to improve the dissolution of tissue at the isthmus level in the posterior teeth (28).

In summary, the reported data may help clinicians understand the variations in the root canal morphologies of C-shaped mandibular molars in order to improve the cleaning and disinfection procedures and, consequently, the outcome of treatments of canals with these anatomies.

Conclusions

Mandibular second molars with C-shaped canals exhibit a similar tridimensional distribution of anatomic variations. The C1 configuration and the distal aspect of the C2 configuration exhibited higher area values, lower roundness values, and greater apical diameters. Possible methods for addressing the enlargement, shaping, cleaning, and disinfection of these canals were discussed in relation to the anatomic findings of this study.

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

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2.2 Article 2 - Effect on finishing instrumentation using niti hand files on volume, surface area and uninstrumented surfaces in C-shaped root canal systems

The article presented in this Thesis was published in the International Endodontic Journal. Annex C contains the permission letter to include a published article from the *the International Endodontic Journal* in this thesis.

Amoroso-Silva P, Alcalde MP, Duarte MA, de Deus G, Ordinola-Zapata R, Freire LG, et al. Effect of finishing instrumentation using niti hand files on volume, surface area and uninstrumented surfaces in C-shaped root canal systems. International Endodontic Journal. 2016. doi: 10.1111/iej.12660

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Effect of finishing instrumentation using NiTi hand files on volume, surface area and uninstrumented surfaces in C-shaped root canal systems

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Abstract

Amoroso-Silva P, Alcalde MP, Hungaro Duarte MA, De-Deus G, Ordinola-Zapata R, Freire LG, Cavenago BC, De Moraes IG. Effect of finishing instrumentation using NiTi hand files on volume, surface area and uninstrumented surfaces in C-shaped root canal systems. *International Endodontic Journal.*

Aim To assess the effect of 90°-oscillatory instrumentation with hand files on several morphological parameters (volume, surface area and uninstrumented surface) in C-shaped root canals after instrumentation using a single-file reciprocation system (Reciproc; VDW, Munich, Germany) and a Self-Adjusting File System (SAF; ReDent Nova, Ra'anana, Israel).

Methodology Twenty mandibular second molars with C-shaped canals and C1 canal configurations were divided into two groups ($n = 10$) and instrumented with Reciproc and SAF instruments. A size 30 NiTi hand K-file attached to a 90°-oscillatory motion handpiece was used as final instrumentation in both groups. The specimens were scanned using micro-computed tomography after all procedures. Volume, surface area increase and uninstrumented root canal surface were analysed using CTAn software (Bruker-microCT, Kontich, Belgium). Also, the uninstrumented root canal surface was calculated for each canal third. All values were compared between groups using the Mann–Whitney test and within groups using the Wilcoxon's signed-rank test.

Results Instrumentation with Reciproc significantly increased canal volume compared with instrumentation with SAF. Additionally, the canal volumes were significantly increased after 90°-oscillatory instrumentation (between and within group comparison; ($P < 0.05$)). Regarding the increase in surface area after all instrumentation protocols, statistical analysis only revealed significant differences in the within groups comparison ($P < 0.05$). Reciproc and SAF instrumentation yielded an uninstrumented root canal surface of 28% and 34%, respectively, which was not significantly different ($P > 0.05$). Final oscillatory instrumentation significantly reduced the uninstrumented root canal surface from 28% to 9% (Reciproc) and from 34% to 15% (SAF; $P < 0.05$). The apical and middle thirds exhibited larger uninstrumented root canal surfaces after the first instrumentation that was significantly reduced after oscillatory instrumentation ($P < 0.05$).

Conclusions The Reciproc and SAF system were associated with similar morphological parameters after instrumentation of mandibular second molars with C-shaped canals except for a higher canal volume increase in the Reciproc group compared to the SAF. Furthermore, the final use of 90°-oscillatory instrumentation using NiTi hand files significantly decreased the uninstrumented canal walls that remained after Reciproc and SAF instrumentation.

Keywords: C-shaped root canal, micro-CT, nickel–titanium, Reciproc, root canal preparation, Self-Adjusting File.

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Introduction

During root canal treatment, the main objective of mechanical instrumentation is to enlarge the root canal, and along with irrigation protocols, remove pulp tissue, layers of infected dentine and biofilms attached to the root canal surface (Gulabivala *et al.* 2005). However, the anatomical complexities of teeth prevent the instruments and irrigant solutions from reaching the entire canal surface (Busquim *et al.* 2015, Versiani *et al.* 2015), which might influence the outcome of root canal treatment.

Advances in endodontic imaging hardware and software (e.g. micro-computed tomography [micro-CT]) have enabled a more accurate determination of the mechanically untreated (noninstrumented) surface area of canals (Gagliardi *et al.* 2015, Peters *et al.* 2015). Three-dimensional (3D) micro-CT data have revealed that over half of dentinal walls remain unprepared, regardless of the instrumentation system used (Paque *et al.* 2010, Zhao *et al.* 2014), and that the ease of debridement decreases as the complexity of root canal anatomy increases (De-Deus *et al.* 2010).

The C-shaped root canal system is an anatomical variation generally found in mandibular second molars that have a higher prevalence (39–44%) in Asian populations (Zheng *et al.* 2011). Teeth with this variation typically have fused roots with a longitudinal groove in the lingual or buccal aspect of the root, and their internal anatomy is characterized by the presence of complex isthmuses that connect mesial and distal canals throughout the length of the root canal (Fan *et al.* 2004a). The complex anatomy of C-shaped canals makes these teeth difficult to clean and shape: previous studies (Yin *et al.* 2010, Solomonov *et al.* 2012) demonstrated large mechanically untreated areas when instrumenting these canals with rotary, manual or self-adjusting file techniques. To achieve better debridement of C-shaped root canals, some authors recommend the combination of two or more instrumentation systems/techniques (Jerome 1994, Yin *et al.* 2010).

Hand files are essential components of the endodontic arsenal; however, studies into the benefits of their use after the instrumentation of C-shaped root canals are scarce. Therefore, this study assessed the effect of finishing instrumentation with hand files using a 90°-oscillatory motion on final root canal instrumentation in combination with two different instrumentation techniques. Mandibular second molars with C-shaped canals were prepared with either a single-file reciprocating system (Reciproc; VDW, Munich, Germany) or a Self-Adjusting File System (SAF; ReDent Nova, Ra'anana, Israel). Changes in dentine volume, surface area increase and the percentage of the uninstrumented canal walls were compared using micro-CT technology. The hypotheses were that:

- the single-file reciprocating system and SAF have similar morphological parameters (volume, surface area and uninstrumented surface) after instrumentation of C-shaped canals;
- finalizing instrumentation with 90°-oscillatory instrumentation using hand files does not improve the above-mentioned morphological parameters in C-shaped canals prepared with either the Reciproc or SAF systems.

Materials and methods

Sample size estimation

The sample calculation was performed using the G*Power v3.1 for Mac (Heinrich Heine, Universität Düsseldorf) by selecting the Wilcoxon–Mann–Whitney test of the *t*-test family. The data of a previous study of C-shaped canals preparation (Solomonov *et al.* 2012) was used, and the effect size in this study was established (=1.60). 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 10 samples per group were indicated as the ideal size required for noting significant differences.

Specimen selection

After ethics committee approval protocol (CEP #703.054), 20 extracted mandibular second molars with fused roots, C-shaped merging-type canals (Gao *et al.* 2006) and an internal C1 canal configuration (Fan *et al.* 2004a), which is defined as an uninterrupted C-shaped canal with no separation or division, were selected from a pool of 88 C-shaped mandibular second molars. All teeth were mounted on a custom attachment and scanned using a high-definition micro-CT scanner (SkyScan 1174v2; Bruker-microCT, Kontich, Belgium). The parameters used were 50 kV, 800 mA, 360° rotation with a step size of 0.8° and an isotropic resolution of 14.1 μm. Images of all specimens were reconstructed using dedicated software (NRecon v.1.6.3, Bruker-microCT) that provided axial cross sections of the inner structure of the roots in

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bitmap format. A 3D evaluation of the initial volume and surface area of the root canal was performed using CTAn v.1.14.4 (Bruker-microCT).

Based on a morphological evaluation, two groups (n = 10) were selected and paired according to the length, initial volume and surface area of the root canals. Then, a coin toss was used to select which group would be treated with the Reciproc or SAF systems. To confirm an even distribution of the group samples, the preoperative volumes and surface areas of the canals were checked for normality assumption (Shapiro–Wilk test) and statistically compared using the nonparametric Mann–Whitney test, confirming an even distribution of the samples ($P > 0.05$).

Root canal instrumentation

Access cavities were prepared in both groups using high-speed diamond burs, and apical patency was determined by inserting a size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) until it reached the apical foramen. Then, a working length (WL) 1 mm shorter than the apical foramen was established. Glide path preparation with PathFile instruments (013, 016, 019; Dentsply Maillefer) was performed in both groups to WL, followed by use of a size 20 K-file to create a free glide path. A single operator with experience of Reciproc and another with experience of the SAF system performed the instrumentation.

Cleaning and shaping with Reciproc

One R25 Reciproc instrument (size 25, .08 taper; VDW) was used per tooth in the Reciproc Silver motor (VDW) according to the manufacturer's instructions. Instrumentation was conducted in three stages (cervical, middle and apical) using an in-and-out pecking motion, with low amplitude and a brushing motion against the lateral walls of the C-shaped root canals. After instrumentation of each third, the file was removed from the canal and cleaned with gauze. Canal irrigation was performed with 5 mL of 2.5% NaOCl (CloroRio, São José do Rio Preto, Brazil) after the instrumentation of each third using a disposable syringe and 27-G NaviTip needles (Ultradent Products, Inc., South Jordan, UT, USA). Once instrumentation was complete, final irrigation with 5 mL of 2.5% NaOCl was performed and 17% ethylenediaminetetraacetic acid (EDTA) (Biodinamica, Ibiporã, Brazil) was used to remove the smear layer. Finally, EDTA was removed with saline solution and dried with paper points (Dentsply Maillefer).

Cleaning and shaping with the Self-Adjusting File System

One 1.5 mm SAF instrument per canal was used for 4 min until it reached WL in a Gentle Power LP handpiece (KaVo Dental GmbH, Biberach, Germany) at a speed of 5000 revolutions per min (rpm) combined with a vibrating RDT3 head (ReDent Nova), resulting in an in-and-out vibration of 5000 vibrations per min with an amplitude of 0.4 mm. Continuous irrigation with 2.5% NaOCl was applied throughout the procedure using a VATEA peristaltic pump (ReDent Nova) at a flow rate of 5 mL/min connected to the hollow SAF file via a silicone tube. Once instrumentation was complete, the smear layer was removed with 17% EDTA, rinsed with saline solution and dried with paper points.

In both groups, the total volume of irrigation per canal was 20 mL of 2.5% NaOCl. After root canal instrumentation, micro-CT and image reconstruction were conducted using the same initial parameters.

90°-oscillatory instrumentation with NiTi hand files

After Reciproc and SAF instrumentation, a size 30 Nitiflex K-file (Dentsply Maillefer; one per canal) attached to a TEP-Y 90° reciprocating motion handpiece (NSK; Nakanishi Inc., Tokyo, Japan) was used to instrument both groups. The canals were flooded with NaOCl, and the file was inserted passively until WL and activated at 400 rpm with an Endo-Mate DT (NSK; Nakanishi Inc.) electric motor for 30 s using in-and-out and brushing motions towards the isthmus area of the C-shaped canal. Final irrigation with 5 mL of 2.5% NaOCl was performed, and 17% EDTA was used to remove the smear layer. The EDTA was removed with saline solution and dried with paper points. Finally, micro-CT and image reconstruction were performed using the same initial parameters.

Two- and three-dimensional analyses

Reconstructed images captured after instrumentation and after 90°-oscillatory instrumentation were geometrically coregistered with the preoperative data sets using the DataViewer software v1.5.2 (Bruker-microCT, Kontich, Belgium), allowing quantitative comparison of

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the morphological parameters before and after shaping the canals with either Reciproc or SAF and after final complementation with oscillatory instrumentation. The analysis included the binarization of the root canals and measurement of the volume (mm^3) and surface area (mm^2) of the full canals using CTAn v.1.14.4. Also, the uninstrumented surface (static voxel) of the root canal length and isolated regions corresponding to the apical (1–3 mm), middle (3–6 mm) and cervical (6–9 mm) thirds were calculated using CTAn v.1.14.4. This software considers a surface voxel to belong to any given structure when the full voxel belongs to it. Therefore, to be counted as 'affected', at least, one full voxel (i.e. 20 μm) must be registered as removed from the preoperative canal model after superimposition (Solomonov *et al.* 2012). All values were calculated by subtracting the scores for the treated canals from those recorded for their untreated counterparts and then converted into percentages.

Statistical evaluation

All the morphological parameters evaluated in this study from all the instrumentation stages were checked for normality using the Shapiro–Wilk test. Due to the absence of normality, the median percentages of volume increase, surface area increase and uninstrumented surface area were compared within groups (Reciproc versus oscillatory) and (SAF versus oscillatory) using the Wilcoxon's signed-rank test. Comparison between groups [(Reciproc versus SAF) and (oscillatory instrumentation from the Reciproc group versus oscillatory instrumentation from the SAF group)] were compared with the Mann–Whitney test. All values were processed using Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA) and expressed as median, minimum and maximum values (ranges). The significance level was set at 5%.

Results

The preoperative volumes (mm^3) and surface areas (mm^2) of the canals were not significantly different between the Reciproc [15.39 (8.06–36.02) mm^3 and 90.97 (56.16–164.3) mm^2, respectively] and SAF [13.56 (9.88–35.26) mm^3 and 104.50 (44.59–143) mm^2, respectively] groups ($P > 0.05$), confirming the even distribution of preoperative samples between groups.

Table 1 shows the percentage volume increase, surface area increase and uninstrumented surface area after use of the Reciproc and SAF instrumentation techniques and after the final oscillatory instrumentation in both groups. Reciproc instrumentation significantly increased canal volume in C-shaped root canals compared with SAF ($P < 0.05$). Additionally, the canal volumes were significantly increased after complementary 90°-oscillatory instrumentation with NiTi hand files (between and within group comparison; $P < 0.05$). Regarding the increase in surface area after all instrumentation protocols, statistical analysis only revealed significant differences in the within groups comparison ($P < 0.05$).

Approximately 28% and 34% of the root canal surface remained uninstrumented after initial instrumentation with Reciproc and SAF, respectively, without statistical significance ($P > 0.05$). However, in the intragroup comparison, 90°-oscillatory instrumentation with NiTi hand files significantly reduced the uninstrumented canal surface area from 28% to 9% (Reciproc) and from 34% to 15% (SAF; $P < 0.05$; Fig. 1). Moreover, after 90°-oscillatory instrumentation, no statistical differences between groups were evident ($P > 0.05$).

Table 2 shows the percentage uninstrumented canal surface in the thirds evaluated. All root canal thirds evaluated had higher percentages of uninstrumented canal surface area, especially the apical third (1–3 mm), after Reciproc and SAF instrumentation, which were significantly reduced after oscillatory instrumentation ($P < 0.05$; Fig. 2).

Discussion

The internal anatomy of C-shaped root canals is complex, featuring many variations along their length

Table 1 Median, minimum and maximum values of root canal volume, area increase and uninstrumented canal area percentages after different instrumentation protocols

	After 1st instrumentation	After oscillatory instrumentation
%Volume increase		
Reciproc	20.08 (6.33–44.20)^{aA}	23.28 (10.49–48.50)^{bA}
SAF	7.39 (4.97–29.82)^{aB}	11.97 (5.33–32.45)^{bB}
% Area increase		
Reciproc	3.74 (1.05–7.25)^{aA}	6.62 (1.51–11.73)^{bA}
SAF	1.72 (0.55–9.33)^{aA}	3.77 (1.17–10.58)^{bA}
% Uninstrumented surface		
Reciproc	28.19 (6.21–63.81)^{aA}	9.65 (4.13–41.49)^{bA}
SAF	34.79 (9.74–57.75)^{aA}	15.01 (4.29–44.14)^{bA}

Different lower-case letters in rows represent significantly differences between the Reciproc and oscillatory complementation or the SAF and oscillatory complementation. Different upper-case letters in columns represent significant differences between the groups.

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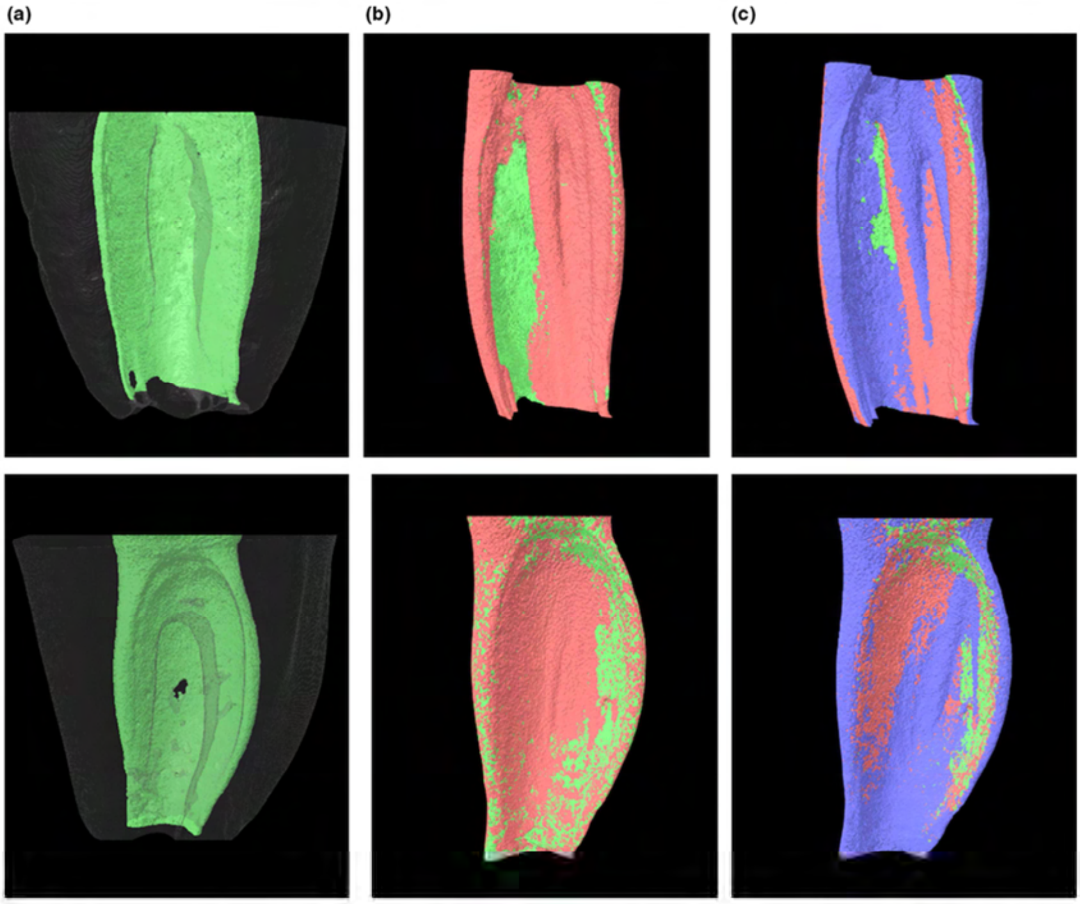

Figure 1 Representative three-dimensional images of mandibular second molars with C-shaped canals before and after root canal preparation with Reciproc (top) or the Self-Adjusting File System (bottom). (a) Preoperative reconstructions. (b) Superimposed root canals before (*green*) and after (*red*) preparation with Reciproc or the Self-Adjusting File System, respectively. (c) Superimposed root canals before (*green*) and after (*red*) preparation with Reciproc or SAF, respectively, and complementary 90°-oscillatory instrumentation with NiTi hand files (*blue*).

(Fan *et al.* 2004b, Amoroso-Silva *et al.* 2015). In this study, a method of sample selection described previously was performed to achieve a well-balanced distribution of specimens. The process was based on the selection of second mandibular molars with C-shaped roots with merging-type canals according to the classification by Gao *et al.* (2006) and an internal C1 cross-sectional configuration (Fan *et al.* 2004a) along the root length (an interrupted C with no separation or division between the canals). Also, similar lengths and internal canal volumes were chosen, resulting in an adequate distribution of specimens between the groups, thus providing a sound basis for statistical comparison of the instrumentation protocols. Furthermore, a major advantage of using micro-CT technology in this study was that the same specimen could be evaluated at different stages of the root canal treatment and served as its own control (De-Deus *et al.* 2016).

Several studies using micro-CT have reported that a high percentage of untreated canal wall is present regardless of the anatomical complexities or instruments used (Peters *et al.* 2010, Yin *et al.* 2010, Paque & Peters 2011, Versiani *et al.* 2013, Zhao *et al.* 2014). In this study, Reciproc and SAF instrumentation yielded a similar percentage of the

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Table 2 Median, minimum and maximum values of uninstrumented surface percentages by thirds after instrumentation protocols used in mandibular second molars with C-shaped root canals

	After 1st instrumentation	After oscillatory instrumentation
% Uninstrumented surface		
Reciproc (1–3 mm)	40.90 (4.25–82.61)^{aA}	11.16 (1.43–33.84)^{bA}
SAF (1–3 mm)	35.56 (1.88–77.79)^{aA}	15.37 (1.88–52.50)^{bA}
Reciproc (3–6 mm)	28.79 (2.51–60.00)^{aA}	8.21 (0.44–31.62)^{bA}
SAF (3–6 mm)	38.52 (13.60–75.55)^{aA}	21.76 (1.42–51.43)^{bA}
Reciproc (6–9 mm)	14.06 (3.68–57.91)^{aA}	3.22 (0.36–43.59)^{bA}
SAF (6–9 mm)	36.24 (8.00–61.59)^{aA}	17.56 (4.29–54.14)^{bA}

Different lower-case letters in rows represent significant differences between the Reciproc and oscillatory complementation or the SAF and oscillatory complementation. Different upper-case letters in columns represent significant differences between the groups.

uninstrumented areas. Therefore, the first hypothesis was partially accepted. In this study, 34% of the walls of C-shaped canals remained unaffected after SAF instrumentation, which is consistent with the findings of a previous study (Solomonov *et al.* 2012). Although the results showed median uninstrumented root canal surface areas (US) of 28% and 34%, higher percentages in the maximum values (Table 1) were observed, suggesting that some C-shaped root canals were difficult to clean and shape with either Reciproc or SAF instruments. This difficulty could relate to the complex anatomy of these teeth and variations in the size or width of the isthmuses that connect the main canals. The C1 internal canal configuration of C-shaped canals can present narrow isthmuses that may limit instrumentation with SAF: the contraction capacity of the file may prevent it from entering the isthmus area and cutting the dentine with the abrasive surface of the file or, in the case of the Reciproc file, decrease its cutting ability. Indeed, a previous study (Siqueira *et al.* 2013) showed that, during debridement, anatomical complexities such as isthmuses are hardly affected by SAF and other rotary instrumentation systems.

Regarding volume changes after root canal shaping with Reciproc, a significant increase in canal volume was evident compared with the SAF group (20% versus 7%). This result can be attributed to the instrument taper, diameter and effective cutting ability of the reciprocating system (Plotino *et al.* 2014). At least three previous studies have found that SAF instruments remove less dentine because of instrument characteristics that include an abrasive metal surface used in a back-and-forth grinding motion on canal walls (Metzger *et al.* 2010, Siqueira *et al.* 2013, Versiani *et al.* 2013). The advantage of an increase in canal volume is that it improves the irrigation process (Boutsioukis *et al.* 2010); however, even though in this study the SAF system did not significantly increase the canal volume, a major advantage of this system is the constant irrigation delivered throughout the instrumentation procedure with the VATEA pump and its hollow file. Furthermore, the use of large file tapers in teeth with this anatomy can remove excessive amounts of dentine, which could weaken the tooth structure (Chai & Thong 2004, Cheung & Cheung 2008) or cause strip perforations towards the C-shaped groove where dentine thickness is minimal (Gao *et al.* 2006). These factors are significant given that both instrument techniques present a similar percentage of uninstrumented canal area.

The second result of this study is that finalizing instrumentation using NiTi hand files at a 90°-oscillatory contra-angle significantly decreases the remaining uninstrumented canal walls and the other studied parameters in C-shaped mandibular molars, rejecting our second hypothesis. Instrumentation with the NSK reciprocating handpiece attached to an electric motor was chosen to standardize the movement kinematics because this is smoother than the motion provided by similar compressed air-driven motors or even manual filing (Wagner *et al.* 2006). Furthermore, NiTi hand files were chosen for their smaller taper (0.02), increased flexibility and reduced dentine-cutting ability compared to stainless steel hand files (Gambill *et al.* 1996), promoting apical size enlargement with only a minor risk of overinstrumentation in the middle and cervical thirds. This was confirmed by the small difference in volume and surface area after the use of these files, suggesting that this is a safe complementary instrumentation technique that does not remove excessive amounts of dentine from the root canal.

A previous study (Amoroso-Silva *et al.* 2015) reported that the apical 3-mm C-shaped root canals with a C1 configuration often have a large area and apical diameter. In the present study, the limitations of the Reciproc and SAF systems in preparing the

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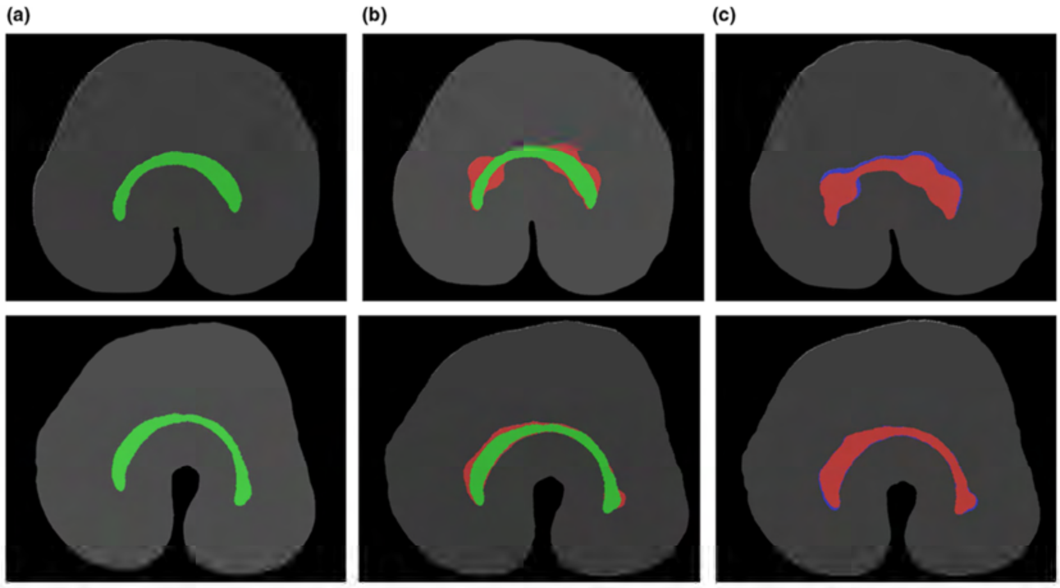

Figure 2 Representative two-dimensional cross sections of superimposed root canals prepared with Reciproc (top) and the Self-Adjusting File System (bottom). (a) Preoperative (*green*), (b) postoperative (*red*) and (c) complementary 90°-oscillatory instrumentation with NiTi hand files (*blue*) at 3 mm from the apex.

apical third of teeth with this anatomy were evident in the uninstrumented surface analysis of different thirds (Table 2). C-shaped and long oval canals in posterior teeth represent difficult clinical situations with which the dentist has to deal during clinical practice. In this study, a possible strategy to enhance root canal instrumentation in C-shaped canals using either the Reciproc or SAF systems is presented. Furthermore, in addition to mechanical instrumentation, it is important to consider complementary irrigation protocols, such as passive ultrasonic irrigation, to remove residual tissue, dentine debris or biofilms from the anatomical irregularities of these teeth persisting after mechanical instrumentation (Burleson *et al.* 2007).

Conclusions

The Reciproc and SAF system were associated with similar morphological parameters after instrumentation of mandibular second molars with C-shaped canals except for higher canal volume increase in the Reciproc group compared to the SAF. Furthermore, the final use of 90°-oscillatory instrumentation using NiTi hand files significantly decreased the uninstrumented canal walls that remained after Reciproc and SAF instrumentation.

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Conflict of interest

The authors have stated explicitly that there are no conflict of interests in connection with this article.

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3 Discussion

3 DISCUSSION

The first report in literature of a C-shaped canal was in 1979 (COOKE; COX, 1979), and after that date, other anatomical studies using histological techniques (MELTON; KRELL; FULLER, 1991), sectioning of the samples (SEO; PARK, 2004), radiographic examination (WANG et al., 2012), dental staining (GULABIVALA et al., 2001), microcomputed tomography (FAN et al., 2004a) among others, have been performed. Currently, the greatest advantage of using the Micro-CT technology over previous techniques is that it provides quantitative and qualitative description of the root canal anatomy (ORDINOLA-ZAPATA et al., 2013), detailed information of the anatomical structures, additional morphological analysis among several other features without destroying the samples (CHEUNG, L.H.; CHEUNG, 2008; FAN et al., 2004a), which allowed us to reuse the specimens for the posterior instrumentation investigation.

In this study, by using micro-CT technology, a detailed description of the morphological and morphometric aspects of fused mandibular second molars evidenced the high variability and complexity of this root canal system. A total of 88 extracted second mandibular molars with fused roots where used in this study. However, after the initial scanning and reconstruction, the qualitative analysis showed that some roots possessed shallow radicular grooves or even no grooves. Moreover, in some cases, no C1, C2 or C3 cross-sectional types could be observed. So, 36 specimens were excluded from the further anatomical analysis. Regarding the cross-sectional classification found in this study, the merging-type canals exhibited a high prevalence of C1 and C4 configurations at the 3-mm apical location, whereas the symmetric C-shaped molars exhibit a high prevalence of the C3 configuration. Additionally, the asymmetric type exhibited a high prevalence of C2 and C3 configurations at the apical third. These results are in agreement with those of a previous study conducted in a Chinese population (FAN et al., 2004a).

Furthermore, it has been reported that large oval shape canals can be difficult to clean and shape properly with manual or rotatory files (DE-DEUS et al., 2010; WU; VAN DER SLUIS; WESSELINK, 2003). Considering the several variations in the C-shaped canal diameters and geometry, including oval canals of the C4 and narrow isthmus in the C1 and C2 configurations, the clinician should pursue the combinations of different techniques that can promote satisfactory debridement of these complex anatomical types. The use of new instruments such as the self-adjusting file can aid in the shaping protocols, leaving less uninstrumented canal area in these type of teeth (SOLOMONOV et al., 2012). Moreover, the use of diverse disinfection procedures such as passive or continuous ultrasonic irrigation (AL-JADAA et al., 2009; BURLESON et al., 2007), and negative apical pressure (HOWARD et al., 2011), can contribute to the removal of debris and dissolution of vital or necrotic tissue from the anatomical complexities of the root canal system.

The second part of this study focused on instrumentation of C-shaped canals. Due to the large variability of the internal anatomy of the C-shaped canals, achieving a well balance distribution of the samples was hard to attain. So, standardizing the sample in only one type of root canal (Merging) and also only one cross-section configuration (C1) yield us 20 specimens with similar anatomical characteristics provided a sound basis for statistical comparison of the instrumentation protocols. Furthermore, the merging type canals with C1 configuration was chosen for the instrumentation analysis because it presented the largest apical diameters and lower roundness values than the rest of anatomies and it is considered a big challenge in terms of cleaning and shaping.

A major advantage of using Micro-CT technology in this analyses was that the same specimen could be evaluated at different stages of the root canal treatment and served as its own control (De-Deus *et al.* 2016). The results of this study revealed that regardless of the instrumentation technique used, both Reciproc and SAF systems had difficulty to clean and shape all the canal surface area leaving high amount of uninstrumented surface areas 28 % vs 34 %. This difficulty might be related to the limitations of both instruments to enter and cut dentine inside the isthmuses and also in the lateral fins of the C1 canal configuration. Furthermore, these values were lower than that of similar studies, (SOLOMONOV et al., 2012; YIN et al., 2010) with C-shaped canals probably because the distribution of the specimens was paired only based on similar morphologies of any anatomical types (Merging, symmetrical and asymmetrical). Certainly, is difficult to collect these type of teeth which possesses a high anatomical variability.

The present instrumentation study was limited to evaluating the hard-tissue changes that occur during all procedures. Even though the wall thickness measurement was not assessed in this study, previous reports (CHAI; THONG, 2004; GAO et al., 2006) related that the dentinal thickness in the lingual surface of the C-shaped molars was minimum due to its deep groove, showing low values of wall thickness of (0.27, 0.74) mm in the apical, (0.45, 0.95) mm middle and (0.85, 1.49) mm cervical portion, respectively. the authors of a previous study do not recommend the enlargement of C-shaped canals above the 30/.06 instrument (CHEUNG, L.H.; CHEUNG, 2008) because diameters and tapers beyond that could potentially weaken the tooth structure and increase the risk of strip perforation during instrumentation (KATO et al., 2014). The results of this study revealed that a possible clinical strategy to achieve better instrumentation results in C-shaped canals is to use NiTi hand files at a 90º-oscillatory contra-angle to finish the instrumentation protocol. It significantly decreased the remaining uninstrumented canal walls in C-shaped mandibular molars and enlarged the canal walls safely with no risk of over-instrumentation.

Furthermore, the results of the anatomical analysis revealed large apical diameters and low roundness values at 3 from the apex. Moreover, (CHEUNG, G.S.; YANG; FAN, 2007) related that his apical area is considered critical in terms of cleanliness due to its high anatomical variability. In this study, this region was particularly hard to instrument by both the instruments as revealed by the Micro-CT instrumentation evaluation. So, it is imperial that after the first instrumentation protocol a manual filling is performed to achieve better debridement in clinical practice.

Finally, when trying to identify a C-shaped molar, the periapical radiography is the most common tool used for diagnosis, but it has the limitation of showing a 2D vision of a structure only. Hence, when a shallow groove is present the C-shaped configuration may not be detectable in the radiograph because of superimposition with the image of the single canal in these types of teeth (FAN et al., 2004b). In contrast, there is less difficulty to identify a C-shaped canal when a deep longitudinal groove is present. Another useful tool of 2D and also 3D imaging alternative would be the cone-beam computed tomography (CBCT), to distinguish accurately the internal and external anatomy of this type of teeth (SILVA et al., 2013). Additionally, the use of surgical operative microscope can be of significant aid to identify and treat a C-Shaped canal (DE CARVALHO; ZUOLO, 2000).

4 Conclusions

4 CONCLUSIONS

- Mandibular second molars with C-shaped canals exhibit a similar tridimensional distribution of anatomical variations. The C1 configuration and the distal aspect of the C2 configuration exhibited higher area values, lower roundness values and greater apical diameters. Possible methods for addressing the enlargement, shaping, cleaning and disinfection of these canals were discussed in relation to the anatomical findings of this study.

- The Reciproc and SAF system presented similar morphological parameters after instrumentation of mandibular second molars with C-shaped canals except for higher canal volume increase in the Reciproc group compared to the SAF. Furthermore, the final use of 90º-oscillatory instrumentation using NiTi hand files significantly decreased the uninstrumented canal walls that remained after Reciproc and SAF instrumentation.

- The combination of two instrumentation systems/techniques proved to be effective in achieving better instrumentation results in C-shaped mandibular second molars.

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Appendixes

APENDIX A - DECLARATION OF EXCLUSIVE USE OF THE ARTICLE IN THESIS

We hereby declare that we are aware of the article *Micro–computed Tomographic Analysis of Mandibular Second Molars with C-shaped Root Canals* will be included in the Thesis of the student (Pablo Andrés Amoroso Silva) and may not be used in other works of Graduate Programs at the Bauru School of Dentistry, University of São Paulo.

Bauru, May 30th 2016.

Pablo Andrés Amoroso Silva
Author

Signature

Ronald Ordinola-Zapata
Author

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Marco Antonio Hungaro Duarte
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James Leo Gutmann
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Aldo del Carpio Perochena
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Clovis Monteiro Bramante
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Ivaldo Gomes de Moraes
Author

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APENDIX B - DECLARATION OF EXCLUSIVE USE OF THE ARTICLE IN THESIS

We hereby declare that we are aware of the article ***Effect of finishing instrumentation using niti hand files on mechanical debridement quality in c-shaped root canal systems*** will be included in the Thesis of the student (Pablo Andrés Amoroso Silva) and may not be used in other works of Graduate Programs at the Bauru School of Dentistry, University of São Paulo.

Bauru, May 30th 2016.

Pablo Andrés Amoroso Silva
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Signature

Murilo Priori Alcalde
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Ronald Ordinola-Zapata
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Marco Antonio Hungaro Duarte
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Bruno Cavalini Cavenago
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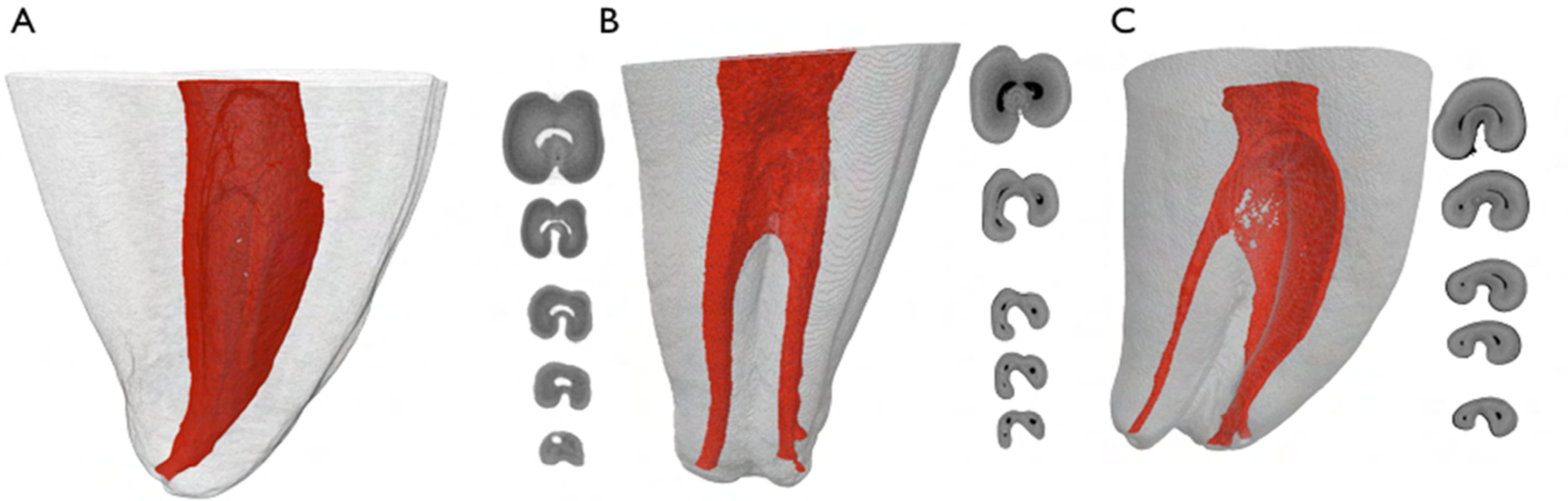
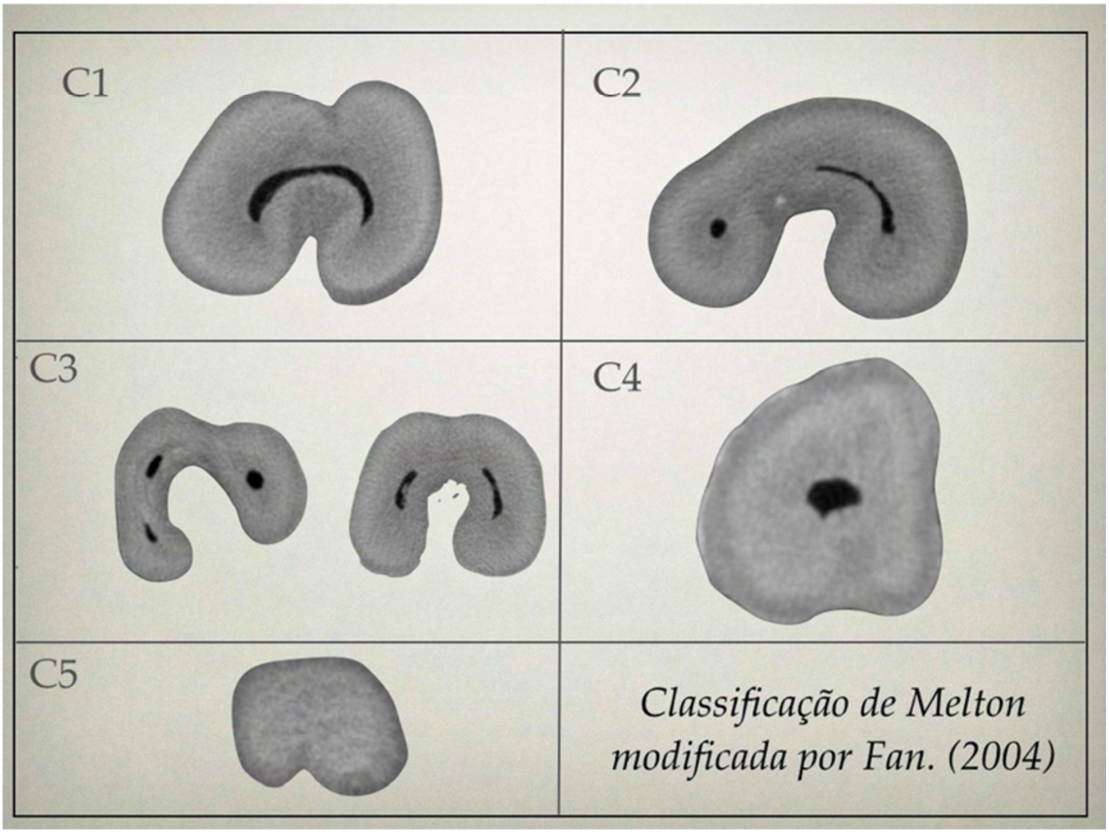
APENDIX C – Representative 3D images and their internal cross-sections at 1,2,3 mm, middle third and cervical third of C-shaped mandibular second molars according to Gao et al: Merging (A), Symmetrical (B), Asymmetrical C).



APENDIX D – Representative 2D images of Melton’s internal cross-sections classification modified by Fan et al.



Annexes

ANNEX A – Ethics committee approval

FACULDADE DE
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PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação da anatomia interna de segundos molares inferiores com raízes fusionadas, da instrumentação e técnicas de retratamento com novos sistemas mecanizados de instrumentação.

Pesquisador: Pablo Andres Amoroso Silva

Área Temática:

Versão: 2

CAAE: 31088014.0.0000.5417

Instituição Proponente: Universidade de Sao Paulo

Patrocinador Principal: MINISTERIO DA EDUCACAO

DADOS DO PARECER

Número do Parecer: 703.054

Data da Relatoria: 25/06/2014

Apresentação do Projeto:

O projeto de pesquisa intitulado como "Avaliação da anatomia interna de segundos molares inferiores com raízes fusionadas, da instrumentação e técnicas de retratamento com novos sistemas mecanizados de instrumentação" da autoria de Pablo Andres Amoroso Silva, sob orientação do Prof. Dr. Ivaldo Gomes de Moraes.

Um total de 88 segundos molares inferiores extraídos com raízes fusionadas e sulcos radiculares, serão selecionados.

Será realizado o estudo da anatomia interna do sistema de canais radicares, a Classificação tridimensional a partir das imagens escaneadas previamente, usando o programa de segmentação e modelagem de superfície automático v.1.12 CTAN (Bruker-microCT) e o software de visualização de dados CTVol v.2.2.1(Bruker-microCT) será utilizado para a visualização e avaliação qualitativa dos espécimes. Será realizada a classificação e análise morfométrica das seções transversais do canal radicular a 1, 2, e 3 mm do ápice radicular. Além disso, o terço médio e a porção cervical da raiz também serão considerados.

Os dentes serão obturados com cimento endodôntico acrescentado de rodamina B, para uma vez tomado presa, ser retratados e avaliar a capacidade de remoção de material obturador com os mesmos sistemas(reciproc e SAF) por meio de Micro-CT. Finalmente estes dentes serão novamente

obturados com cimento endodôntico acrescentado de corante de fluorescina e cortados com maquina ISOMET para serem avaliados na microscopia confocal de varredura a laser a interface cimento/dentina com a finalidade de mensurar os resíduos de cimento que não foram removidos quando do retratamento. Para obter os resultados dos estudos anatômicos, o intervalo dos valores médios, mínimos e máximos serão processados
mediante o programa Prisma 5.0 (GraphPad Software Inc, La Jolla, CA, USA).Os demais resultados serão submetidos aos testes de D'Agostino e Pearson para verificação de distribuição normal. Em caso de ausência de normalidade, será empregado o teste não- paramétrico de Kruskal-Wallis. Havendo normalidade será empregado o teste paramétrico de ANOVA. Para todos os testes será considerado o nível de significância de 5%.

Objetivo da Pesquisa:
Analisar os aspectos morfológicos e morfométricos da anatomia interna do sistema de canais radiculares com formato em C, dos segundos molares inferiores com raízes fusionadas, por meio de micro tomografia computadorizada (micro-CT;)
Avaliar a área, volume e espessura mínima de dentina radicular remanescente nas paredes do canal radicular antes e após a instrumentação dos canais dos
três tipos anatômicos de molares em C, (simétrico, assimétrico e convergente), por meio do sistema de instrumentação mecanizada com movimento Reciproco (Reciproc) complementada pela instrumentação manual, e da Self Adjusting File (SAF) com complementação de instrumentação manual;
Analisar a capacidade de retratamento (remoção do material obturador), de ambos os sistemas, por meio de microtomografia computadorizada;
Avaliar por meio de microscopia confocal a quantidade de cimento remanescente após a remoção do material (retratamento), nas paredes de dentina utilizando o sistema de instrumentação reciproca e SAF. Além disso, comparar o restante do material junto com a nova obturação.

Avaliação dos Riscos e Benefícios:
instrumentação manual, e da Self Adjusting File (SAF) com complementação de instrumentação manual;
Analisar a capacidade de retratamento (remoção do material obturador), de

ambos os sistemas, por meio de microtomografia computadorizada;
Avaliar por meio de microscopia confocal a quantidade de cimento remanescente após a remoção do material (retratamento), nas paredes de dentina utilizando o sistema de instrumentação reciproca e SAF. Além disso, comparar o restante do material junto com a nova obturação.

Comentários e Considerações sobre a Pesquisa:

A pesquisa em questão apresenta descrição clara do desenho e dos procedimentos que serão utilizados no projeto, apresentando pertinência e valor científico do estudo proposto.

Considerações sobre os Termos de apresentação obrigatória:

toda documentação necessária a aprovação do projeto de pesquisa foi devidamente apresentada.

Recomendações:

Projeto de pesquisa anteriormente analisado pelo CEP e considerado pendente pois:
Na justificativa apresentada pelo pesquisador, para a dispensa do termo de consentimento Livre Esclarecido, solicitou-se que o mesmo substituísse a citação " na Resolução n° 196 de 10 de outubro de 1996", por Resolução 466 de 12 de dezembro de 2012".
A carta de encaminhamento ao CEP, fazia referencia ao Professor Drº Flavio Augusto Cardoso de Faria com Coordenador do CEP/FOB. Solicitado a correção deste documento com adequação ao Nome da Coordenadora atual deste Comitê.

Conclusões ou Pendências e Lista de Inadequações:

O pesquisador reapresentou o Projeto para análise onde esclareceu as dúvidas citadas no parecer anterior, sendo assim o parecer favorável pela aprovação do projeto de pesquisa "Avaliação da anatomia interna de segundos molares inferiores com raízes fusionadas, da instrumentação e técnicas de retratamento com novos sistemas mecanizados de instrumentação"

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

Esse projeto foi considerado APROVADO na reunião ordinária do CEP de 25.6.2014, com base nas normas éticas da Resolução CNS 466/12. Ao término da pesquisa o CEP-FOB/USP exige a

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BAURU, 30 de Junho de 2014

Assinado por:
Izabel Regina Fischer Rubira Bullen
(Coordenador)

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ANNEX B – Permission letter to include a published article from the Journal of Endodontics in this thesis

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