UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

VICTOR DE MORAES CRUZ

Evaluation of irrigation diffusibility by different irrigation techniques: Analysis in µCT

Avaliação da difusibilidade de irrigante por diferentes técnicas de agitação: Análise em µCT

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Dissertação constituída por artigo apresentada a Faculdade de Odontologia de Bauru da Universidade de São Paulo para obtenção do título de Mestre em Ciências no Programa de Ciências Odontológicas Aplicadas, na área de concentração Endodontia.

Orientador: Prof. Dr. Rodrigo Ricci Vivan

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ABSTRACT

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INTRODUCTION: This ex vivo study objective was evaluate the irrigant penetration by different methods agitation, measuring the your diffusion in the root canal of mesiobuccal roots of maxillary molars.

MATERIAL AND METHODS: Conventional irrigation (syringe and needle), Endoactivator (Dentsply Maillefer, Ballaigues, Switzerland), Easy Clean (Easy Equipamentos, Belo Horizonte, MG, Brazil), RinsEndo (Dürr Dental GmbH, Bietigheim-Bissingen, Germany), PUI, CUI and Xp-Clean (MK Life, Porto Alegre, Brazil) were tested. Seventy maxillary molars were used. The teeth were scanned in computerized micromotomography (SkyScan 1174) in two moments. The initial scanning was performed to determine the total root canals volume. The different irrigation agitation protocols were performed and then scanned again to obtain the canals volume filled by the irrigant. Statistical analysis with a significance level of 5%.

RESULTS: The PUI, CUI and Xp-Clean groups presented a percentage of total filling significantly higher than the conventional groups, Endoactivator and RinsEndo ($P < 0.05$). No significant difference was found between the EasyClean group and the other groups ($P < 0.05$). In the apical filling there was no significant difference between the groups ($P < 0.05$).

CONCLUSIONS: Based on the proposed methodology and results, it can be concluded that PUI, CUI and XP-Clean improve the canals filling. Regarding apical filling, the methods presented the same results.

Keywords: endodontics; irrigation; computerized microtomography.

RESUMO

RESUMO

Avaliação da difusibilidade de irrigante por diferentes técnicas de agitação: Análise em µCT

INTRODUÇÃO: O objetivo deste estudo foi avaliar *ex vivo* o efeito de diferentes métodos de agitação do irrigante na difusão do mesmo pelo canal radicular de raízes mésio-vestibulares de molares superiores.

MATERIAL E MÉTODOS: Foram testados irrigação convencional (seringa e cânula), Endoactivator (Dentsply Maillefer, Ballaigues, Suíça), Easy Clean (Easy Equipamentos, Belo Horizonte, MG, Brazil), RinsEndo (Dürr Dental GmbH, Bietigheim-Bissingen, Alemanha), IUP, IUC e Xp-Clean (MK Life, Porto Alegre, Brasil). Foram utilizados 70 molares superiores extraídos. Os dentes foram escaneados em micromotomografia computadorizada (SkyScan 1174) em dois momentos. O escaneamento inicial foi realizado para determinar o volume total dos canais. Foram realizados os diferentes protocolos de agitação do irrigante e, então, escaneados novamente para obtenção do volume do canal preenchido pelo irrigante. Análise estatistica com nível de significância de 5%. RESULTADOS: Os grupos IUP, IUC e Xp-Clean apresentaram porcentagem de preenchimento total significativamente maiores que os grupos convencional, Endoactivator e RinsEndo ($P<0.05$). Não foi encontrada diferença significativa entre o grupo EasyClean e os demais grupos ($P<0,05$). No preenchimento apical não houve diferença significante entre os grupos ($P<0,05$).

CONCLUSÕES: Baseado na metodologia proposta e nos resultados encontrados, pode-se concluir que IUP, IUC e XP Clean melhoram o preenchimento total do canal. Com relação ao preenchimento apical, os métodos apresentaram os mesmos resultados.

Palavras-chave: endodontia; irrigação; microtomografia computadorizada.

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1 INTRODUCTION

1 INTRODUCTION

The anatomical complexity of the root canal system is described by several authors (WEINE *et al.,* 1969; VERTUCCI, 1974, 1984, 2005; TEIXEIRA *et al.,* 2003; KIM *et al.,* 2013; MARCELIANO-ALVES *et al.,* 2016; DE FREITAS *et al.,* 2017). The irrigation in endodontics is a vital step for the correct root canal system cleaning and disinfection (ZEHNDER *et al.,* 2006; HAAPASALO *et al.,* 2010; ORDINOLA-ZAPATA *et al.,* 2014; ALVES *et al.,* 2015; VIVAN *et al.,* 2016).

The permanency of necrotic tissues, microorganisms, and their products, and dentin residues from instrumentation directly interfere with the treatment quality (PRADO *et al.,* 2013; FREIRE ET AL. 2015; TOPÇUOĞLU *et al.,* 2015). In anatomically complex cases, removing undesirable products lays only in irrigation because they are inaccessible to instruments (PETERS *et al.,* 2001). The complex areas such as isthmus are the major problems. Narrow and irregular extensions still contain microorganisms and dentin debris after the physical-mechanical preparation (ADKOCK *et al.,* 2011; NEELAKANTAN *et al.,* 2016). Thus, incomplete cleaning may lead to failure (RICCUCI *et al.,* 2010; ZEHNDER et al. 2015). Accordingly, two main aspects should be observed: the irrigant diffusion throughout the root canal and the penetration into areas that are inaccessible to the instruments (DE GREGÓRIO *et al.,* 2010; SPOORTHY *et al.,* 2013; KANUMURU *et al.,* 2015).

The traditional irrigation method, cannula, and syringe (HOLLIDAY et al. 2014), evolved by advances in the manufacturing process of instruments, therefore, more flexible and smaller-caliber cannulas permitted irrigation in curvature situations (ZMENER *et al.,* 2009). However, this method is still limited to cleaning the apical third and isthmus areas (CHOW *et al.,* 1983; WU *et al.,* 1995; THOMAS *et al.,* 2014). Among the possibilities to optimize irrigation, the Endoactivator, a sonic device (Dentsply Maillefer, Ballaigues, Switzerland), improves irrigation through the transmission of vibrations by flexible polymer inserts. They are presented in three different diameters (15/.02, 25/.04 and 35/.04) that promote irrigant agitation within the root canal and making cleaning more effective than conventional irrigation (MANCINI *et al.,* 2005, 2013), increasing the organic material dissolution (CONDE *et* *al.,* 2016). Besides, the RinsEndo® device (Dürr Dental GmbH, Bietigheim-Bissingen, Germany) is a mechanical system that uses pressure-suction technology for root canal irrigation (7.2 ml/min) with 96 action cycles per minute (1.6 Hz). It proved to be superior to conventional irrigation increasing the irrigant penetration in dentin (HAUSER *et al.,* 2007) and reducing the bacteria number (McGILL *et al.,* 2008), however some studies pointed it to be more ineffective than the dynamic conventional irrigation (VIVAN *et al.,* 2010), and controversial when compared to other techniques such as passive ultrasonic irrigation PUI (RÖDIG *et al.,* 2010; CACHOVAN *et al.,* 2013; TOLJAN *et al.,* 2016). PUI used at a very high frequency (>20Khz) lead the insert moves the irrigant through an acoustic transmission phenomenon promoting solution displacement (AHMAD et al, 1987). Another technique, the acoustic hydrodynamic cavitation uses negative pressure in the opposite displacement direction face of the insert at high speed (JENSEN *et al.,* 1999; PLOTINO *et al.,* 2007; MACEDO *et al.,* 2014). This negative pressure vaporizes the water producing many bubbles, resulting in a phenomenon called transitory cavitation. The bubbles collapse causing a shock wave against the canal walls favoring waste and small material fragments release. The phenomenon produced is designated erosion (cavitation corrosion) (REVERO et al.; 1993), advantageous in anatomically complex areas (VAN DER SLUIS *et al.,* 2010).

More new appliances and techniques appeared with some specific benefits. The continuous ultrasonic irrigation (CUI) has gained prominence because besides promoting better irrigant penetration to lateral canals and the apical third (CASTELO-BAZ *et al.,* 2016), it favors constant irrigant renewal, preventing saturation of organic tissue dissolution or instrumentation subproducts.

A plastic (acrylonitrile-butadiene-styrene) rotary file called Easy-Clean (Easy Equipment, Belo Horizonte, MG, Brazil), similar to an endodontic rotating file was introduced as a complementary agitation option with a tip Nº 25 and taper 0.04 recommended to be used in the reciprocating movement. It showed good results in the cleaning of lower molars and mesial roots canals (KATO *et al.,* 2016). Recent studies show good results after its use in continuous rotation promoting cleaning similar to passive ultrasonic irrigation (DUQUE *et al.,* 2017).

Additionally, the XP-Clean irrigant agitation device manufactured in conventional NiTi with a 0.25 mm diameter and taper .02 sinuous and eccentric shape, (MK Life, Porto Alegre, RS, Brazil) came to be used after instrumentation. However, there are no studies on its efficacy compared to other agitation methods.

In order to demonstrate the effects and compare the cleaning capacity of different irrigation techniques several methodologies have been developed, including histological sections (NADALIN *et al.,* 2014, 2017), fluid computational dynamics reconstructions (CFD) (BOUTSIOUKIS *et al.,* 2010; SHEN *et al.,* 2010; WANG et al. 2015), blocks, prototyped teeth and artificial grooves (VAN DER SLUIS *et al.,* 2005; 2006; CAPAR *et al.,* 2014) and radiopaque solutions use in vivo (VERA et al. 2011; 2011; 2012). Nonetheless, these experimental models are limited to image evaluation. None of them allows a three-dimensional irrigant diffusion evaluation or a quantitative comparison of preserved root canals without changing their characteristics. Most of the techniques need teeth cleavage for observation, therefore, the images are analyzed in an ordinal qualitative manner through scores assignment thus they ignore anatomical condition or avoid radiopaque substance identification.

To overcome these limitations, the experimental model described by Versiani *et al.,* 2015, is performed with very close ideal conditions because it preserves morphology and measures the volume region of interest, allowing a more reliable quantitative irrigation effectiveness. Thus, a study comparing the different irrigant agitation techniques still becomes pertinent. Microtomography allows quantifying the percentage of the volume of the canal filled by the irrigant, both in the canal as a whole and in the most critical regions such as the apical third. The purpose of this study was to approach different methods of irrigant agitation filling capacity to the root canal system by micro-CT.

2 Article

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The article presented in this Dissertation was formatted according to the Journal of Endodontics instructions and guidelines for article submission.

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RESULTS: The PUI, CUI and Xp-Clean groups presented a percentage of total filling significantly higher than the conventional groups, Endoactivator and RinsEndo (P <0.05). No significant difference was found between the EasyClean group and the other groups (P <0.05). In the apical filling there was no significant difference between the groups (P <0.05). CONCLUSIONS: Based on the proposed methodology and results, it can be concluded that PUI, CUI and XP-Clean improve the canals filling. Regarding apical filling, the methods presented the same results.

Keywords: endodontics; irrigation; computerized microtomography.

INTRODUCTION

Root canals disinfection and cleaning is a determining factor for successful endodontic therapy (1). To achieve canal cleaning, (2) associated the instrumentation to irrigation but there were large areas of the channels that the instrumentation could not reach, thus, the cleaning of these areas was achieved only by irrigation. Irrigation is of vital importance in the optimization of canals disinfection (1,3,4,5,6). The traditional method of irrigation, performed with cannula and syringe, is extremely limited for the apical region and areas such as isthmus (7,8,9,10).

Among the possibilities of irrigation optimizing, some options appeared in endodontic practice. Among them, Endoactivator as a sonic device (Dentsply Maillefer, Ballaigues, Switzerland) (11,12), the RinsEndo® device (Dürr Dental GmbH, Bietigheim-Bissingen, Germany) which is a mechanical system that uses pressure-suction technology to move the irrigant (13,14,15,16,17,18), the passive ultrasonic irrigation technique (PUI) (19,20,21,22) and the continuous ultrasonic irrigation (CUI) which was highlighted by its constant irrigant renewal. In addition, other options for rotary instruments are the Easy Clean file (Easy Clean Equipments, Belo Horizonte, MG, Brazil) which is made of plastic polymer, very similar to an endodontic rotary file and introduced as a complementary option for agitation (23,24) and the XP-Clean agitation file with sinuous shape and manufactured in NiTi (25\.02) (MK LIFE, Porto Alegre, RS, Brazil) which came to be used after the instrumentation.

To compare the effects and cleaning capacity of different irrigation techniques, several methodologies have been developed, but these experimental models are limited to evaluation of two-dimensional interest areas then end up destroying or changing the sample morphology and fluid conditions. To overcome these drawbacks, the micro-CT appears as a good alternative because it keeps the sample unaltered during the analysis, and coming very close to the ideal working conditions by monitoring the three-dimensional irrigant diffusion along the root canal.

The real behavior of the agitated irrigation solution with different methods using micro-CT methodology is not yet known. Micro-CT allows comparing different agitation techniques, to quantify filled canal volume percentage by the irrigant, both in the channel as a whole and in the most critical regions such as the apical third.

We aimed to compare by microtomography the filling capacity of the root canal system of different agitation methods. The null hypothesis was that doesn't have to differences in the total or apical volume filling percentage.

MATERIAL AND METHODS

Tooth selection criteria

The project was approved by the local Human Ethics Commission (Process Number: 03931118.7.0000.5417).

Seventy human maxillary first molars extracted with mesio-vestibular root with a single flattened canal, moderate curvature of 10 to 20 degrees (25) and complete apex were selected and individually stored in containers, immersed in 0.1% thymol solution. The teeth were scanned (SkyScan 1174) to measure the volumes. After that, a statistical analysis was performed to verify that there was no difference in volume in the distribution of the sample, in order to ensure anatomical standardization. Teeth with obstructions and/or resorption were excluded.

Preparation of root canals

The mesio-vestibular root canal of the specimens was explored with K file #10 and #15 (Dentsply Maillefer, Ballaigues, Switzerland), and instrumented up to Mtwo file 40/.04. Irrigation was performed with 10 mL of NaOCl (2.5%) by means of syringe and NaviTip cannula (Ultradent Products Inc., South Jordan, UT, USA) until maximum needle penetration, in entry and exit movements. The working length was established at 1 mm from the length of the canal. The canals were treated with 17% ethylenediaminetetraacetic acid for 5 minutes followed by 3 mL NaOCl 2.5% and finally inactivation with saline solution. The root canals were aspirated using a Capillary Tip Purple (Ultradent Products Inc., South Jordan, UT, USA) coupled to a high-powered suction pump for 1 minute and a 40.02 paper cone was used for 5 seconds. At the end, the mesio-vestibular root apex of each specimen was sealed with epoxy resin, creating a closed system, allowing channel recapitulation but preventing extrusion of the irrigating fluid through the apical foramen during irrigation protocols.

Irrigation methods

In all groups, care was taken to always keep the pulp chamber filled with the irrigant during the entire time the irrigation protocol was performed. TEREBRX 35 Contrast Solution (CS) was used for all groups so that it could be analyzed later through computed microtomography.

Conventional irrigation (syringe and cannula)

Irrigation was performed with 5 mL of contrast solution (CS) performing in and out movement with total time of 60s. Excess irrigation was aspirated with the use of Capillary Tip cannula, coupled to a vacuum pump, to aspirate the surplus.

Easy Clean in rotary action

The Easy Clean file (Easy Equipment, Belo Horizonte, MG, Brazil) was positioned at 2mm from the working length, coupled to a low speed electric motor driven at 10,000 rpm (Beltec, Araraquara, Brazil), activating it for a total time of 60s.

Passive Ultrasonic Irrigation (PUI)

The Irrisonic E1 ultrasonic insert (Helse Dental Technology, Santa Rosa de Viterbo, Brazil) was used with a Varios 350 ultrasonic device (NSK, Shinagawa, Tokyo), at 10% power in G function, positioned at 2 mm from working length, and positioning the transducer in the vestibulo-palatal direction, activating it for total time of 60s.

Continuous Ultrasonic Irrigation (CUI)

The CS was placed in a disposable syringe coupled to the VistaFlow ultrasonic insert and a Varios 350 (NSK, Shinagawa, Tokyo) performing the filling with SC initially and then the activation of the set positioned 2 mm from the working length, and positioning the transducer in the vestibulo-palatal direction, performing the activation for 60s, with an average flow of 5 mL per minute.

RinsEndo Activator

Irrigation was performed with the RinsEndo system (Durr Dental GmbH & Co. KG, Bietigheim-Bissingen, Germany) connected to a 30G cannula (Durr Dental GmbH & Co. KG) of the system itself, positioned at the root canal entrance and activated for 60s.

Endoactivator (Dentsply Maillefer, Ballaigues, Switzerland)

The Small file (15/.04) was inserted at 2 mm from the CT coupled to the device at a maximum power of 10000cpm (166.67Hz) for 60s.

XP-Clean (Mk Life, Porto Alegre, Brazil)

The root canals were filled with the CS, and then the XP-Clean instrument was positioned at 2 mm from the CT, and activated at 800 rpm for 60s.

Volumetric analysis in computed microtomography

Each specimen was scanned two times using computed microtomography (SkyScan 1174, Billerica, Massachusetts, USA): after root canal preparation (E1, without CS) and after agitation protocols (E2, with CS). The teeth were scanned at 50 kV, 800 µA, with an isotropic pixel size of 16.8 µm, performed by 360-degree rotation around the vertical axis, camera exposure time of 6000 ms, rotation step of 0.8°, and average of frames of 2. The X-rays were filtered with an aluminum filter of 0.05 mm and Flat Field was performed at the beginning of each day, before scanning to correct variations in pixel sensitivity of the camera. The images will be reconstructed using NRecon v.1.6.3, (Bruker microCT) with a beam hardening correction of 15%, smoothing of 2 and an attenuation coefficient range of -0.013-0.11.

The contrast solution used was Terebrix 35 (350 mg/ml of Meglumine ioxitalamate), physically and chemically similar to Ioditrast 76 (Meglumine and sodium diatriazote 370 mg/ml) used in the methodology described by Versiani et al. 2015, but with slightly lower surface tension. Precautions were taken to avoid extravasation, avoiding contamination of the external surface of the root, which could cause false negative.

The different levels of contrast of SC, non-irrigated areas and dentin provide excellent distinction of volumetric extensions in specimens. Irrigation volume and empty areas were measured with the CTAnalyzer (v1.1.1.0) software, using a binarization value of 85(+/-5). The total filling volume of each technique was calculated, evaluating the total filling (10mm) and the apical portion (3mm).

Statistical Analysis

The normality test for analysis of all groups was Shapiro-Wilks. The initial pairing between the groups was done through the distribution of samples in seven groups and analysis through the Anova test to ensure that there was no significant initial difference between the groups.

The data obtained after the agitation protocols of the total volume were analyzed with the Anova test and the apical filling, for not presenting normality, was analyzed with the Kruskal-Wallis and Dunn tests. The level of significance adopted in all tests was 95% ($p>0.05$).

RESULTS

The mean and standard deviation values of total filled volume percentage by the contrast solution are presented in Table 1 and Graph 1. In addition, the percentage of completion of the apical portion was calculated, and these data are also shown in Table 2 and Graph 2.

The total volume filling analysis showed that PUI, CUI and XP-Clean presented a significantly higher percentage of filling than the Conventional, Endoactivator and RinsEndo groups ($P < 0.05$). No significant difference was found between the EasyClean and the other groups ($P<0.05$). There was no significant difference between all groups ($P<0.05$). The EasyClean group was the only group where the irrigant arrived at the apical all specimens portion.

DISCUSSION

The study objective was to quantify utilizing computed microtomography the irrigant penetration percentage by different agitation methods - Conventional, Endoactivator, PUI, CUI, EasyClean, RinsEndo and XP-Clean. The null hypothesis was partially rejected since in the total root canal filling percentage PUI, CUI and XP Clean methods presented best results. Regarding apical filling, the methods presented similar results.

Even with the ex vivo study limitations, this was the first time that different irrigant agitation methods were evaluated three-dimensionally by computed microtomography means. Before that, Versiani *et al.* (10) confirmed the possible use of this methodology in irrigant filling analysis. Also, computed microtomography is a non-destructive method enabling the analysis of the same specimen without destroying it or deforming it. The root canals preparation was standardized, both in tip and taper (26) so that only the agitation method influence is evaluated with the lowest possible bias. The radiographic contrast allowed to map the irrigant scattering into the root canal spaces (10).

However, speculation about the real irrigation performance in clinical situations by different methods should be done with caution due to the inherent characteristics of an ex vivo model, the operator, temperature variations, behavior of the irrigant, and the physical-chemical differences between the NaOCl and the contrast solution. Additionally, the external surface of each root was sealed with epoxy resin to create a closed system and to obtain the " vapor lock effect" in order to simulate a clinical condition during irrigation (27,28). Future randomized clinical trials to evaluate postoperative healing and pain are necessary. Accordingly PUI, CUI and XP Clean showed significant differences when compared with Conventional, Endoactivator and RinsEndo ($p<0.05$) demonstrating that these methods were able to improve irrigant percentage penetration.

In the CUI and PUI groups, the movement occurs through the insert promoting acoustic cavitation and causing almost instantaneous temporary bubbles formation that ruptures inside the root canal during the canal filling promoting a great irrigant displacement and waves that go against the adjacent surfaces. It differs from other methods that also may induce the formation of new bubbles through the static cavitation but are not able to rupture the canals (29,30,31). Ahmad *et al.* (19) describe another phenomenon, denominated acoustic transmission that consists of a constant flow of fluid driven by the acoustic streaming, those are also reported as ultrasonic agitation dominant effect.

The XP-Clean, due to the sinuous design and the helical instrument movement, promotes agitation and may allow the stocked bubbles to pull out, then allowing the deepest entry of irrigant into the channel. Regarding the irrigant percentage penetration in the apical third, it wasn’t demonstrated a significant difference among the groups. This probably occurs due to the lack of adequate irrigant flow and the phenomenon denominated "capillarity" that occurs in narrow spaces and modify the behavior of the liquids, limiting the hydraulic force promoted inside the canal surfaces (32).

The results provide evident information regarding irrigant propagation ability, which reinforces the idea that μ-CT itself is a tool of great precision and importance for future approaches on the behavior of irrigation solutions. This enables a better understanding of the pros and cons of each technique in different root anatomy and allows to use them in the optimization of root canal cleaning and decontamination. Randomized clinical studies are indicated to analyze clinical efficiency of these protocols in endodontic practice.

CONCLUSION

Based on the proposed methodology and the results, it is concluded that PUI, CUI and XP Clean improve the total filling of the channel. Irrigant apical filling are similar among the analyzed methods.

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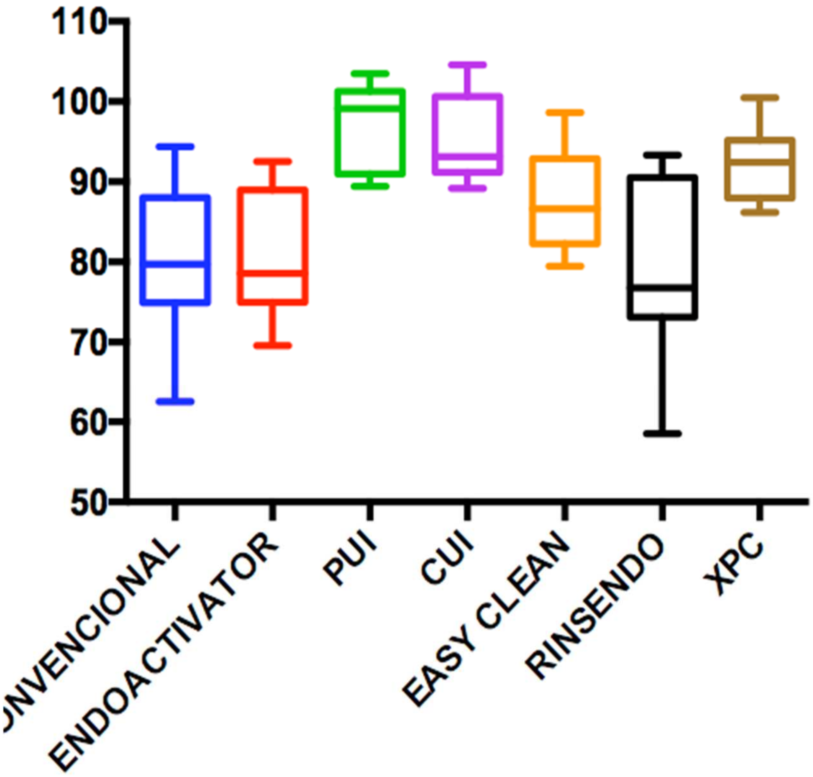
Table 1. Mean and standard deviation of Percentage (%) canal filled by CS. Different letters represent statistically significant differences (p<0.05) between groups.

Method	*Conventional*	*EndoActivator*	*PUI*	*CUI*	*Easy Clean*	*RinsEndo*	*XP Clean*
Mean/SD	80,18±9,433[a]	80,82±7,948[a]	97,42±5,079[b]	95,07±5,552[b]	87,65±6,503[ab]	78,87±10,70[a]	92,37±4,567[b]

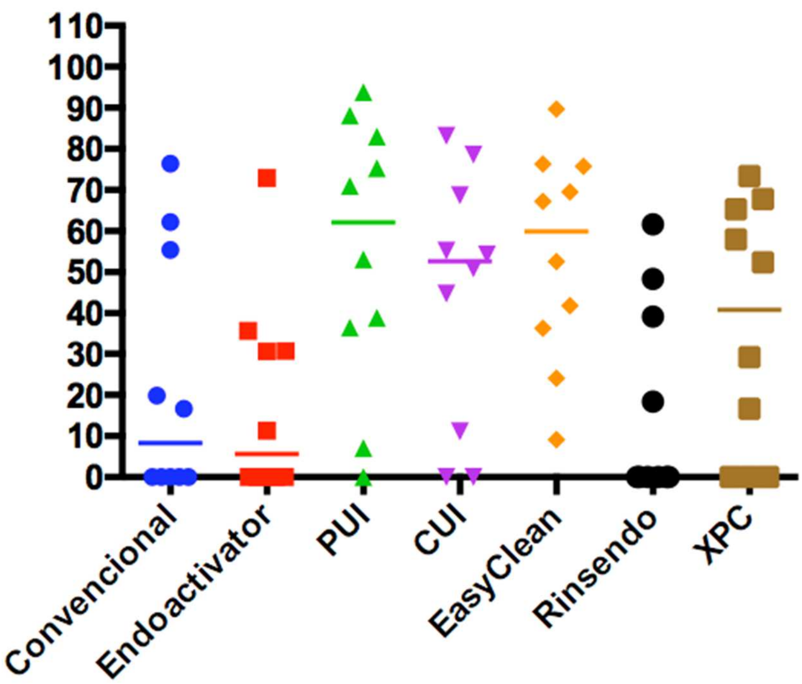
Table 2. Median, minimum and maximum of Percentage (%) apical portion (3mm) of canal filled by CS. Different letters represent significant differences (p<0.05) between groups.

Method	*Conventional*	*EndoActivator*	*PUI*	*CUI*	*EasyClean*	*RinsEndo*	*XP Clean*
Median/mínimum e maximum	8,313 0,0-76,40[a]	5,646 0,0-72,95[a]	62,06 0,0-93,80[a]	52,62 0,0-83,23[a]	59,91 9,087-89,68[a]	0,0 0,0-61,68[a]	40,80 0,0-73,35[a]

Graph 1. Minimum values (%), Percentile 25, Mean, Percentile of 75 and Maximum of the total filling of the canal.



Graph 2. - Median and individual values of percentage of apical filling.



3 DISCUSSION

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The knowledge of irrigation solutions penetration and disinfection inside the root canal system using complementary methods is still limited and unpredictable (VERSIANI *et al.,* 2015; ALVES *et al.,* 2016).

Considering that the main irrigation limitation is the inability to eliminate microorganisms and their products for not reaching anatomical complexities areas.

Complementary approaches for adequate disinfection improve the endodontic treatment prognosis (HAAPASALO *et al.,* 2010; VERA *et al.,* 2012).

We analyzed by μ-CT the irrigant penetration level of conventional syringe and cannula method compared to complementary methods of agitation including Endoactivator, PUI, CUI, EasyClean, RinsEndo, and XP-Clean. Even knowing the limitations of an ex vivo study, this is the first time that six different irrigant agitation methods are evaluated in a 3D model by microtomography.

A significant difference in the irrigant penetration percentage occurred and the best performance in the total volume filling was in PUI, CUI, and XP-Clean. However, there was no difference in irrigant penetration percentage in EasyClean group related to other groups. Also, there was no significant difference among groups in the apical region. Accordingly, the irrigation methods show an equal deficiency while diffusing the irrigant to the apical region. MOORER & WESSELINK (1982) established that the complementary irrigant agitation can increase its dispersion in the root canal system. Besides, JIANG et al. (2010) show that there is an improvement in the root canal cleaning and disinfection by improving the fluid movement. This study partially confirms these premises, since the agitation of the irrigant increases the root canal filling. However, improvement in the apical region was not evident.

Specimens were standardized prior to group division based on morphological and anatomical aspects of the mesiobuccal root canal system. In addition, the root canals preparation was standardized both in apical diameter and taper (LEE *et al.,* 2004) so that only the agitation method influence could be evaluated with the least possible bias. The density and surface tension were tested (VERSIANI *et al.,* 2015), and the behavior of the radiographic contrast (Ioditrast® 76, Justesa, Mexico) was very similar to the NaOCl solution, allowing mapping the spread of the irrigant into the root canal space in the computed microtomography images. In this study, the contrast medium used presented 22% lower surface tension than NaOCl 2.5%. It is worth noting that in our study the same solution was used for all groups, eliminating biases regarding substance variability.

However, speculations about the real clinical behavior in different irrigation methods should be carefully approached, due to the inherent characteristics of an ex vivo study. The technique of their operator, temperature knowledge of differences between NaOCl and contrast solution may affect the results. Future randomized clinical trials to evaluate postoperative healing and pain are necessary. The external surface of each root was sealed with epoxy resin to create a closed system and to obtain the "vapor lock" effect in order to simulate the clinical condition during irrigation (SENIA *et al.,* 1971; VERA *et al.,* 2011; VERA *et al.,* 2012; ALVES *et al.,* 2016).

The irrigant penetration analysis showed good capacity to improve solution penetration through a agitation techniques, which coincide with previous studies (VERA *et al.,* 2011; VERSIANI *et al.,* 2015). Regarding agitation method, PUI, CUI, and XP Clean showed a significant difference when compared to the conventional, Endoactivator and RinsEndo ($p<0.05$), demonstrating that these methods can improve solution penetration and allow higher canal filling. In the CUI and PUI groups, the movement occurs through the insert promoting acoustic cavitation and causing almost instantaneous temporary bubbles formation that ruptures inside the root canal during the initial canal filling promoting a great irrigant displacement and waves that go against the adjacent surfaces. It differs from other methods that also may induce the formation of new bubbles through the static cavitation but are not able to rupture the canals (Martin et al 1980, Cunningham et al 1982, Martin & Cunningham 1985). Ahmad et al (1987) describe another phenomenon, denominated acoustic transmission that consists of a constant flow of fluid driven by the acoustic streaming, those are reported as ultrasonic agitation dominant effect.

The XP-Clean, due to the sinuous design and the helical instrument movement, promotes agitation and may allow the stocked bubbles to swirl, then allowing the deepest entry of irrigant into the channel. Regarding the irrigant percentage penetration in the apical third, it wasn’t demonstrated a significant difference among the groups. This probably occurs due to the lack of adequate irrigant flow and the phenomenon denominated "capillarity" that occurs in narrow spaces and modify the behavior of the liquids, limiting the hydraulic force promoted inside the canal surfaces (SUSIN *et al.,* 2010).

Another option to evaluate the penetration of these irrigation methods would be in situ, however, the high radiation required for μ-CT exams makes it unfeasible. Also, in vivo models need to be performed in selected standardized samples with adequate anatomy also with the required pre- and post-scanning Cone-Beam Computed Tomography to analyze the results. Those factors represent an ethical problem due to the radiation exposure needed (PAQUÉ *et al.,* 2009). Besides, the quality and resolution of images using μ-CT are extremely superior to those of CBCT.

Although the limitation mentioned in the previous paragraph, the μ-CT technology has many advantages over other types of analyses since it is a non-destructive method that allows canals to be analyzed before and after procedures, obtaining very accurate quantitative results. Micro-CT enables researchers to obtain previously unknown results such as changes in the morphology of the canals, amount of accumulated or extruded debris, in addition to the tracking of the irrigation solution along with the endodontic treatment phases (VERSIANI *et al.,* 2015).

Our results provide evident information regarding irrigant propagation ability, which reinforces the idea that μ-CT itself is a tool of great precision and importance for future approaches on the behavior of irrigation solutions. This enables a better understanding of the pros and cons of each technique in different root anatomy, and allows using them in the optimization of root canal cleaning and decontamination.

4 CONCLUSIONS

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Based on the proposed methodology and the results, it is concluded that PUI, CUI and XP Clean improve the total filling of the canals. The irrigant apical filling is similar among the analyzed methods.

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