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Microbial leakage in root-end fillings materials after retro-preparation by two ultrasonic tips

ABSTRACT

Aim: the purpose of this study was to evaluate the microbial leakage of root-end fillings materials (ProRoot, MTA-Angelus and Portland cement) after retro-preparation by two ultrasonic tips (Osada and CVD).
Methodology: seventy maxillary anterior human teeth had their root canals prepared using crown-down technique. The apical 3 mm was resected and the root-end cavity was prepared. The samples then were randomly distributed in 6 groups and two controls groups. In order to make the fixation of the teeth, a platform was used, containing the upper chamber and the lower chamber with Brain Heart Infusion broth (BHI). Approximately 3 mm of the apical part of the root were stored in a BHI culture medium. The samples were inoculated with 0.1 ml of the microbial suspension *(E. faecalis + S. aureus + P. aeruginosa + B. subtilis + C. albicans)* for 60 days.
Results: all groups showed a certain degree of microbial leakage. There were no statistically significant differences among the experimental groups, both in relation to the retrofilling materials and ultrasonic tips.
Conclusion: the present experiment supports previous information from studies about the acceptable sealing quality of MTA and Portland cement when used in root-end fillings.

Key words:
Root-end fillings, root-end preparation, microbial leakage, ultrasonic, MTA.

INTRODUCTION

Several species of microorganisms can colonize the root canal system and affect the periodontal tissues causing periapical destruction. The endodontic treatment involves sanitization process, cleaning and shaping and three-dimensional sealing of root canal system. When these objectives are reached, in most of the cases, the endodontic treatment is successful.
However, several factors inherent to the treatment, as the presence of periapical lesion associated to instrument breakage, zips, root perforations, intraradicular posts can lead to failure of root canal treatment or result in a great risk for the maintenance of the tooth. In some cases when the retreatment is not sufficient or not able to solve the problem, the periapical surgery is also the therapeutic option. Several Authors have reported a success rate higher than 80% in periapical surgery (1, 2). The root-end filling in general is recommended considering that most of the failures are due to inadequate preparation and root canal obturation.
Different investigations indicated the relevance of the cavity preparation in relation to the root-end filling (3-5), as well as to biological, physical and chemical characteristics of the retrofilling materials (6-11). Other studies about root-end cavity preparation compare the use of burs with ultrasonic tips and the shape, depth and uniformity of the cavities, removal of debris and smear layer (3, 4, 12, 13). Clinical studies have shown that the ultrasonic tips produce deeper root-end cavities, parallel to the long axis of the canal and with less smear layer and also provide a more conservative surgical access (3, 4).
Chailertvanitkul et al. (5) compared the coronal leakage of the Super-EBA, used as root-end filling material, after preparing the root-end cavities with burs or ultrasonic tips, using two different types of microorganisms. The group prepared with ultrasonic tips showed less leakage than the group prepared with burs.
Lee et al. (14) and Torabinejad et al. (15) discussed the use of a new material – MTA – in the sealing of the communications between the root canal and the periodontal tissues. Several reports using different methodologies showed that MTA has excellent sealing properties and biocompatibility (6-10, 16). These studies also indicate the need to obtain good microbial control, which maintains a better condition of the periapical tissues, allowing the apical periodontal tissue regeneration. Wucherpfenning & Green (17), Estrela et al. (18) and Holland et al. (19) showed behavior similar between MTA and Portland cement.
Thus, the aim of this study was to eva-

luate the microbial leakage in root-end fillings, in relation to the cavity preparation, made with two ultrasonic tips (Osada and CVD) and the type of root-end filling material (ProRoot, MTA-Angelus and Portland cement).

MATERIALS AND METHODS

Seventy maxillary anterior human teeth, extracted due to different reasons and obtained from the teeth bank of the University of Taubaté (SP, Brazil) were used as the experimental sample. The use of these teeth was appreciated and approved by the Ethics Committee. After being radiographed, teeth with incomplete rhizogenesis, calcifications, internal and external resorption, fracture lines, curved root (or dilacerated) and prepared and/or filled root canals were excluded. Then, the selected teeth were immersed and maintained for 30 minutes in 5.0% sodium hypochlorite, in order to make the previous microbial control. The sizes of the samples were standardized through the resection of part of the coronal structure, keeping a length of 15 mm. After establishing this pattern, a working length of 14 mm was adopted. The root canals were prepared up to a size 50 file (K-file, Maillefer, Switzerland), 1 mm short of the apical foramen, using the crown-down technique. Three milliliters of a 2.5% sodium hypochlorite were used as an irrigating solution, after the use of each file. Then, the root canals were dried and filled with a solution of EDTA (17%) for 5 minutes to remove the smear layer. After concluding the removal of the smear layer, the apical 3 mm of each root was resected with a Zekrya® bur (Dentsply Maillefer-Switzerland) in high speed, under water spray, in an angle of 90 degrees in relation to the long axis of the tooth and a root-end cavity prepared to a depth of 3 mm (20).

Then, the samples were randomly distributed in 6 experimental groups with 10 samples each. Five additional samples were used as positive and negative controls. In group 1, the samples were prepared with Osada ultrasonic tips and retro-filled with the gray Portland cement (CPII F 32, Goania, GO, Brazil), while the teeth in group 2 were prepared with Osada ultrasonic tip and retro-filled with MTA – Angelus®, (Angelus Soluções Odontológicas Ltda, Londrina, PR, Brazil). In group 3, the samples were prepared with the use of Osada ultrasonic tip and retro-filled with ProRoot® (Dentsply Tulsa Dental, Oklahoma - USA), while in group 4, they were prepared with CVD ultrasonic tip and retro-filled with gray Portland cement (CPII F 32, Goiania, GO, Brazil). In group 5, the teeth were prepared with CVD ultrasonic tip and retro-filled with MTA – Angelus®, (Angelus Soluções Odontológicas Ltda, Londrina, PR, Brazil). In group 6, the samples were prepared with a CVD ultrasonic tip and retro-filled with ProRoot® (Dentsply Tulsa Dental, Oklahoma - USA). The teeth in the negative control group were treated just like the procedure for the root canal preparation. The root-end filling was made with the Portland cement. In the positive control, the teeth were treated the same way as the root canal preparation, except that the canals were not retro-filled.

A platform was designed in order to make the tooth's fixation, taking into consideration other experimental models (21). The structure of this platform was made with 10 ml glass vials (Wheaton do Brasil S.A., São Bernardo do Campo, SP), rubber stoppers with a diameter of 20 mm (Adnaloy Artefatos de Borracha Ltda., São Paulo, SP) and 1.5 ml Eppendorf tubes (Cral, Comércio de Artigos para Laboratório Ltda., São Paulo, SP). An 11 mm-diameter steel drill was used to perforate the rubber stoppers. Then, approximately 5 mm of the Eppendorf tubes' tip was cut using the carborundum disk. The tooth was introduced in the Eppendorf mounting and adapted until it reached the best cervical third fitting, in order to obtain a minimum of 8 mm. A cervical line was made in the tube-tooth limit to identify the best position to adapt the tooth inside the tube when making the final platform mounting. After that step, all samples were identified and the teeth, tubes and stoppers were autoclaved. At this time, the root-end fillings were made. A gutta-percha cone was fitted in the canal, 3 mm away from the apical foramen, in order to avoid the dislodgement of the filling material inside the canal. The root-end filling materials were inserted in the cavities using the Map System and were condensed with a Bernabé-type condenser.

Further, the tooth was fitted to the tube until it reached the cervical limit line so that the internal impermeabilization could be made between the Eppendorf tube and the tooth with nail polish. After that procedure, the impermeabilization of the apical portion was made using two layers of cyanoacrylate (Super Bonder®, Henkel Loclite Adesivos Ltda., Itapevi, SP, Brazil), with an interval of an hour. The samples were kept in room temperature until they got dry. Then, the tube-tooth portion was sealed with an epoxy-resin layer (Durepóxi®, Alba Química Indústria e Comércio Ltda., Boituva, SP) to ensure the proper sealing. A layer of nail polish over the impermeabilized root and epoxy-resin surface was used after the previous procedures. The samples were kept in an incubator at 37º C for 24 hours to ensure the complete setting of the impermeabilizing agents.

After 24 hours, the teeth were stored for 30 minutes in a 5% sodium hypochlorite to keep the perfect microbial control. The samples were then inoculated in sterilized tubes containing 8 ml of Brain Heart Infusion® culture media (BHI, Difco Laboratories, Detroit, MI, USA) and sodium thiossulfate and tween 80 as neutralizers, both in a concentration of 1%. Therefore, approximately 3 mm of the root apical portion was stored in the culture media. An additional caution was taken to ensure the sealing between the rubber stopper and the glass platform, with additional impermeabilization (cyanoacrylate and nail polish) being performed. To guarantee the aseptic control of the set (platform and culture media) during the described procedures, the teeth were kept for 24 hours in a bacteriological incubator at 37ºC (20).

A microbial suspension was prepared in 5 ml of sterilized distilled water from a culture incubated for 24 hours and a turbidity equivalent to 1 McFarland standard (3 x 10^8 cells/ml). One ml of each microbial suspension was withdrawn to prepare a solution containing the five indicating microorganisms of the American Type Culture Collection (*E. faecalis* (ATCC 29212) + *S. aureus* (ATCC 6538) + *P. aeruginosa* (ATCC 27853) + *B. subtilis* (ATCC 6633) + *C. albicans* (ATCC 10231). From this solution, 0.1 ml was employed to prepare a new microbial suspension in 8 ml of BHI. Then, 0.1 ml of this new solution was used to make the microbial inoculation of the samples. This microbial inoculation was made every seven days, with a culture of 24 h, during 60 days. After the inoculation of the microbial suspensions, the samples were kept in a bacteriological incubator at 37°C. The turbidity in the culture media was checked during all the experimental period in the bottom of the glass vial containing the tooth apex, which indicated the presence or lack of microorganisms, characterizing the microbial leakage through the root-end filling. The microscopic analysis (Gram stain) was performed in the samples randomly selected from the contaminated tubes so that the contamination would have the same biological indicators used in the inoculation process. The results were written down in a data sheet, being daily interpreted by two calibrated observers, following the directions of a double blind experiment (20).

RESULTS

Table 1 shows the experimental period (days) and mean rank of the microbial leakage. Data were analyzed statistically using the Kruskal-Wallis test that evaluated the differences between the groups of root-end filling cements using the same ultrasonic tips in the retrocavity preparation. The Mann-Whitney test was adopted to analyze the results of the experimental groups, with the various types of ultrasonic tip used in the root-end cavity preparation.
The results showed no statistically significant differences among the experimental groups, when comparing the microbial leakage period of time, both in relation to the retrofilling materials and ultrasonic tips. All groups showed a certain degree of microbial leakage.

DISCUSSION

New materials, equipment and techniques have been introduced to the dentist community providing lots of benefits to the population. Some investigations employing different methodologies are required to prove their efficacy. Thus, it is relevant to emphasize the evolution in the periapical surgery with the introduction of modern techniques for the preparation of the root-end cavity using ultrasonic tips and the use of MTA as a root-end filling material. Although, the success the parendodontic surgery is directly related to the shaping, disinfection and three-dimensional obturation of the root canal systems should be also highlighted.
Many materials, such as amalgam, gutta-percha, zinc oxide-eugenol cement and its derivates and calcium hydroxide-based cements, Cavit®, composite resin, gold foil and glass ionomer have been used in the root-end filling (10, 22-24). However, none of these materials show ideal biological and physical and chemical properties. Mineral trioxide aggregate (MTA) was developed at the Loma Linda University (USA) with the specific goal of sealing communications between the rot canal and the external surface of the tooth (14). Due to its excellent biological properties and sealing capacity investigated through previous studies (6-8, 14-16), this material has been indicated as the first choice in root-end fillings. Different studies demonstrated the similarity of chemical elements between MTA with Portland cement (17-19, 25)
While carrying out a study about the influence of the apical preparation and these materials on the periapical surgery, it is important to investigate whether there is a difference in the quality of the apical sealing when using the Osada or CVD ultrasonic tips to prepare the root-end cavities. The root-end filling materials Portland cement, MTA-Angelus and Pro-Root were also analyzed.
The results show no statistically significant difference among them in terms of leakage on polymicrobial markers. However, there was microbial leakage in all materials tested in some of the samples during the experimental period of 60 days (Table 1).
Most of the evaluated samples did not show leakage during the observation period. This finding supports the ones found in several other studies that have shown the excellent sealing capacity of these materials, when used to seal the communications between the root canal and the periodontal tissues (8, 14, 26).
The current study analyzed the sealing capacity of various materials - Portland cement, MTA-Angelus and ProRoot - using a conventional experimental model on a polymicrobial culture as a leakage marker.
The comparison to others studies resulted difficult, particularly because of the different variables in the methodologies used, such as: methods of coronal or apical leakage, microbial or non-microbial markers, period between the root-end filling and the exposure to the markers, leakage length, period of time required for the leakage, apicectomy angle, types of root-end cavity preparations and different root-end filling materials (3-5, 10, 14).

Materials	n	Minimum (days)	Maximum (days)	Mean Rank	p
Osada® (Portland cement)	10	39	> 60	16,45	not significant
Osada® (MTA-Angelus®)	10	32	> 60	15,15	
Osada® (Pro-Root MTA®)	10	3	> 60	14,90	
CVD® (Portland cement)	10	42	> 60	14,50	
CVD® (MTA-Angelus®)	10	26	> 60	13,90	
CVD® (Pro-Root MTA®)	10	13	> 60	18,10	

Tab. 1 - *Minimum and maximum period (days) and mean rank of the microbial leakage, considering the preparation of retrocavities and root-end filling materials (Kruskal-Wallis test; Mann-Whitney test).*

Dyes, radioisotopes, electrochemical methods, endotoxins and other substances have been employed as markers to evaluate the leakage. Wu & Wesselink (27) comparing some of the data concerning the linear measurements of the dye leakage after the gutta-percha lateral condensation, after several studies, have observed a range of 0,12 mm to 9,25 mm. These Authors have questioned whether this high level of variations in the results is reliable and if any conclusion may be reached as the evaluation of the modern techniques. The Authors have also questioned if the quantitative methods that measure the fluid volume that may penetrate within the filled root canal are not more valuable than the semi-quantitative methods that investigate the linear penetration and that only indicate the cracks length between the filling and the root canal walls.

The present study model tried to simulate, as much as possible, clinical situation and deserves some considerations. The adopted study model was designed based on observations and changes in studies previously presented (21, 26, 28, 29). The microbial markers used in this work included important microorganisms with different structural characteristics.

The use of an ultrasonic tip as a variable aimed to compare the degree of leakage while using Osada and CVD tips. The results have shown that there were no statistically significant differences in relation to the microbial leakage, when comparing the Osada and CVD tips, regardless the type of root-end filling material used.

It is important to highlight that many studies *in vitro* do not simulate the real surgical conditions. In these cases, the tips of conventional high and low speed burs and even mini hand-pieces, are too large in relation to the surgical cavities and the use of a bur within these bony cavities is complicated. In most of the cases, it is impossible to prepare the cavities in the long axis of the tooth. Ultrasonic tips specifically designed to prepare apical cavity represent one of the greatest technical evolution in endodontic surgery. In addition to be small and easy to use, they produce preparations that follow the long axis of the root canal, deeper cavities, more parallel cavities and a reduced amount of smear layer, when compared to apical cavities prepared with burs (13, 30, 31).

The relevance of the root-end materials properties indicate once that the failure of the endodontic treatment may be directly related to the improper apical sealing. Therefore, a root-end material with good sealing qualities is critical to the periapical surgery success. Researches have shown that MTA is a material that presents superior biological and physical and chemical properties in relation to the other materials employed so far. However, Saidon et al. (25) highlight the high cost of MTA. As the Portland cement is a cheap material and apparently presents similar properties when compared to MTA, it is only reasonable to consider the Portland cement as a possible substitute for the MTA in endodontic applications. Due to this observation, the Portland cement was included in this investigation, since current and future scientific evidences may provide the introduction of a less expensive material. Holland *et al.* (7) have investigated the reaction of rat subcutaneous connective tissue to implanted dentin tubes filled with mineral trioxide aggregate, Portland cement or calcium hydroxide. The results showed that the action mechanism of MTA and Portland cement stimulated the hard tissue deposition and that it has some similarity with the mechanism of calcium hydroxide. So, up to now, MTA is the material that best meets the biological properties of a good root-end filling material because it allows the total repair of the injured tissues through the regeneration of the periodontal tissue of apical insertion, including the cement that covers the apicectomized root surface and the root-end filling material, periodontal material and alveolar bone. This is more likely to occur when the root canal is retro-filled with a material that results in a properly sealing, avoiding the recontamination by the remaining bacteria and their products.

Therefore, the results of the present experiment support the previous studies about the sealing quality of MTA and Portland cement used in root-end fillings. However, it should be emphasized that retrospective and prospective studies and clinical observations should be carried out to confirm the behavior of these materials. Additional studies using different methodologies should also be performed to evaluate other variables.

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