Effectiveness of hand files and the MTwo R system in bacterial reduction in endodontically treated teeth with chronic apical periodontitis

Marcos Sergio **ENDO**[1]
Fernanda Graziela Corrêa **SIGNORETTI**[1]
Nair Narumi Orita **PAVAN**[2]
Frederico Canato **MARTINHO**[1]
Brenda Paula Figueiredo de Almeida **GOMES**[3]

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ABSTRACT

Objective: The aim of this *in vivo* study was to compare the efficacy of two techniques used to remove gutta-percha: hand files and MTwo R system, assessing their efficacy to reduce microbial load after chemo-mechanical preparation of root-filled teeth with post-treatment apical periodontitis. **Methods:** Thirty single root-filled teeth with periapical lesion were divided into two groups. In one group (n = 15), gutta-percha was removed with hand files, whereas the other group (n = 15) was prepared with MTwo R files. After removing the gutta-percha, the first sample was obtained (S1). Subsequently, chemo-mechanical preparation was carried out and a second sample was collected (S2). Bacterial load was determined by means of culture techniques. Statistical analysis was performed by means of Wilcoxon and Mann-Whitney tests. **Results:** At S1, all canals were positive for bacteria in both hand file and MTwo R groups, with medians of 5.14×10^3 (range 20 - 1.7×10^5) and 3.4×10^2 (range 20 - 3.14×10^3), respectively. At S2, the bacterial load reduced in both groups ($P < 0.05$). **Conclusion:** MTwo R was significantly more effective in reducing intra-canal bacteria during endodontic retreatment.

Keywords: Endodontics. Retreatment. Microbiology

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[1]PhD in Clinical Dentistry, State University of Campinas (UNICAMP).
[2]PhD in Pharmaceutical Sciences, State University of Maringá (UEM).
[3]PhD in Restorative Dentistry, University Dental Hospital of Manchester.

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Contact address: Marcos Sergio Endo
Rua Campos Sales, 133 – Apto 702 – Maringá/PR – Brazil — CEP: 87020-080
E-mail: marcossendo@gmail.com

Introduction

One of the major factors associated with endodontic treatment failure is the persistence of microbial infection within the root canal system and/or periradicular area.[1,2] Post-treatment apical periodontitis is a consequence of residual root infection, which may be radiographically undetectable, persisting or developing as a defense mechanism to prevent the systemic spread of bacteria and/or their by-products into other sites of the body.[3]

Clinical studies reveal that, despite thorough mechanical instrumentation and disinfection of the root-canal system, microorganisms might recolonize it both at the end of the treatment procedure and at subsequent treatment sessions. Such residual organisms are likely to play a role in treatment failure.[4] As in occurs in the first treatment session, root canal retreatment aims at eliminating or substantially reducing the microbial load from the root canal[5]

Removing all root filling material is a prerequisite of non-surgical retreatment, as it allows subsequent cleaning, shaping and filling of the root canal system.[6] Complete removal of material from the canal and access to the apical foramen during retreatment are mandatory for proper cleaning and refilling.[7] The techniques used to remove gutta-percha are varied and include the use of hand or rotary instruments with or without heat and solvents and/or ultrasound. The use of nickel-titanium (NiTi) rotary systems in endodontic retreatment has been proposed due to providing safety, efficiency, and speed in removing gutta-percha and sealers.[8,9] MTwo R rotary files (VDW, Munich, Germany) have been recently introduced with a view to removing semisolid filling material. No *in vivo* studies have been conducted to investigate the efficacy of these instruments in reducing bacterial content. In general, the quantification of endodontic bacteria relies on traditional cultivation techniques which are of great importance for studying microbial diseases.

The purpose of this *in vivo* study was to compare the efficacy of two techniques for removal of gutta-percha: hand files and Mtwo R system. It assessed the efficacy of both methods in reducing the bacteria load after chemo-mechanical preparation of root-filled teeth with post-treatment apical periodontitis.

MATERIAL AND METHODS

Patient selection

Thirty patients were selected from the School of Dentistry of Piracicaba. All of them were in need of nonsurgical endodontic retreatment. This research was approved by the Institutional Review Board of the School of Dentistry of Piracicaba, and all patients signed an informed consent form. A detailed medical and dental history was provided by each patient. Patients who had undergone antibiotic therapy during the previous 3 months or had any kind of systemic disease were excluded from the study. Patients' age ranged from 19 to 65 years old. All teeth selected had been previously root-filled and showed radiographic evidence of apical periodontitis. Root canal treatment failure was determined on the basis of clinical and radiographic examinations. All teeth had undergone root canal treatment more than 2 years earlier, and all patients were asymptomatic.

Microbial sampling

The teeth were isolated with a rubber dam and had the crown a disinfected with 30% H_2O_2 (v/v) for 30 seconds, followed by 2.5% NaOCl also for 30 seconds. Subsequently, 5% sodium thiosulphate was used to inactivate the disinfectant agents.[10,11] A swab sample was taken from the surface and streaked onto blood agar plates to test for disinfection. An access cavity was prepared with sterile high-speed diamond burs under irrigation with sterile physiological solution. Before entering the pulp chamber, the access cavity was disinfected according to the same aforementioned protocol, and sterility was again checked by taking a swab sample from the cavity surface and streaking it onto blood agar plates. Aseptic techniques were employed throughout root canal treatment and sample acquisition. The samples (pre- and post-clinical procedures) were collected with three sterile paper points which were consecutively placed into each canal until reaching the total length calculated based on the preoperative radiograph. The paper points were kept in place for 60 seconds and then pooled in a sterile tube containing 1 mL VMGA III transport medium.[10] The samples were transported to an anaerobic workstation (DonWhitley Scientific, Bradford, UK) at the microbiology laboratory within 15 minutes.

Clinical procedures

The same endodontic specialist performed all retreatment procedures, including the sampling procedures (Fig 1). The tooth was anesthetized and after

accessing the pulp chamber, the root filling was removed by means of two different crown-down techniques. The thirty patients were randomly divided into two groups. In one group, root filling was removed by hand files, whereas in the second group it was removed with rotary Mtwo retreatment (R) files (VDW, Munich, Germany). The working length was established radiographically and with the aid of an electronic apex locator (Novapex, Forum Technologies, Rishon le-Zion, Israel) at the apical foramen. No solvent or any other chemical substance was used to remove gutta-percha, and bucco-lingual and mesio-distal radiographs of each tooth were taken to confirm such a removal.

Hand file group: The root filling material was removed by means of Gates-Glidden drills (Dentsply Maillefer, Ballaigues, Switzerland) of sizes #5 (1.3 mm), #4 (1.1 mm), #3 (0.9 mm), and #2 (0.7 mm) until reaching a 6-mm length, which was shorter than the working length and endodontic files. Irrigation with saline solution was performed in order to remove any remaining material and to moisten the canal prior to sample collection. A #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was placed into the full length of the root canal calculated on the basis of the pre-operative radiograph. The working length (at apical foramen) was confirmed by the apical locator (Novapex, Forum Technologies, Rishon le-Zion, Israel). Apical preparation was performed by using K-files ranging from #40 to #45, followed by step back instrumentation, which ended after the use of three files larger than the last file used for the apical preparation.

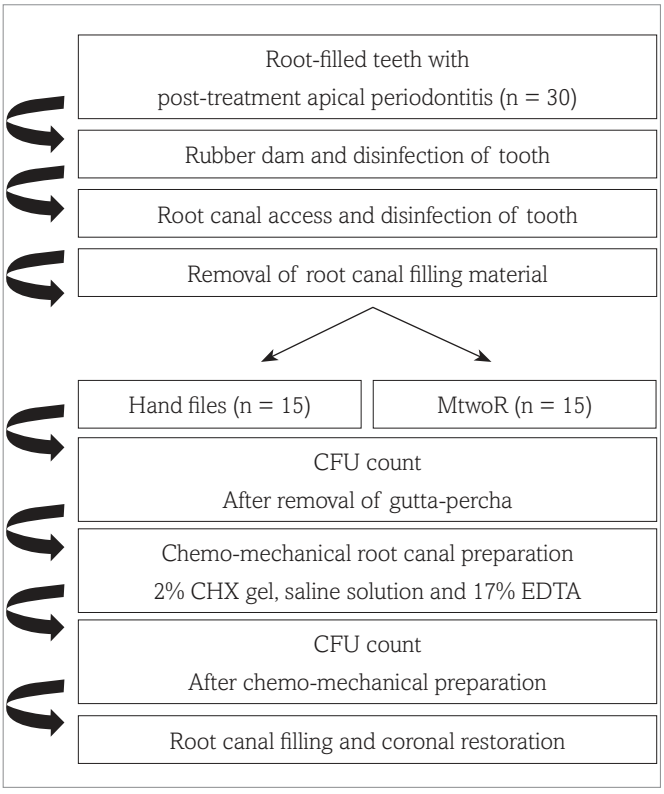

Figure 1. Clinical procedures and sampling (S1 and S2).

Rotary Mtwo R group: The root canal filling material was removed by means of MTwo R files (VDW, Munich, Germany). A #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was used to explore the root canal. A #15 MTwo R file with taper of 0.05 (21 mm) was first used up to the working length (at the apical foramen), followed by a #25 MTwo R file with taper of 0.05 (21 mm), both in round- tripping with a lateral pressing movement. Progression of the rotary files was performed by slightly applying apical pressure and frequently removing the files to inspect the blade and clean the debris. The normal shaping sequence was then used in a circumferential filing motion while pressing against the root canal walls (e.g.: MTwo of size #30 with taper of 0.05; size #35 with taper of 0.04, and size #40 with taper of 0.04). Instrumentation with MTwo files was performed by using an electric motor (VDW, Munich, Germany) operated according to the manufacturer's instructions.

All root canals were irrigated with a syringe (27-gauge needle) containing 1 mL of an auxiliary chemical substance (2% chlorhexidine gel) (Endogel, Itapetininga, SP, Brazil) before the use of each instrument and immediately rinsed with 4 mL of saline solution. Chlorhexidine (CHX) gel consisted of gel base (1% natrosol) and CHX gluconate at pH 7.0. Natrosol gel (hydroxyethyl cellulose) is a nonionic, highly inert and water-soluble agent. After instrumentation, CHX activity was inactivated with 5 mL of a solution containing 5% Tween 80 and 0.07% (w/v) lecithin during a 1-minute period. The solution was removed with 5 mL of saline solution. Retreatment was deemed complete when the last file reached the working length, with no filling material covering the instrument, and canal walls were smooth and free of visible debris. Furthermore, a close inspection under high magnification with dental operating microscope

(D F Vasconcellos S/A, São Paulo, Brazil) showed complete removal of gutta-percha.

After root canal preparation was finished, the canal was irrigated with 17% EDTA (5 mL) for 3 minutes and then rinsed with 5 mL of saline solution. Subsequently, the second (post-chemo-mechanical preparation) sample was collected with three paper points, and transported in VMGA III. Finally, all teeth were filled with vertically and laterally compacted gutta-percha cones (Konne, Belo Horizonte, MG, Brazil) and Endomethasone® sealer (Septodont, Saint-Maur-des-Fossés, France). Access cavities were restored with 2 mm of Coltosol® (Coltène Whaledent, Cuyahoga Falls, OH) and Filtek Z250® (3M Dental Products, St Paul, MN, USA).

Culture technique

Inside the anaerobic workstation, the transport media, containing glass beads with a diameter of 3 mm to facilitate mixing and homogenization of the sample, were shaken thoroughly in a mixer for 60 seconds (Agitador MA 162 – MARCONI, São Paulo, SP, Brazil). Serial 10-fold dilutions were made up to 1/104 in fastidious anaerobe broth (FAB, Laboratory M, Bury, UK) and 50 µL of each serial dilution were plated onto several media by using sterile plastic spreaders.

Fifty µL of the serial dilutions $1:10^2$, $1:10^3$ and $1:10^4$ were plated into 5% defibrinated sheep blood fastidious anaerobe agar (FAA, Laboratory M, Bury, UK) by using sterile plastic spreaders. Also, 600 µL of hemin and 600 µL of menadione were added in order to culture non-selectively obligate anaerobes and facultative anaerobes. The plates were incubated in an anaerobic atmosphere (80% N_2, 10% H_2, 10% CO_2) at 37°C for 14 days. After incubation, the total colony forming unit (CFU) was counted with a stereomicroscope at 16x magnification (Zeiss, Oberkoren, Germany).

Statistical analysis

Data were assessed after removal of gutta-percha and chemo-mechanical preparation. Subsequently, they were submitted to statistical analysis by means of Wilcoxon and Mann-Whitney U tests, with significance level set at 5%.

Results

Sterility samples collected from the external and internal surfaces of the crown and its surrounding structures showed no microbial growth.

The culture technique showed that the number of CFU differed considerably between patient samples (Table 1). Microorganisms were detected in all initial samples. The initial (S1) bacterial load in the hand file group ranged from 20 CFU/mL to 1.7 x 10^5 (median of 5.14 x 10^3), whereas in the MTwo R group it ranged from 20 to 3.14 x 10^3 CFU/mL (median of 3.4 x 10^2 CFU/mL). After chemo-mechanical root canal preparation, the number of CFU decreased drastically in all cases (Wilcoxon, signed-rank test; $P < 0.05$). In S2, the post-treated amount of CFU ranged from 0 to 1.96 x 10^3 CFU/mL (median 20 CFU/mL) in the hand file group and from 0 to 1.4 x 10^2 CFU/mL (median 0) in the MTwo R group.

Bacterial reduction ranged from 60.26% to 100% in both groups. In the hand file group, 33.3% of the cases (n = 5) were free of cultivable bacterial after chemo-mechanical preparation, whereas in the MTwo R group, 86.67% of the cases (n = 13) had such a result. Significant difference was found in the percentage of reduction between hand file (median 99.61%) and MTwo R (median 100%) groups (Mann-Whitney test, $P = 0.0076$).

DISCUSSION

The culture procedure used for CFU count in the present work had been previously published in studies using necrotic root canals.[12,13] After 14 days of anaerobic incubation, the samples allowed both facultative and possibly slow-growing anaerobic microorganisms to recolonize. Culture is a widely used method to assess the antimicrobial efficacy of root canal procedures against viable bacteria in root canal infection.[14] Correlations between absence of bacteria and favorable treatment outcomes have been reported elsewhere.[15] Bacteriological assessment was chosen for the present study because of the importance of canal disinfection in apical periodontitis treatment success.

Samples positive for the presence of microorganisms in root-filled teeth ranged from 35 to 100%.[16] In S1, the occurrence of microorganisms in root-filled teeth associated with periradicular lesions detected by culture (100%) was similar to the findings by Gomes et al[11] and Siqueira & Rôças.[17] The median value of initial CFU samples in this study contained 5.14 x 10^3 CFU/mL (hand file group) and 3.4 x 10^2 CFU/mL (MTwo R group), whereas others

Table 1. Bacterial load (CFU/mL) and percentage reduction determined for root canal samples of 30 root-filled teeth with post-treatment apical periodontitis, before and after chemo-mechanical preparation with either hand files or MTwo R system.

Hand files (Group 1)				MTwo R (Group 2)			
Samples	Before (S1)	After (S2)	Reduction (%)	Samples	Before (S1)	After (S2)	Reduction (%)
1	1.54×10^5	1.96×10^3	98.73	16	3.14×10^3	0	100
2	2.8×10^2	102	64.29	17	6×10^2	0	100
3	3.4×10^2	20	94.12	18	1.58×10^3	0	100
4	4.48×10^3	1.78×10^3	60.27	19	40	0	100
5	20	0	100	20	1.7×10^3	0	100
6	2.6×10^2	20	92.31	21	3.02×10^3	40	98.68
7	5.14×10^3	20	99.61	22	20	0	100
8	104	80	99.20	23	8.2×10^2	0	100
9	1.6×10^5	3×10^2	99.81	24	60	0	100
10	4×10^2	0	100	25	1.26×10^3	1.4×10^2	88.89
11	8.34×10^3	1.58×10^3	81.06	26	20	0	100
12	1.264×10^4	40	99.68	27	3.4×10^2	0	100
13	1.7×10^5	0	100	28	1.2×10^2	0	100
14	9.44×10^3	0	100	29	80	0	100
15	8.4×10^2	0	100	30	40	0	100
Median	5.14×10^3 (c)	20 (d)	99.61 (A)	Median	3.4×10^2 (e)	0 (f)	100 (B)

Different letters (A,B) indicate significance difference (Mann-Whitney test, $P < 0.05$); capital letter indicates differences for different techniques of root filling material removal.

Different letters (c,d) indicate significance difference (Wilcoxon test, $P < 0.05$); lower case letter indicates the same group.

Different letters (e,f) indicate a significance difference (Wilcoxon test, $P < 0.05$); lower case letter indicates the same group.

studies by Schirrmeister et al[16] and Blome et al[18] found 3.5×10^3 CFU/mL and 2.6×10^5 CFU/mL, respectively. Regardless of the treatment technique, there was substantial bacterial reduction after instrumentation and irrigation. After chemo-mechanical preparation, the median value of CFU in the present study ranged from 20 CFU/mL (hand file group) to 0 CFU/mL (MTwo R group) compared to Schirrmeister et al[16] (median 0 CFU/mL) and Blome et al[18] (median 6.4×10^3 CFU/mL). The different results found for the number of microorganisms might be due to specific environmental and nutritional conditions as well as to different protocols followed during endodontic retreatment.

It is likely that current sampling techniques only identify organisms in the main branches of the root canal system, in other words, they cannot sample areas beyond the apical end-point of preparation and filling or in lateral canals, canal extensions, apical ramifications, isthmuses and within dentinal tubules. Care should be taken when interpreting the results because a negative culture result does not mean that there are no microorganisms present. In fact, bacteria may not be soaked up by the paper point and thereby not be detected, or they may even be uncultivable.[16] Studies have shown that none of the retreatment procedures are able to completely clean root canal walls,[8] particularly in the apical third where microorganisms generally persist.

Adequate working length control is important in treating teeth with apical periodontitis[19] because bacterial contamination may extend to the apical few millimeters of the root canals.[1] Allowing a critical number of microorganisms within the root canal may result in persistent periradicular inflammation after endodontic therapy.[1] Conventional radiographic measurements can be deceiving, as the apical foramen is not located at the apex in more than 60 percent of teeth.[20] Modern

electronic apex locator has been shown to be reliable in measuring the actual working length[21] in its whole extension, including apical foramen decontamination. In the present study, the strategies used to clean the most apical portion of the main canal involve preparation of the apical foramen, use of a patency file, and foramen enlargement.

Clinical follow-up studies have reported that chemo-mechanical procedures reduce microorganisms in the root canal system[22] thus allowing healing of periradicular tissues. Instrumentation using stainless-steel hand K-files was chosen as a comparative technique because it is frequently taught in dental schools and is commonly used by many practitioners. In the present study, no failure such as perforation, blockage, or deviation was observed in the use of stainless-steel hand K-files or MTwo R files. Both groups showed statistically significant reduction in CFU count after chemo-mechanical preparation. However, the MTwo R group presented better results compared to the hand files group. Moreover, studies showed that mechanical instrumentation with NiTi was significantly more rapid than that with hand files.[8,23] Authors suggested that the active tip and cutting blades of NiTi rotary files provide not only quickness[8] for retreatment, but also safety of instruments[23] and less apical extrusion.[6] These retreatment instruments can easily progress into the filling material as plasticized gutta-percha offers less resistance, and consequently they can open way to other instruments to be used for subsequent instrumentation.[9] For this reason, it was probably easier to reach the working length with NiTi instruments rather than with hand files.

Chlorhexidine digluconate (CHX) irrigation is more effective on gram-positive than on gram-negative bacteria and should be additionally used during endodontic retreatment in which high proportions of gram-positive bacteria (such as *E. faecalis*) are to be expected in the root canal system.[24] It possesses broad-spectrum antibacterial activity, biocompatibility with periodontal tissues, and substantivity.[25] CHX has shown excellent antibacterial efficacy *in vitro*.[26] In previous studies, 2% CHX was effective in reducing or completely eliminating *E. faecalis* from the root canal space and dentinal tubules, showing residual antimicrobial activity.[25] Thus, CHX is recommended for use during endodontic retreatment, especially to eliminate pathogens, such as *E. faecalis*.[24] EDTA is recommended as adjuvant in root canal therapy due to its ability to remove the smear layer.[24] Furthermore, EDTA can detach biofilms from the root canal walls.[24]

There has been a shift toward single-visit endodontic therapy, even for endodontic retreatment with radiographically demonstrable apical radiolucencies. Single-visit root canal treatment has become common practice and offers several advantages, such as reduced flare-up rate,[27] good patient acceptance, and practice management considerations. Based on the clinical outcomes, no significant difference between post-instrumentation and inter-appointment samples with antibacterial calcium hydroxide dressing was observed.[16,18]

Conclusion

This study indicated that engine-driven rotary instruments using MTwo R was significantly more effective in reducing intra-canal bacteria load in the root canal after chemo-mechanical preparation during endodontic retreatment, thereby allowing a favorable host response to healing periapical tissues.

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