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Comparação *in vivo* de HyFlex CM e ProTaper Next na Remoção de Bactérias e Endotoxinas de Infecções Endodônticas

Dissertação apresentada à Faculdade de Odontologia de Araçatuba da Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, como parte dos requisitos para a obtenção do Título de Mestre em Ciência Odontológica – Área de Concentração Endodontia.

Orientador: Prof. Associado Rogério de Castilho Jacinto
Coorientador: Prof. Assint. Dr. Frederico Canato Martinho

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RESUMO

O objetivo deste estudo clínico foi avaliar a eficácia de dois sistemas rotatórios: HyFlex CM e ProTaper Next na remoção de bactérias cultiváveis e endotoxinas de canais radiculares infectados. Vinte e quatro canais radiculares de molares e pré-molares com necrose pulpar e lesão periapical foram selecionados e divididos aleatoriamente em 2 grupos: HyFlex CM (n = 12); e ProTaper Next (n = 12). As amostras foram coletadas antes e após o preparo biomecânico e inoculadas em frascos específicos. A irrigação foi realizada com hipoclorito de sódio a 2,5%. Um teste turbidimétrico LAL (Pyrogent 5000 – Lonza, Walkersville, MD, EUA) foi utilizado para quantificar endotoxinas. Cultura microbiológica foi utilizada para determinar a contagem de unidades formadoras de colônias bacterianas (UFC/mL). Os dados coletados foram analisados estatisticamente usando SigmaPlot 12.0 para Windows (Systat Software Inc., San Jose, CA). Foi realizado o teste estatístico de Two-Way ANOVA e o nível de significância foi de 5%. Nas coletas antes do preparo biomecânico, bactérias cultiváveis e endotoxinas foram evidenciadas em 100% das amostras. A análise de cultura revelou que não houve uma diferença estatisticamente significativa na redução bacteriana entre os dois sistemas de instrumentação. As endotoxinas estavam presentes em 100% dos canais após a instrumentação e não houve diferença estatística entre os dois sistemas na redução de endotoxinas. Assim, concluímos que ambos os sistemas de instrumentação foram eficazes na redução de bactérias e endotoxinas de canais radiculares com infecção endodôntica primária e que não houve diferença estatística entre eles. Contudo, nenhum sistema foi capaz de eliminar 100% das bactérias e seus subprodutos.

Palavras-chave: Endotoxinas. Bactérias. Endodontia. Desinfecção.

Dias-Machado CA. ***In Vivo*** **Comparison of HyFlex CM and ProTaper Next in Bacteria and Endotoxin Removal from Endodontic Infections.** 2019. 39 f. Dissertation (Master's Degree in Endodontics) - Araçatuba School of Dentistry, São Paulo State University (UNESP), 2019.

ABSTRACT

The aim of this clinical study was to compare the effectiveness of two rotary systems: HyFlex CM and ProTaper Next on the removal of cultivable bacteria and endotoxins from primarily infected root canals. Twenty-four root canals of molars and premolars with pulp necrosis were selected and randomly divided into 2 groups: HyFlex CM (n = 12); and ProTaper Next (n = 12). Samples were collected before and after the biomechanical preparation and inoculated in specific flasks. Irrigation was performed using 2.5% sodium hypochlorite. A kinetic turbidimetric lysate assay of limulus amoebocytes was used to quantify endotoxins. Microbiological culture was used to determine the count of bacterial colony forming units (CFU/mL). Data collected were statistically analyzed using SigmaPlot 12.0 for Windows (Systat Software Inc, San Jose, CA). The Two-Way ANOVA statistical test was performed and the level of significance was 5%. In the samples before the biomechanical preparation, cultivable bacteria and endotoxins were evidenced in 100% of the cases. The culture analysis revealed that there was no statistically significant difference in the bacterial reduction between the two instrumentation systems. Endotoxins were present in 100% of the canals after instrumentation, and there was no statistical difference between the two systems in endotoxin reduction. Thus, it was concluded that both instrumentation systems were effective in reducing root canal bacteria and endotoxins with primary endodontic infection and that there was no statistical difference between them. However, no system was able to eliminate 100% of the bacteria and their by-products.

Keywords: Endotoxins. Bacteria. Endodontics. Disinfection.

TABLE LIST

LIST OF ABBREVIATIONS

CFU	Colony forming units
CM	Controlled memory
EU	Endotoxin units
HCM	HyFlex CM
LAL	Limulus Amebocyte Lysate
LPS	Lipopolysaccharide
PTN	ProTaper Next
VMGA	Viability Medium Göteborg Anaerobically
WL	Working Length

SUMMARY

1 INTRODUCTION*

One of the fundamental objectives of the endodontic therapy is the reduction of bacteria and their byproducts, which are responsible for root canal infections and development of apical diseases.[1] Byproducts released from the outer layer of the membrane of gram-negative bacterial species such as Lipopolysaccharides (LPS) are released during division or rupture of the bacterial cells.[2,3] LPS are detected in 100% of the root canals with primary infections, and play a key role in triggering inflammation and subsequent release of inflammatory mediators,[4] so that in addition to the elimination of bacteria, endotoxins should also be removed during endodontic instrumentation.

The use of nickel-titanium rotary files has become a standard technique for providing more rapid procedures,[5,6] more centered preparations,[7,8] and less apical extrusion of debris.[9] Due to its super elasticity, it has advantages over stainless steel files such as flexibility and a lower number of steps. The flexibility of these files reduces the incidence of procedural errors, increasing success rates in endodontic treatment compared to conventional techniques.[10,11,12] HyFlex CM are NiTi rotary instruments with controlled memory (CM) (Coltene-Whaledent, Altstätten, Switzerland) produced from a single thermal process[13] and changes in Ni amounts (CM includes 52.1% Ni, versus 54.2-56.2% Ni by weight of the conventional alloys). This treatment gives HyFlex CM (HCM) instruments greater flexibility compared to conventional NiTi instruments.[14]

ProTaper Next (PTN) (Dentsply Sirona, Ballaigues, Switzerland) is a system consisting of three instruments made of a single NiTi alloy and M-wire alloy manufactured through a heat treatment process, and incorporates a variable conical design and a single mass of displacement of rotation, which improves the resistance and flexibility throughout its active part.[15]

Several studies have evaluated the physical properties of memory-based files, such as flexibility and resistance.[15,16,17] Previous studies have shown that the preparation of root canals with rotary systems is capable of achieving a reduction of more than 90% of the bacteria and the endotoxin load of infected root canals.[18] However, to date there is no instrumentation system that has proven to be 100% effective.

*The manuscrispt is according to the guidelines for authors of Brasilian Oral Research (Attachment C)

Increasingly, there is a need for instruments with higher cleaning capacity, as well as flexibility, durability, ease of use and safety. PTN and HCM are rotary instruments made by thermomechanical treatment of Ni-Ti alloys, representing the M-wire and CM-wire alloys, respectively. In view of the fact that the HCM instruments are made of CM-controlled memory wires, having a superior flexibility compared to other instruments made of M-wire, and the PTN incorporate a variable taper design and a single mass rotational displacement, it is believed that these systems can reach all the walls of the root canal, improving the ability to remove bacteria and endotoxins.

Therefore, the objective of this study was to compare the effectiveness of the HCM and PTN systems for the removal of cultivable bacteria and endotoxins from root canals. The null hypothesis tested is that there are no differences in bacterial and endotoxin reduction between the two systems tested.

2 METHODOLOGY

Twenty-four patients requiring primary endodontic treatment were included in the present study. A detailed dental history was obtained from each patient. Those who had received antibiotic treatment during the last 3 months or who had any systemic disease were excluded. The Human Research Ethics Committee of Araçatuba Dental School approved the research protocol describing the sample collection for this investigation (CAAE: 55513016.8.0000.5420), and all volunteer patients signed an informed consent form.

Only molars or pre-molars with primary endodontic infection and without periodontal pockets deeper than 4 mm were selected. Only the palatal canal of each tooth was sampled. None of the patients reported spontaneous pain. Teeth that could not be isolated with a rubber dam and teeth in which the paper point could not be introduced in the canal were excluded. The following clinical/radiographic features were found in root canals with primary endodontic infections investigated: pain on palpation (10/24), tenderness to percussion (13/24), and a radiolucent area greater than 3 mm in size (09/24).

Files, instruments, and all materials used in this study were treated with Cobalt^{60} gamma radiation (20 kGy for 6 hours) for sterilization and the elimination of pre-existing endotoxins (IPEN; Instituto de Pesquisas Energéticas e Nucleares, São Paulo, SP, Brasil). The method used for disinfection of the operative field was previously described.[19,20,21] Briefly, the teeth were isolated with a rubber dam, and the crown and surrounding structures were disinfected with 30% hydrogen peroxide (volume/volume for 30 seconds) followed by 2.5% sodium hypochlorite (NaOCl) for the same period of time, and then inactivated with 5% sodium thiosulfate. The sterility of the external surfaces of the crown was checked by taking a swab sample from the crown surface and streaking it onto blood agar plates, which were then incubated both aerobically and anaerobically.

A 2-stage access cavity preparation was made without the use of water spray but under manual irrigation with sterile/apyrogenic saline solution and using a sterile/apyrogenic high-speed diamond bur. The first stage was performed to promote a major removal of contaminants, including carious lesions and restorations. In the second stage, before entering the pulp chamber, the access cavity was disinfected according to the protocol previously described. Also, sterility of the internal surface of

the access cavity was checked by taking a swab sample from the access cavity surface, and analyzed. All procedures were performed aseptically. The first endotoxin sampling was taken by introducing sterile/apyrogenic paper points (size #15, Dentsply Sirona) into the full length of the canal, which was determined radiographically and retained in position for 60 seconds for sampling. Immediately afterwards, the sample was placed in a pyrogen-free glass and immediately suspended in 1 mL limulus amebocyte lysate (LAL) water, according to the endotoxin dosage by using a kinetic turbidimetric LAL (Lonza, Walkersville, MD) assay. This sampling procedure was repeated with 3 paper points that were pooled in a sterile tube containing 1 mL Viability Medium Göteborg Anaerobically (VMGA III) transport medium[22] for microbial cultivation.

After accessing the pulp chamber and subsequent first endotoxin sampling, teeth were randomly divided into 2 groups: HCM (n = 12) and PTN (n = 12). After the first sampling, the root canal length was determined from the preoperative radiograph and confirmed using an apex locator (Root ZXII Mini, J. Morita Corp., Tokyo, Japan). The root canals were then prepared according to the group selection.

All instruments were set into permanent rotation with a 6:1 contra-angle handpiece (Sirona, Bensheim, Germany) powered by a torque-limited electric motor (VDW.Silver Reciproc motor, VDW). The preparation sequences were as described below.

2.1 Group HCM

HCM instruments were used according to the manufacturer's instructions. The first instrument was used in an in-an-out pecking motion of about 3 mm in amplitude with apical pressure. After 3 pecking motions, the instrument was removed from the canal and cleaned. Next, a size #15 K-type file was taken to the working length (WL) to check whether the canal was patent. These procedures were repeated until the HCM instrument reached the WL (−1 mm). All HCM instruments were used to the WL of the canals in a gentle in-and-out motion. The instrumentation sequence was as follows: file Step 1 #25/08 at two thirds of the WL, followed by the file Step 2

#20/04, Step 3 #25/04, Step 4 #20/06, Step 5 #30/04, and Step 6 size #40/04 at the WL.

2.2 Group PTN

PTN instruments were used according to the manufacturer's instructions in a gentle in-and-out motion. Afterward, the instrument was removed from the canal and cleaned. Next, a size #15 K-type file was taken to the WL (−1 mm) to check whether the canal was patent. The instrumentation sequence was as follows: X1 instrument at two thirds of the WL, X1 instrument at the WL (−1 mm) (taper = 04, size #16), X2 instrument at the WL (−1 mm) (taper = 06, size #25), X3 at the WL (−1 mm) (taper = 075, size #30).

For both groups irrigation was performed with disposable syringes and 30-G NaviTip needles (Ultradent, South Jordan, UT) by using 5 mL 2.5% NaOCl solution between files. Before the second sampling after instrumentation, NaOCl was inactivated with 5 mL sterile 0.5% sodium thiosulfate during a 1-minute period, which was then removed with 5 mL sterile/apyrogenic water. Next, a new sampling procedure was performed as described previously. After instrumentation and sample collection, the root canals received calcium hydroxide intracanal medication and after 14 days were filled with Gutta-Percha (Dentsply Sirona [PTN] or VDW [HCM]) and FillApex MTA (Angelus, Londrina, Paraná, Brazil), and then restored with composite resin (3M, Maplewood, Minnesota, EUA).

2.3 Determination of Cultivable Bacterial Counts (Culturing Procedure)

The method used for culture procedures in the present study was previously reported.[21,23,24] Briefly, the transport media containing the root canal samples were thoroughly shaken for 60 seconds (Vortex; Marconi, Piracicaba, São Paulo, Brazil). Serial 10-fold dilutions were made up to 10^{-4} in tubes containing Brain Heart Infusion broth (BHI; Himedia, Mumbai, Maharashtra, India). Fifty microliters of the serial dilutions were plated onto 5% defibrinated sheep Brain Heart Infusion agar (BHI agar; Kasvi, São José dos Pinhais, PR, Brazil) by using sterile plastic spreaders

to culture nonselectively obligate anaerobes and facultative anaerobes. The plates were incubated at 37°C in anaerobic atmosphere for up to 14 days. After this period, colony-forming units (CFU/mL) were visually quantified for each plate.

2.4 Determination of Endotoxin Concentration (Kinetic Turbidimetric LAL Assay)

The kinetic turbidimetric LAL assay Pyrogent-5000 (Lonza, Walkersville, MD, EUA) used for quantification of endotoxins was previously described.[25,26] Briefly, 100 mL apyrogenic water (reaction blank), 5 standard endotoxin solutions (0.01–100 endotoxin units [EU]/mL), root canal samples, and positive controls (each root canal sample contaminated with a known concentration of endotoxin [10 EU/mL]) were added to a 96-well apyrogenic plate. The tests were performed in duplicate. The plate was incubated at 37°C ± 1°C for 10 minutes in the microplate reader BioTek ELx808 (Lonza, Walkersville, MD, EUA), which was coupled to a microcomputer by means of the WinKQCL software. Next, 100 µL Pyrogent-5000 reconstituted reagent was added to each well. After the beginning of the kinetic test, the software continuously monitored absorbance at 340 *nm* in each microplate well, and automatically calculated the log/log linear correlation between the reaction time of each standard solution and the corresponding endotoxin concentration.

2.5 Statistical Analysis

The data collected (CFU/mL and endotoxin concentrations EU/mL) were statistically analyzed by using Sigma Plot 12.0 for Windows (Systat Software Inc, San Jose, CA). The Shapiro-Wilk test showed that the variables studied had normal distribution. The data also presented a homogeneous distribution. A comparison between different sampling times and the root canal treatment groups was performed by using the Two-Way ANOVA test. The significance level was always set at 5% ($P < .05$).

3 RESULTS

Table 1 shows the number of CFU/mL and the concentration of Endotoxins (EU/mL) found before (S1) and after (S2) the instrumentation with PTN or HCM. Concerning the bacterial culture, the presence of bacteria was verified in 100% of the initial samples (S1) in the two systems. At S2, there was no bacterial growth in 4 of 12 PTN samples and 5 of 12 HCM samples. Bacterial culture analysis revealed no statistically significant difference between the two instrumentation systems (p=0.226) in the reduction of root canal bacteria with primary endodontic infections. In relation to endotoxin concentrations, its presence was detected in 100% of the samples collected before and after root canal instrumentation. However, there was no statistically significant difference between the two instrumentation systems in reducing endotoxin concentration (p=0.240).

Table 1 - Effectiveness of PTN and HCM for the removal of cultivable bacteria (CFU/mL) and endotoxins (EU/mL) from primarily infected root canals

	Cultivable bacteria (CFU/mL)		Endotoxins (EU/mL)	
groups	Before treatment (S1)[a]	After treatment (S2)[b]	Before treatment (S1)[a]	After treatment (S2)[b]
PTN	3.6×10^6 ($2.0 \times 10^4 – 3.0 \times 10^7$)	4.4×10^4 ($0 – 4.0 \times 10^5$)	27.50 (2.34 – 100)	2.95 (0.24 – 7.63)
HCM	1.2×10^6 ($5.0 – 8.6 \times 10^6$)	1.7×10^3 ($0 – 1.7 \times 10^4$)	26.38 (1.63 – 122)	1.82 (0.29 – 7.10)

Source: prepared by the author
CFU, colony-forming units; EU, endotoxin units; PTN, ProTaper Next; HCM, HyFlex CM.
Media and range values of cultivable bacteria and endotoxins found in primarily infected root canals.
Different letters indicate significant statistical differences between initial and final sampling (Fisher LSD Method, $P < 0.001$).

Table 2 - Percentage reduction values of cultivable bacteria and endotoxins found in primarily infected root canal after PTN or HCM instrumentation

groups	Cultivable bacteria	Endotoxins
PTN	98,7%	89,2%
HCM	99,8%	93,1%

Source: prepared by the author
PTN, ProTaper Next; HCM, HyFlex CM.

4 DISCUSSION

The results demonstrated that the two instrumentation systems were effective in the reduction of bacteria and endotoxins from root canals with primary endodontic infections. The data are in accordance with other studies that also demonstrated bacterial reduction above 95% with mechanized systems.[24,27] However, none of the systems was able to completely eliminate bacteria and endotoxins.

In our study, we observed the presence of negative culture in the samples in part of the patients after the instrumentation. Although CFU/mL counting is a reliable method to evaluate the cleaning ability of endodontic instrumentation, one should take into account several limitations that may have led to samples with negative bacterial cultures, which does not mean that bacteria were not present. Thus, these negative cultures could be a consequence of a very low level of bacteria that possibly could not be detected; limitations related to the sampling procedures, to the culture techniques, or to the presence of bacteria that cannot yet be cultured.[28]

It is known that pulp and periapical diseases are mainly caused by bacteria and their byproducts.[1] Endotoxins are related to the triggering of inflammation and subsequent release of inflammatory mediators.[4,29] In the present study, the detection of endotoxins was performed in 100% of the samples, in accordance with previous studies.[23,30,31,32] Endodontic files systems reduced endotoxins by more than 89%, with no statistical difference between them. However, endotoxins were detected in all cases after instrumentation, regardless of the system used. The endotoxin values found are in agreement with previous studies that used the Pyrogent-5000 turbidimetric LAL kinetic test (Lonza).[33,34]

LAL tests for the quantification of endotoxins use a coagulation cascade that is activated by the presence of endotoxin.[25] In the chromogenic tests (chromogenic endpoint [QCL test] and kinetic chromogenic [KQCL test] assays) this presence is represented by the yellow color intensity of the samples while in the turbidimetric test (Pyrogent-5000) the measurement is by turbidity. In both, higher amount of endotoxins, more yellowish or turbid the solutions with the samples are presented.

According to Martinho et al.[25] the turbidimetric kinetic method is one of the most useful tests to quantify the endotoxins of root canal infections, being an

accurate test with good reproducibility, as well as the KQCL test. Whereas, QCL test is limited in relation to its sensitivity.[25]

In the present study, molars and pre-molars with primary endodontic infections and chronic apical periodontitis were used, in order to obtain a sample with similar microbiological characteristics. To perform the initial collection without previous instrumentation, the palatine canal was chosen due to its larger anatomy. However, in some cases, dentinal removal was necessary in order to allow the introduction of the paper cone until the apparent tooth length. In these cases, Gattes-Gliden drill was used to remove dentin only at the orifice entrance.

The PTN files presents larger taper compared to the HCM files, and its decentralized core design promotes less contact of the instrument with the root canal walls,[35] favoring a greater extrusion of debris in the coronal direction.[36] HCM has a great flexibility when compared in to other instruments made of super-elastic wires, which could suggest a lower cleansing of large root canals. Instruments with great flexibility have their cutting power diminished since it deforms easily in the walls of the root canals against slight pressure.[37]

On the other hand, the HCM system presents a greater number of instruments than the PTN systems and consequently the greater the need for irrigation during the biomechanical preparation since the root canals were irrigated with each file change. Studies have shown that the number of instruments does not influence root canal cleansing, but a sequence with more instruments provides more volume irrigation, contributing to decontamination.[33] Another difference between the two systems is the final tip diameter of the last instrument used for each system: while HCM presents size #40/04 (step 6), PTN has size #30/075 (X3), which promotes further widening of the zone suggesting greater cleanliness of this region.[34] Nevertheless, no statistical difference was found in the reduction of bacteria and endotoxins between the two systems of instrumentation and none of the systems were able to eliminate 100% of the bacteria and their byproducts.

Given the results of the present study, where bacteria and endotoxins were not completely eliminated regardless of the system used, it is important to highlight the relevance of using intracanal medication as an aid in the disinfection of infected root canals. Recent studies showed that calcium hydroxide-based intracanal medication reduces 99.5% of microorganisms in persistent infections, in addition to

reducing pro-inflammatory cytokines.[38] Other studies confirm the ability of calcium hydroxide-based medication to eliminate endotoxins at different time periods of action.[39]

5 CONCLUSION

Thus, it could be concluded that both systems were in the same way able to eliminate large amounts of bacteria and endotoxins from the root canals with primary endodontic infections, although remnants of endotoxins were found in all cases.

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Laboratory of Microbiology of Piracicaba Dental School - State University of Campinas (UNICAMP).

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ATTACHMENTS

ATTACHMENT A - Clinical Record

UNIVERSIDADE ESTADUAL PAULISTA
"Júlio de Mesquita Filho"

CÂMPUS DE ARAÇATUBA – FACULDADE DE ODONTOLOGIA

DISCIPLINA DE ENDODONTIA

FICHA DE ANAMNESE

1. Identificação

Nome completo:		
Naturalidade:		Estado Civil:
Sexo: ( )M ( )F	Data de Nascimento: ___/___/___	Local:

Endereço Residencial:		
CEP:	Cidade:	Estado:

2. Questionário

Nome do Médico:		
Endereço:		
CEP:	Cidade/Estado:	Fone:
Quando foi seu último exame físico completo?		
Está sob cuidado médico? ( ) Sim ( ) Não	Desde Quando?	
Por quê?		
Está tomando algum medicamento? ( ) Sim ( ) Não		
Qual?		

Toma periodicamente substâncias que afetam a saúde? ( ) Sim ( ) Não
Qual?
É alérgico a algum medicamento ou substância? ( ) Sim ( ) Não
Qual?
É alérgico a metais ou ao látex? ( ) Sim ( ) Não
É alérgico aos compostos que contém iodo? ( ) Sim ( ) Não
É alérgico a antibióticos, anestésicos ou outro tipo de medicamento? ( ) Sim ( ) Não
Cite:
Está grávida ou crê que possa estar? ( ) Sim ( ) Não
Utiliza algum anticoncepcional? ( ) Sim ( ) Não
Qual?
Está sendo tratado para enfermidades cardíacas? ( ) Sim ( ) Não
Sofre de sopro no coração? ( ) Sim ( ) Não
Usa marca-passo ou válvula cardíaca artificial? ( ) Sim ( ) Não
Tem história de doença cardíaca na família? ( ) Sim ( ) Não
Teve alguma enfermidade? ( ) Sim ( ) Não
Qual?
Já sofreu alguma intervenção cirúrgica? ( ) Sim ( ) Não
Qual?
Esteve sob tratamento com radiação ou quimioterapia para combater algum tumor? ( ) Sim ( ) Não
Tem pressão alta? ( ) Sim ( ) Não
Tem pressão baixa? ( ) Sim ( ) Não
Tem febre reumática? ( ) Sim ( ) Não
Tem alterações no sangue, como anemia ou leucemia? ( ) Sim ( ) Não
Qual?
Sangrou excessivamente depois de cortar-se ou ferir-se? ( ) Sim ( ) Não
Tem algum problema de estômago? ( ) Sim ( ) Não
Tem problemas renais? ( ) Sim ( ) Não

Tem problemas hepáticos? ( ) Sim ( ) Não
É diabético? ( ) Sim ( ) Não
Tem história de diabetes na família? ( ) Sim ( ) Não
Sofre de asma? ( ) Sim ( ) Não
Tem epilepsia ou ataque nervoso? ( ) Sim ( ) Não
Tem ou teve alguma doença venérea? ( ) Sim ( ) Não
Foi-lhe diagnosticado ser HIV positivo (AIDS)? ( ) Sim ( ) Não
Teve ou tem hepatite? ( ) Sim ( ) Não
Qual delas?
Teve ou tem tuberculose? ( ) Sim ( ) Não
Fuma, mastiga tabaco ou consome outra variedade do tabaco? ( ) Sim ( ) Não
Consome bebidas alcoólicas? ( ) Sim ( ) Não
Qual a frequência e que tipo?
É usuário de drogas (cocaína, maconha ou outro tipo)? ( ) Sim ( ) Não
Utiliza habitualmente substâncias controladas? ( ) Sim ( ) Não
Está sob tratamento psiquiátrico? ( ) Sim ( ) Não
Tem alguma enfermidade, condição ou problema não mencionado neste questionário? ( ) Sim ( ) Não
Explique:
Existe alguma informação ainda não esclarecida que deseja manifestar? ( ) Sim ( ) Não
Gostaria de falar confidencialmente com o dentista sobre algum problema? ( ) Sim ( ) Não

3. Queixa principal

DADOS SOBRE A DOR

1 – Localização	( ) Localizada	( ) Difusa
2 – Manifestação	( ) Provocada	( ) Espontânea

3 - Duração	( ) Curta	( ) Longa	
4 - Frequência	( ) Intermitente	( ) Contínua	
5 – Intensidade	( ) Leve	( ) Moderada	( ) Severa

Certifico que as informações prestadas são exatas.

_____ _____

Assinatura do Paciente Assinatura do Responsável

Data: _____/_____/_____.

Observações:_____

ATTACHMENT B - Informed Consent Form

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Título da Pesquisa: “Análise da efetividade de procedimentos endodônticos na redução de microrganismos e endotoxinas”.
Nome do (a) Pesquisador (a): Camila Ambrosio Dias Machado
Nome do (a) Orientador (a): Prof. Adj. Rogério de Castilho Jacinto

1. **Natureza da pesquisa**: o(a) sr.(a) está sendo convidado (a) a participar desta pesquisa que tem como finalidade avaliar a eficácia dos sistemas mecânicos para a remoção de bactérias cultiváveis e endotoxinas de canais radiculares.

2. **Participantes da pesquisa**: Trinta e seis pacientes que procurarem o serviço de Triagem da Faculdade de Odontologia de Araçatuba com necessidade de tratamento endodôntico primário serão incluídos no presente estudo. Serão selecionados apenas molares superiores com infecção endodôntica primária, com ausência de sintomatologia dolorosa espontânea, e ausência de bolsa periodontal com profundidade maior que 4 mm.

3. **Envolvimento na pesquisa**: ao participar deste estudo o(a) sr.(a) permitirá que o (a) pesquisador (a) realize o tratamento endodôntico proposto, e colete amostras microbiológicas do sistema de canais radiculares. O(a) sr.(a) tem liberdade de se recusar a participar e ainda se recusar a continuar participando em qualquer fase da pesquisa, sem qualquer prejuízo para o(a) sr.(a) nome do participante. Sempre que quiser poderá pedir mais informações sobre a pesquisa através do telefone do (a) pesquisador (a) do projeto e, se necessário através do telefone do Comitê de Ética em Pesquisa.

4. **Sobre as entrevistas**: será realizada uma entrevista para avaliar as condições sistêmicas do paciente, como parte do protocolo clínico da instituição. Além disso, será questionado ao paciente de uso prévio de antibióticos (3 meses), pois em caso

afirmativo poderá influenciar nos resultados e o paciente será eliminado da pesquisa.

5. **Riscos e desconforto**: a participação nesta pesquisa não infringe as normas legais e éticas. Os riscos ou desconfortos causados aos pacientes estão relacionados ao tratamento endodôntico em geral como dor pós-operatória, agudização do processo infeccioso, fraturas de lima, desvio do canal e sobreinstrumentação. A técnica de coleta de amostra com cone de papel absorvente estéril apresenta riscos mínimos, como um pequeno desconforto durante a coleta. A área que receberá tratamento e o dente envolvido estarão sob efeito de anestesia local, entretanto você poderá sentir dor durante o tratamento endodôntico. Este desconforto poderia ocorrer independentemente de ser realizada a coleta. Os procedimentos adotados nesta pesquisa obedecem aos Critérios da Ética em Pesquisa com Seres Humanos conforme Resolução nº. 466/12 do Conselho Nacional de Saúde. Nenhum dos procedimentos usados oferece riscos à sua dignidade.

6. **Confidencialidade**: todas as informações coletadas neste estudo são estritamente confidenciais. Somente o (a) pesquisador (a) e seu (sua) orientador (a) (e/ou equipe de pesquisa) terão conhecimento de sua identidade e nos comprometemos a mantê-la em sigilo ao publicar os resultados dessa pesquisa.

7. **Benefícios**: ao participar desta pesquisa o(a) sr.(a) não terá nenhum benefício direto. Entretanto, esperamos que este estudo resulte em informações importantes sobre qual tratamento apresenta maior efetividade na remoção de microrganismos e seus produtos (endotoxinas), de forma que o conhecimento que será construído a partir desta pesquisa possa tornar o tratamento endodôntico mais efetivo, onde pesquisador se compromete a divulgar os resultados obtidos, respeitando-se o sigilo das informações coletadas, conforme previsto no item anterior.

8. **Pagamento**: o(a) sr.(a) não terá nenhum tipo de despesa para participar desta pesquisa, bem como nada será pago por sua participação.

Após estes esclarecimentos, solicitamos o seu consentimento de forma livre para participar desta pesquisa. Portanto preencha, por favor, os itens que se seguem: Confiro que recebi via deste termo de consentimento, e autorizo a execução do trabalho de pesquisa e a divulgação dos dados obtidos neste estudo.
Obs: Não assine esse termo se ainda tiver dúvida a respeito.

Consentimento Livre e Esclarecido

Tendo em vista os itens acima apresentados, eu, de forma livre e esclarecida, manifesto meu consentimento em participar da pesquisa.

_____
Nome do Participante da Pesquisa

_____
Assinatura do Participante da Pesquisa

_____
Camila Ambrosio Dias Machado

_____
Prof. Associado Rogério de Castilho Jacinto

Pesquisador(a): Camila Ambrosio Dias Machado (18) 997199853
Orientador(a): Prof. Associado Rogério de Castilho Jacinto (18) 36362890
Coordenador(a) do Comitê de Ética em Pesquisa: Prof. Dr. André Pinheiro de M. Bertoz
Vice-Coordenador(a): Prof. Dr. Aldiéres Alves Pesqueira
Telefone do Comitê: (18) 36363234
E-mail cep@foa.unesp.br

ATTACHMENT C - Journal Regulations

ISSN 1807-3107 *online version*

INSTRUCTIONS TO AUTHORS

Presentation of the manuscript

The manuscript text should be written in English and provided in a digital file compatible with "Microsoft Word" (in DOC, DOCX, or RTF format).

All figures (including those in layouts/combinations) must be provided in individual and separate files, according to recommendations described under the specific topic. Photographs, micrographs, and radiographs should be provided in TIFF format, according to the recommendations described under the specific topic.

Charts, drawings, layouts, and other vector illustrations must be provided in a PDF format individually in separate files, according to the recommendations described under the specific topic.

Video files may be submitted as per the specifications, including the author's anonymity (for purposes of evaluation) and respect for the patient's rights.

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Title page (compulsory data)

- This must indicate the specialty* or research field focused on in the manuscript.

*Anatomy; Basic Implantodontology and Biomaterials; Behavioral Sciences; Biochemistry; Cariology; Community Dental Health; Craniofacial Biology; Dental Materials; Dentistry; Endodontic Therapy; Forensic Dentistry; Geriatric Dentistry; Imaginology; Immunology; Implantodontology – Prosthetics; Implantodontology – Surgical; Infection Control; Microbiology; Mouth and Jaw Surgery; Occlusion; Oral Pathology; Orthodontics; Orthopedics; Pediatric Dentistry; Periodontics; Pharmacology; Physiology; Prosthesis; Pulp Biology; Social/Community Dentistry; Stomatology; Temporomandibular Joint Dysfunction.

- Informative and concise title, limited to a maximum of 110 characters, including spaces.

- Names of all authors written out in full, including respective telephone numbers and email addresses for correspondence. We recommend that authors collate the names present in the Cover Letter with the profile created in ScholarOne™, to avoid discrepancies.
- The participation of each author must be justified on a separate page, which should meet the authorship and co-authorship criteria adopted by the International Committee of Medical Journal Editors, available at http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html
- Data of institutional/professional affiliation of all authors, including university (or other institution), college/program, department, city, state, and country, presented according to internal citation norms established by each author's institution. Verify that such affiliations are correctly entered in ScholarOne™.

Abstract: This should be presented as a single structured paragraph (but with no subdivisions into sections) containing the objective of the work, methodology, results, and conclusions. In the System if applicable, use the Special characters tool for special characters.

Keywords: Ranging from 3 (three) to 5 (five) main descriptors should be provided, chosen from the keywords registered at http://decs.bvs.br/ or http://www.nlm.nih.gov/mesh/MBrowser.html (no synonyms will be accepted).

Main Text

Introduction: This should present the relevance of the study, and its connection with other published works in the same line of research or field, identifying its limitations and possible biases. The objective of the study should be concisely presented at the end of this section.

Methodology: All the features of the material pertinent to the research subject should be provided (*e.g.*, tissue samples or research subjects). The experimental, analytical, and statistical methods should be described in a concise manner, although in detail, sufficient to allow others to recreate the work. Data from manufacturers or suppliers of products, equipment, or software must be explicit when first mentioned in this section, as follows: manufacturer's name, city, and country. The computer programs and statistical methods must also be specified. Unless the objective of the work is to compare products or specific systems, the trade names of techniques, as well as products, or scientific and clinical equipment should only be cited in the "Methodology" and "Acknowledgments" sections, according to each case. Generic names should be used in the remainder of the manuscript, including the title. Manuscripts containing radiographs, microradiographs, or SEM images, the following information must be included: radiation source, filters, and kV levels used. Manuscripts reporting studies on humans should include proof that the research was ethically conducted according to the Helsinki Declaration (*World Medical Association,* http://www.wma.net/en/30publications/10policies/b3/). The approval protocol number issued by an Institutional Ethics Committee must be cited. Observational studies should follow the STROBE guidelines (http://strobe-statement.org/), and the check list must be submitted. Clinical Trials must be reported according to the CONSORT Statement standard protocol (http://www.consort-statement.org/); systematic reviews and meta-analysis must follow the PRISMA (http://www.prisma-statement.org/), or Cochrane protocol (http://www.cochrane.org/).

Clinical Trials

Clinical Trials according to the CONSORT guidelines, available at www.consort-statement.org. The clinical trial registration number and the research registration name will be published along with the article.

Manuscripts reporting studies performed on animals must also include proof that the research was conducted in an ethical manner, and the approval protocol number issued by an Institutional Ethics Committee should be cited. In case the research contains a gene registration, before submission, the new gene sequences must be included in a public database, and the access number should be provided to BOR. The authors may use the following databases:

- GenBank: http://www.ncbi.nlm.nih.gov/Genbank/submit

- EMBL: http://www.ebi.ac.uk/embl/Submission/index.html
- DDBJ: http://www.ddbj.nig.ac.jp

Manuscript submissions including microarray data must include the information recommended by the MIAME guidelines (Minimum Information About a Microarray Experiment: http://www.mged.org/index.html) and/or itemize how the experimental details were submitted to a publicly available database, such as:

- ArrayExpress: http://www.ebi.ac.uk/arrayexpress/
- GEO: http://www.ncbi.nlm.nih.gov/geo/

Results: These should be presented in the same order as the experiment was performed, as described under the "Methodology" section. The most significant results should be described. Text, tables, and figures should not be repetitive. Statistically relevant results should be presented with enclosed corresponding p values.

Tables: These must be numbered and cited consecutively in the main text, in Arabic numerals. Tables must be submitted separately from the text in DOC, DOCX, or RTF format.

Discussion: This must discuss the study results in relation to the work hypothesis and relevant literature. It should describe the similarities and differences of the study in relation to similar studies found in literature, and provide explanations for the possible differences found. It must also identify the study's limitations and make suggestions for future research.

Conclusions: These must be presented in a concise manner and be strictly based on the results obtained in the research. Detailing of results, including numerical values, etc., must not be repeated.

Acknowledgments: Contributions by colleagues (technical assistance, critical comments, etc.) must be given, and any bond between authors and companies must be revealed. This section must describe the research funding source(s), including the corresponding process numbers.

Plagiarism

BOR employs a plagiarism detection system. When you send your manuscript to the journal it may be analyzed-not merely for the repetition of names/affiliations, but rather the sentences or texts used.

References: Only publications from peer-reviewed journals will be accepted as references. Unfinished manuscripts, dissertations, theses, or abstracts presented in congresses will not be accepted as references. References to books should be avoided.

Reference citations must be identified in the text with superscript Arabic numerals. The complete reference list must be presented after the "Acknowledgments" section, and the references must be numbered and presented in Vancouver Style in compliance with the guidelines provided by the International Committee of Medical Journal Editors, as presented in Uniform Requirements for Manuscripts Submitted to Biomedical Journals (http://www.ncbi.nlm.nih.gov/books/NBK7256/). The journal titles should be abbreviated according to the List of Journals Indexed in Index Medicus (http://www.ncbi.nlm.nih.gov/nlmcatalog/journals). The authors shall bear full responsibility for the accuracy of their references.

Spelling of scientific terms: When first mentioned in the main text, scientific names (binomials of microbiological, zoological, and botanical nomenclature) must be written out in full, as well as the names of chemical compounds and elements.

Units of measurement: These must be presented according to the International System of Units (http://www.bipm.org or http://www.inmetro.gov.br/consumidor/unidLegaisMed.asp).

Footnotes on the main text: These must be indicated by asterisks and restricted to the bare minimum.

Figures: Photographs, microradiographs, and radiographs must be at least 10 cm wide, have at least 500 dpi of resolution, and be provided in TIFF format. Charts, drawings, layouts, and other vector illustrations must be provided in a PDF format. All the figures must be submitted individually in separate files (not inserted into the text file). Figures must be numbered and consecutively cited in the main text in Arabic numerals. Figure legends should be inserted together at the end of the text, after the references.

Characteristics and layouts of types of manuscripts

Original Research

Limited to 30,000 characters including spaces (considering the introduction, methodology, results, discussion, conclusion, acknowledgments, tables, references, and figure legends). A maximum of 8 (eight) figures and 40 (forty) references will be accepted. The abstract can contain a maximum of 250 words.

Layout - Text Files

- Title Page
- Main text (30,000 characters including spaces)
- Abstract: a maximum of 250 words
- Keywords: 3 (three)-5 (five) main descriptors
- Introduction
- Methodology
- Results
- Discussion
- Conclusion
- Acknowledgments
- Tables
- References: maximum of 40 references
- Figure legends

Layout - Graphic Files

- Figures: a maximum of 8 (eight) figures, as described above.

EXAMPLES OF REFERENCES

Journals

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