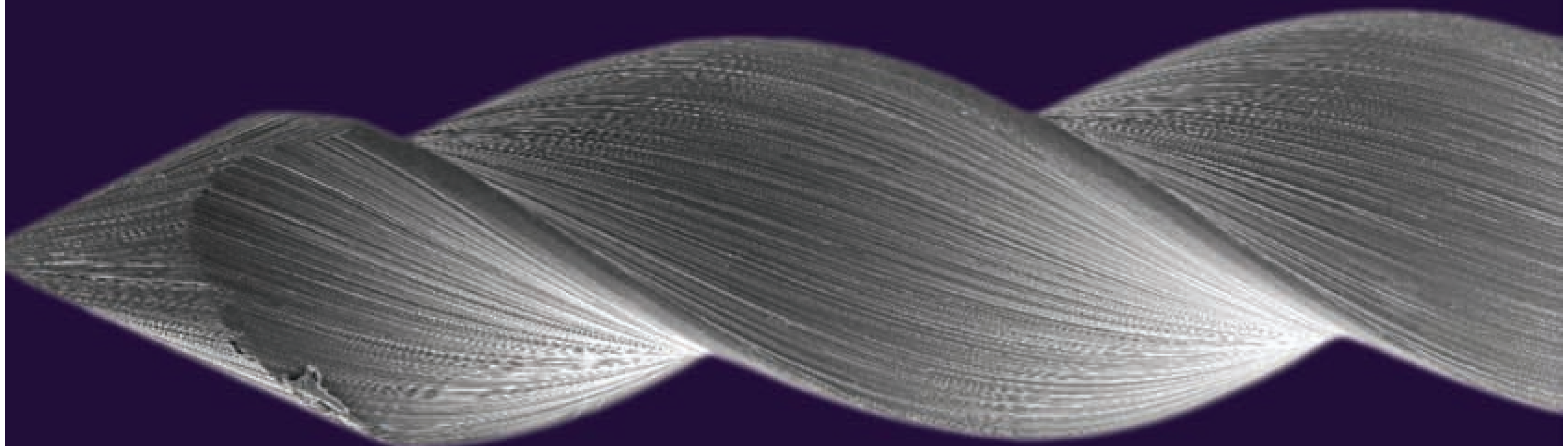
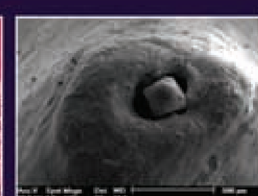
Dental Press

Endodontics





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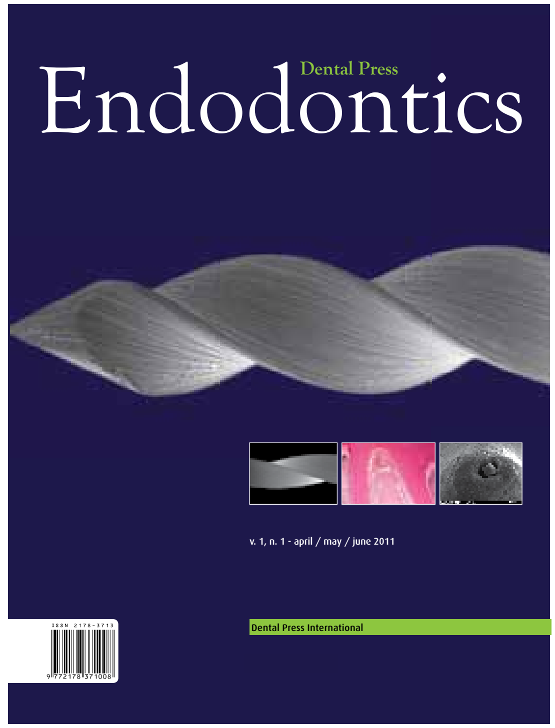

Dental Press Endodontics

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Revolution in scientific information

Mankind is experiencing constant change, which causes direct repercussions in its essence. The industrial revolution was a remarkable event. The society witnesses, at the present time, the revolution of information in different segments. The speed and the way in which this information has been promoted is fantastic. The scientific globalization encourages the different levels of an academic structure.

The challenge of the moment requires a careful selection of storage and a proper interpretation. Brazil is experiencing a very favorable moment in science, within which we can mention its quality and its acceptance by the international community. Moreover, the stimulus to the development of a major project, to the entire community that brings together endodontics as a magna specialty, is born from a careful and well structured programming. The realization of this project came from the opportunity afforded by Dr. Laurindo Furquim, publisher of Dental Press, with the creation of the Dental Press Endodontics.

Therefore, the challenge of disseminating endodontic science is launched with the creation of the Dental Press Endodontics, which is composed by a team of renowned professors, researchers and specialists in endodontics in Brazil and internationally. Endodontic scientific information certainly will have a new vehicle facilitator and promoter, able to improve clinical decisions supported by scientific evidence. The Dental Press Endodontics allow the reader to renew concepts and experience the revolution in scientific informations.

Carlos Estrela
Editor-in-chief

Case report

Informação científica e profissio

Dental Press Journal of Othodontics
160 páginas; bimestral
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Desde: 1996

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136 páginas; trimestral

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Revista Dental Press de Periodontia e Implantologia
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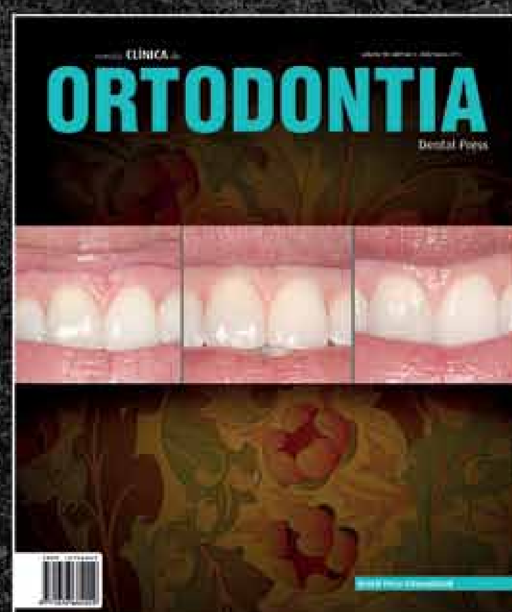
Desde: 2007



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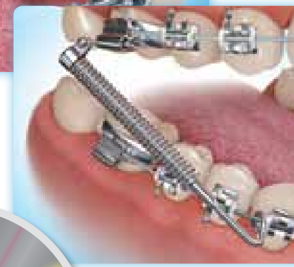

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Edmar Gontijo
Presidente do Evento

23 de junho (Quinta - feira)

08:00 às 09:00h

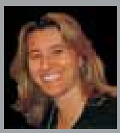
FLEXIBILIDADE EM INSTRUMENTOS ENDODÔNTICOS DE NiTi: FATORES DETERMINANTES E SEU SIGNIFICADO CLÍNICO
Profª. Ana Cecília Diniz Viana de Castro
(Belo Horizonte - MG)

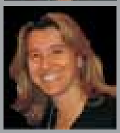
09:00 às 10:00h

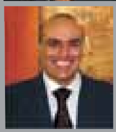
RESPONSABILIDADES LEGAIS DO CIRURGIÃO DENTISTA EM ENDODONTIA.
Prof. Ricardo Nazar
(Belo Horizonte - MG)

10:00 às 11:00h

COMPETÊNCIA EMPREENDEDORA EM ODONTOLOGIA
Prof. Trajano Arantes de Oliveira
(Porto Alegre - RS)

11:00 às 12:00h

CRITÉRIOS DE ESCOLHA DE SOLUÇÕES ANESTÉSICAS
Profª.Juliana Cama Ramacciato
(Campinas-SP)

14:00 às 15:00h

CONTROLE DA DOR E INFECÇÃO EM ODONTOLOGIA
Profª.Juliana Cama Ramacciato
(Campinas-SP)

15:00 às 16:00h

CONQUISTANDO NOVAS POSSIBILIDADES COM MTA. DAS PERFURAÇÕES AO SELAMENTO DA CAVIDADE PULPAR.
Prof. Carlos Alberto Spironelli Ramos
(Londrina - PR)

16:00 às 17:00h

OTIMIZAÇÃO DO TRATAMENTO ENDODÔNTICO EM MOLARES COM O SISTEMA ROTATÓRIO DE NRT
Prof. Érico de Mello Lemos
(São Paulo - SP)

17:00 às 18:00h

SISTEMA PRODESIGN S: UMA NOVA PROPOSTA
Prof. Henrique Bassi
(Belo Horizonte - MG)

24 de junho (Sexta - feira)

08:00 às 09:00h

MICROSCÓPICA OPERATÓRIA & TOMOGRAFIA DO TIPO CONE BEAM NA ENDODONTIA: PREVISIBILIDADE & PRODUTIVIDADE CLÍNICA.
Prof. Carlos A. F. Murgel
(Campinas- SP)

09:00 às 10:00h

FRATURA DOS INSTRUMENTOS ENDODÔNTICOS MECANIZADOS DE NiTi. CONSIDERAÇÕES CLÍNICAS:
Prof. Hélio Pereira Lopes
(Rio de Janeiro - RJ)

10:00 às 11:00h

TRATAMENTO DE DENTES COM INFECÇÃO E LESÕES PERIAPICAIS EM UMA ÚNICA SESSÃO
Prof. Ruy Hizatugu
(São Paulo - SP)

11:00 às 12:00h

COMO AGILIZAR A PRÁTICA CLÍNICA EMPREGANDO OS MAIS RECENTES SISTEMAS ROTATÓRIOS.
Prof. Gilson Sydney
(Curitiba-PR)

13:00 às 17:00h

INOVAÇÕES EM ENDODONTIA
Prof. Michael J. Scianamblo, DDS
(Califórnia-EUA)

ENCONTRO DE PROFESSORES DE ENDODONTIA

17:00 às 19:00h


A COMISSÃO ORGANIZADORA DO “CANAL 2011”, CONGRESSO INTERNACIONAL DE ENDODONTIA CONVIDA TODOS OS PROFESSORES DE ENDODONTIA PARA PARTICIPAREM DE UM ENCONTRO VISANDO PROMOVER UMA TROCA DE IDEIAS SOBRE O MOMENTO ATUAL DO ENSINO DA ENDODONTIA. NÃO ESPERAMOS MUDANÇAS IMEDIATAS, MAS DEVEMOS DISCUTIR IDEIAS QUE NOS CONDUZAM A UMA MELHOR PREVISIBILIDADE DE RESULTADOS. CONTAREMOS COM A PRESENÇA DOS PROFESSORES: HÉLIO PEREIRA LOPES (RJ), RUY HIZATUGU (SP),GILSON SYDNEY (PR) E FRANK FERREIRA SILVEIRA (MG).
A COORDENAÇÃO DOS TRABALHOS FICARÁ A CARGO DO PROF. CARLOS MURGEL (SP).
A CADA PROFESSOR SERÃO CONCEDIDOS VINTE MINUTOS PARA FAZER CONSIDERAÇÕES SOBRE OS TEMAS ABAIXO E, POSTERIORMENTE, TODOS OS PROFESSORES PRESENTES TERÃO OPORTUNIDADE DE LEVANTAR QUESTÕES DE MODO A ESTIMULAR A TROCA DE IDEIAS.
CONTAMOS COM A PRESENÇA DE TODOS OS PROFESSORES.
TEMAS:
1 - SITUAÇÃO ATUAL DO ENSINO DA ENDODONTIA. 2 - COMO PREPARAR O ALUNO PARA O MERCADO DE TRABALHO.
3 - O QUE SE DEVE MELHORAR NO ENSINO DA ENDODONTIA. 4 - CONSIDERAÇÕES FINAIS.
DATA : DIA 24 JUNHO 2011, SEXTA FEIRA.
HORÁRIO: 17 ÀS 19 HORAS, NO AUDITÓRIO DO TEATRO SESIMINAS.

25 de junho (Sábado)

08:00 às 12:00h

IRRIGAÇÃO E DESINFECÇÃO DO CANAL RADICULAR.SUCESSO E INSUCESSO NO TRATAMENTO ENDODÔNTICO.
Prof. Luc Van Der Sluis
(Amsterdam-Holanda)

CATEGORIA	
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Profª. Giane Borges
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Orthodontic treatment does not cause pulpal necrosis

Alberto **CONSOLARO**

Professor, Bauru Dental School (USP) and Postgraduate Professor of Ribeirão Preto Dental School (USP).

Consolaro A. Orthodontic treatment does not cause pulpal necrosis. Dental Press Endod. 2011 apr-june;1(1):14-20.

Introduction

The dental pulp has an arborized vascular system and its only blood source is represented by a delicate artery that penetrates the apical foramen. Seldom there is a vascular communication of the pulp with the periodontal ligament through the lateral canals and accessories from the lateral and apical foramina.

Connective tissue has many functions such as filling in the spaces between the organs, ducts and other structures. Another important function of connective tissue is the support of specialized cells in organs such as liver, kidney, pancreas and glands. In these organs, the connective tissue support is called stroma and the specialized cells parenchyma. The connective tissue can, besides filling and supporting, assume very specialized roles as in the dental pulp, which provides sensitivity and form dentin.

There are different kinds of connective tissues such as fibrous, bone, cartilage, adipose and others. They are the only vascularized tissues and they get blood to nourish and keep the cells alive and functional. Just as the vessels, there is a conjunctive plot of neural threads.

During compression and massage of soft tissues of the body, major shifts in centimeters can occur without breakdown of connective tissue structures, especially of vessels and nerves. This elasticity of the connective tissue is caused by the presence of collagen and elastic fibers in the extracellular matrix, especially when subjected to forces applied gradually and slowly. When there are sudden movements of conjunctive structures, there may be disruptions of vessels and nerves. When this occurs bruises are formed clinically characterized by reddish-purple spots. These hematomas can occur after pinching, biting and hitting, i.e., sudden traumatic and intense actions on the fibrous connective tissues.

Dental traumatism, occlusal trauma and orthodontic movement: are different in their effect the in periodontal tissue

Although the terms traumatism and trauma account for the deleterious action of physical agents, as forces on the tissues, their characteristics are not always equal in intensity and frequency. The dental traumatism can break vessels and promote ruptures, characterized by the application of sudden and intense forces on the teeth. Differently from the occlusal trauma, in which the forces are intense, small extension, short duration, but constantly repeated. On the other hand, the forces of tooth movement are very light, even the most intense and prolonged ones slowly applied on the teeth, so that they gradually disappear within hours or days. In short, although they are caused by forces, dental traumatism, occlusal trauma and orthodontic movement are not alike regarding the characteristics of the forces applied and their effects on connective tissues.

In dental traumatism the forces act abruptly with rupture of connective structures, including vessels and nerves. When a force apparently light acts on the tooth, depending on its angle of incidence and location in which it acted, there may be a resultant of forces in the apical third of the tooth root with rupture of the vascular and nerve bundle that enters the pulp. An example of dental traumatism is the concussion, with no clinically detected mobility and pain, if any, is easily controlled with common analgesics, lasting several hours or even 2 to 3 days.[2,3] Apparently, the tooth gets back to normal, but within time the pulp may show its damage with the presence of calcium metamorphosis of the pulp or pulp aseptic necrosis, both clinically revealed by coronary darkening in an apparently healthy tooth.

In occlusal trauma the death of cell and the structural rupture are minimized by the quick length and repetitiveness of the process, although it is for a long time. In this case, there is no structural damage to vascular and nervous bundle of pulp, nor fast aging of the pulp. The periodontal lesions are light and subtle. The periodontal structure must be acknowledged as an example of an organization to receive the strong forces of chewing. The periodontal fibers are organized in a space with an average thickness of 0.25 mm, but even so during chewing the teeth do not touch the bone.

In the orthodontic movement the forces applied to the tooth structure, even the most intense, gradually disappear in the surrounding tissues. The plasticity of the connective tissue of the periodontal ligament, plus the deflection capacity of the bone crest and the rotation that happens in the tooth socket promote a slow and gradual adaptation of the surrounding tissues. The orthodontic movement is limited to a maximum of 0.9 mm at the crown during the first hours[1] providing no conditions for the structural rupture of vessels and nerves to happen.

There should not be a comparison among the tissue effects induced by dental traumatism, occlusal and orthodontic movement, as they are different situations. In the apical third of root the induced orthodontic tooth movement is confined practically to the compression of the periodontal ligament, because the bone deflection in the periapical bone is much smaller and the tooth hinge axis is near the apex. The forces are absorbed and dissipated slowly, without rupturing vessels. Small movements are naturally absorbed by fibrous and elastic connective tissue.

Another important information concerns the duration in which the orthodontic forces are active: 2 to 4 days. After this time these forces are dissipated and the reorganization of the periodontal structures begins with resorption of the periodontal bone surface, cell migration for reorganization with the production of new collagen fibers.[1] After 15 to 21 days the periodontal ligament and other structures are ready for a new cycle of events by the reactivation of the orthodontic appliance. In other words, the induced tooth movement is achieved in cycles of 15 to 21 days, the tooth does not move all the time. In the orthodontic movement forces are mitigated by the collagen and elastic fibers, without damaging the structures that carry blood and sensitivity to the pulp.

With each activation period of orthodontic appliances — from 15 to 21 days — the periodontal tissues reorganize themselves and return to normal. The ultimate effects of orthodontic treatment on the structures and position of teeth are the sum of all cycles from 15 to 21 days. The forces and the effects were not continuous and unceasing. Sometimes the question is: when there is rotation of the tooth around its long axis, as in giroversion, vascular and nerve bundles get twisted around themselves, does it not compromise the blood supply to the pulp? No, they are not twisted, because the tissues reorganize themselves in each period of 15 to 21 days, they return to their normal position and relationship. When the new cycle of movement is established by a new activation, the vessels and nerves are in normal relationship with no change in their shape. Tissues constantly renew its structures, remodel and adapt themselves well to new positions and structural relationships.

Consolaro,[4] in his investigation of Masters in 2005, and Massaro et al[9] in 2009, examined microscopically the pulp of 49 first molars of rats under induced tooth movement after 1, 2, 3, 4, 5, 6 and 7 days. Resorption was detected in the external surfaces of the root, indicating the efficiency of the applied forces. However, no morphological changes was detected in the pulp tissues (Figs 1-6).

Synopsis for endodontists of the induced tooth movement, or does intense force increase the chance of pulpal necrosis by orthodontic movement?

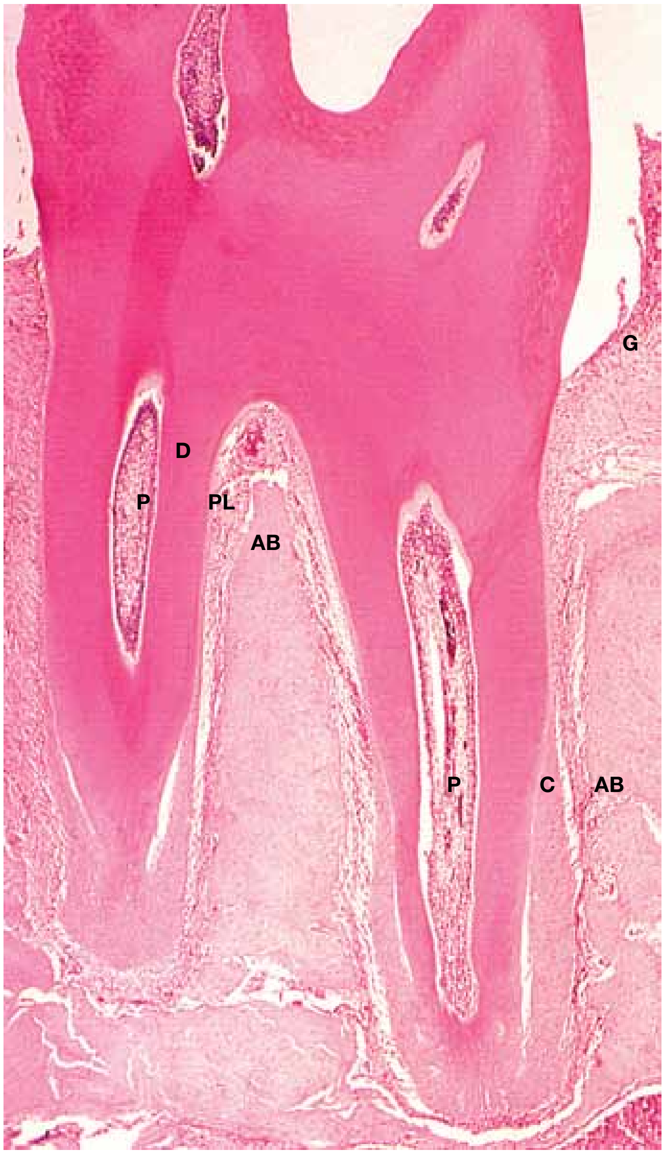

Figure 1. Rat's molar 7 days after been moved. P = pulp, D = dentin, C = cementum; PL = periodontal ligament, AB =alveolar bone, G = gum. (HE; 4X).

Figure 2. Area of compression of the periodontal ligament (PL) of the rat's molar 7 days after been moved. The arrow indicates the direction of the applied force and the narrowing of the periodontal space. Despite the compression of periodontal ligament, cells and fibers are present in the area, as well as cementoblasts, osteoblasts and also the clasts (circles). The morphological pattern of normal dental pulp is highlighted (P). D = dentin, C = cementum; AB = alveolar bone. (HE, 25X).

The orthodontic forces compress a certain segment of the periodontal ligament, because the teeth are bent on the alveolar bone crest or on the apical third on the opposite side (Figs 1 and 2). The compressed blood vessels reduce the amount of blood to the cells of that local: they momentarily stop the production and renewal of the extracellular matrix, including collagen; and get disorganized (Figs 3, 4 and 5). In some cases the cells migrate to surrounding areas still vascularized. Only the extracellular matrix in some areas that have been strongly affected by hypoxia remains in the local. These areas turn into a glassy aspect to the optical microscope and are, therefore, called hyaline areas (Fig 6).

In this segment of the compressed periodontal ligament and with reduction of blood support, there will

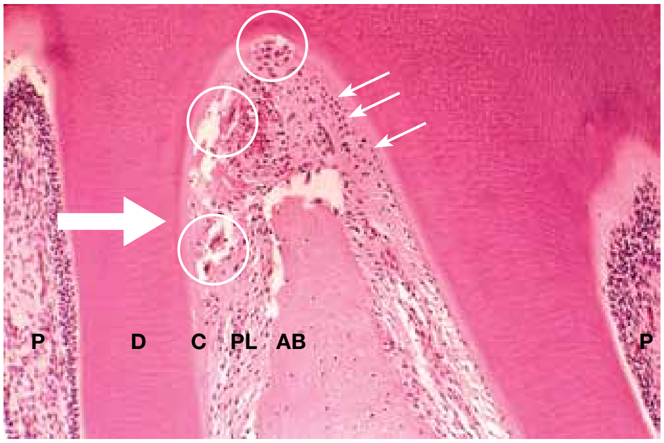

Figure 3. Area of compression of the periodontal ligament (PL) of the rat's molar 7 days after been moved. The larger arrow indicates the direction of the applied force. The small arrows indicate the cementoblasts, which are absent in the area of pressure, indicating efficiency of the applied force. Despite the compression of periodontal ligament, cells and fibers are present in the area, as well as cementoblasts, osteoblasts and also the clasts (circles). It is important to notice the morphological pattern of normal dental pulp (P). D = dentin, C = cementum; AB = alveolar bone. (HE, 25X).

be an increased local production of cellular mediators produced by metabolic stress and by the mild induced inflammation. The periodontal ligament is alive, metabolically viable, with blood supply and with clasts sufficiently activated to resorb the periodontal bone surface of the tooth socket (Fig 5). The periodontal bone resorption occurs in front of the compressed periodontal ligament and therefore it is nominated frontal bone resorption (Fig 5). Gradually, over few days, the tooth will be displaced to one side of the tooth socket, reoccupying its new place, and ligament cells restore the average thickness of 0.25 mm. In the process, especially in the apical region, vascular rupture does not occur in tissues that enter into the root canal. From this normal restored relationship, the periodontal ligament and surrounding tissues will be reorganized in a few days. After 15 to 21 days it is ready to reactivate the appliance as the tissues return to normal.

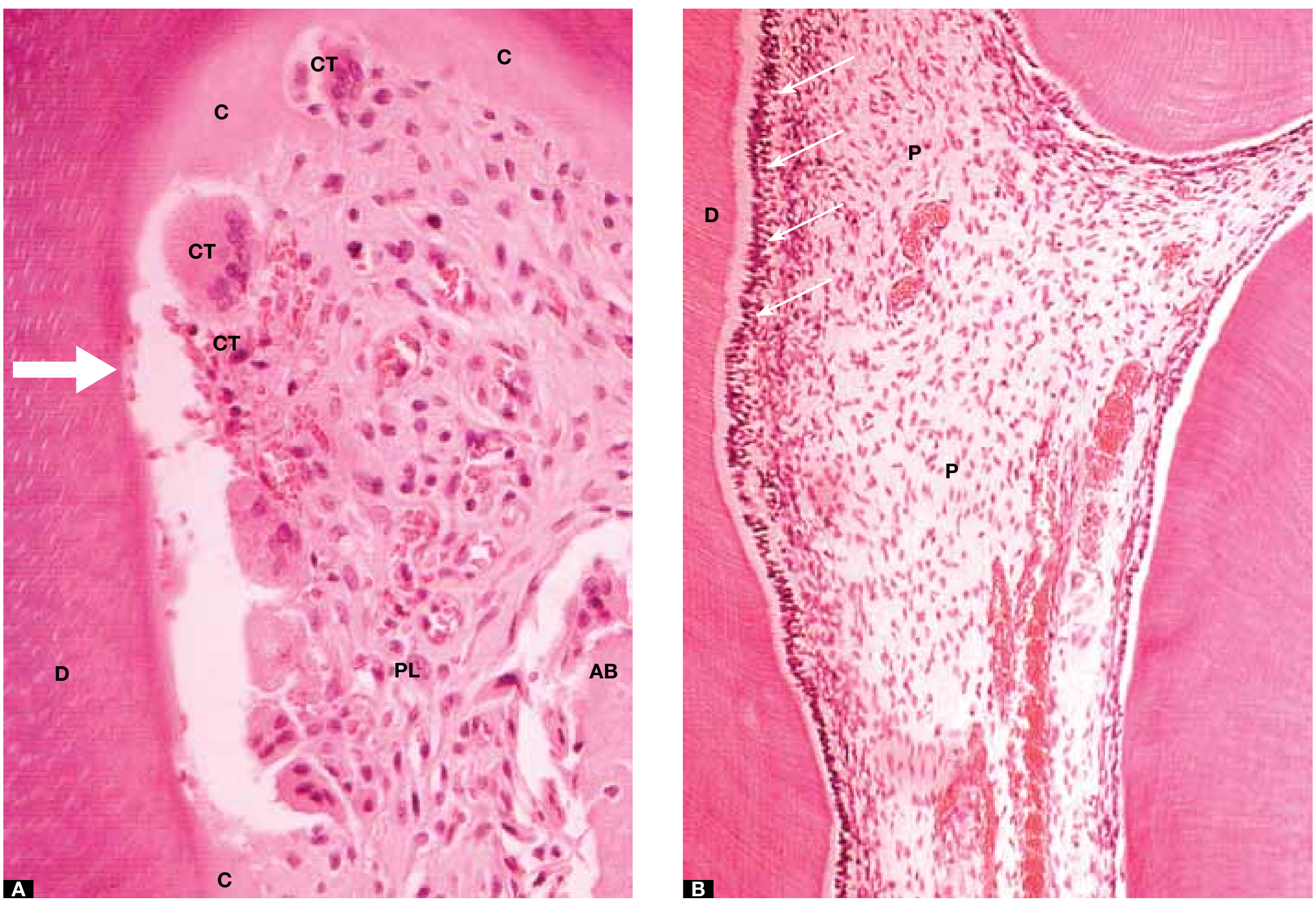

Figure 4. A) Area of compression of the periodontal ligament (PL) of rat's molar 7 days after been moved. The arrow indicates the direction of the applied force. The clasts (CT) in the root surface indicate efficiency of the applied force. In **B**, there is the morphological pattern of normal dental pulp with odontoblastic layer (small arrows). D = dentin, C = cementum; AB = alveolar bone. (HE, 40X).

The displacement of the root apex is very small and slow, the connective tissue is elastic enough to withstand much larger displacements. Besides having elastic fibers, the extracellular matrix of connective tissue display a gel between the cells and fibers, damping forces and applied displacements, without cell death and vascular rupture (Figs 1 and 2).

When a very intense force, as the one applied to the teeth that act as support for jaw expander appliance, acts on the tooth there will not be an effective movement of the tooth in the tooth socket. A very intense force collapses the blood vessels, interrupts normal vascularization in that periodontal segment. The local cells die, or, more often, flee to surrounding areas, including inflammatory and clast cells (Fig 6). Without blood supply there will be no cell activity in the periodontal surface of the alveolar bone. That is, the compressed periodontal segment gets hyaline in these conditions and without any cell activity (Fig 6).

When the vascularization is restored due to the gradual dissipation of excessive force applied, the neighboring cells will change from center to the periphery, resorbing and remodeling the hyalinized area of the periodontal ligament. Therefore, the tooth will

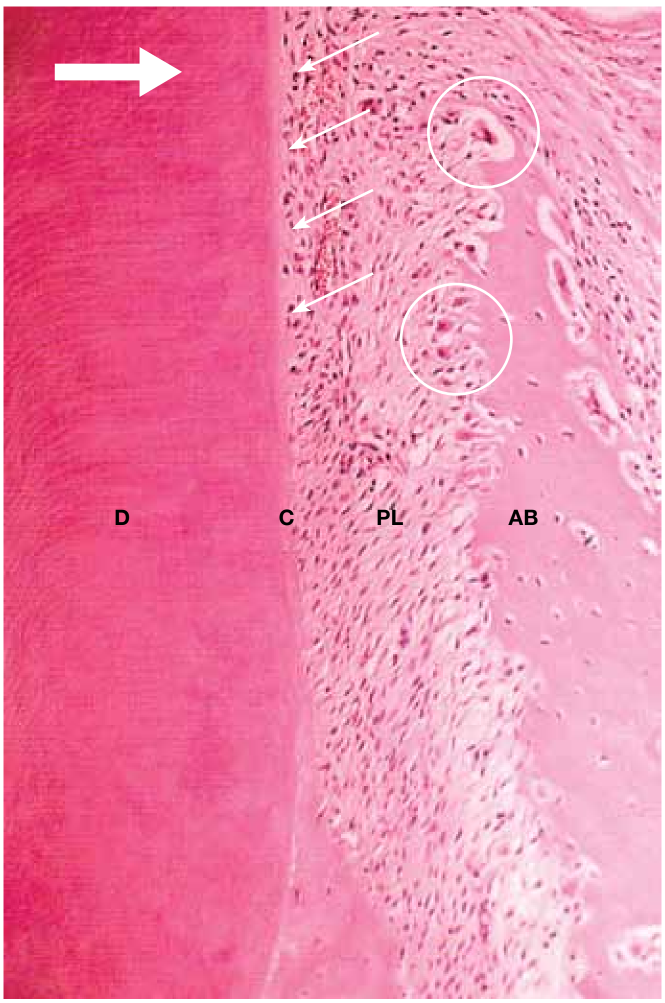

Figure 5. Area of compression of the periodontal ligament (PL) of the rat's molar 6 days after been moved with typical *frontal bone resorption*. The larger arrow indicates the direction of the applied force. The small arrows indicate the cementoblasts. Despite the compression of periodontal ligament, cells and fibers are present in the area, as well as cementoblasts, osteoblasts and clasts (circles). D = dentin, C = cementum; AB = alveolar bone. (HE, 25X).

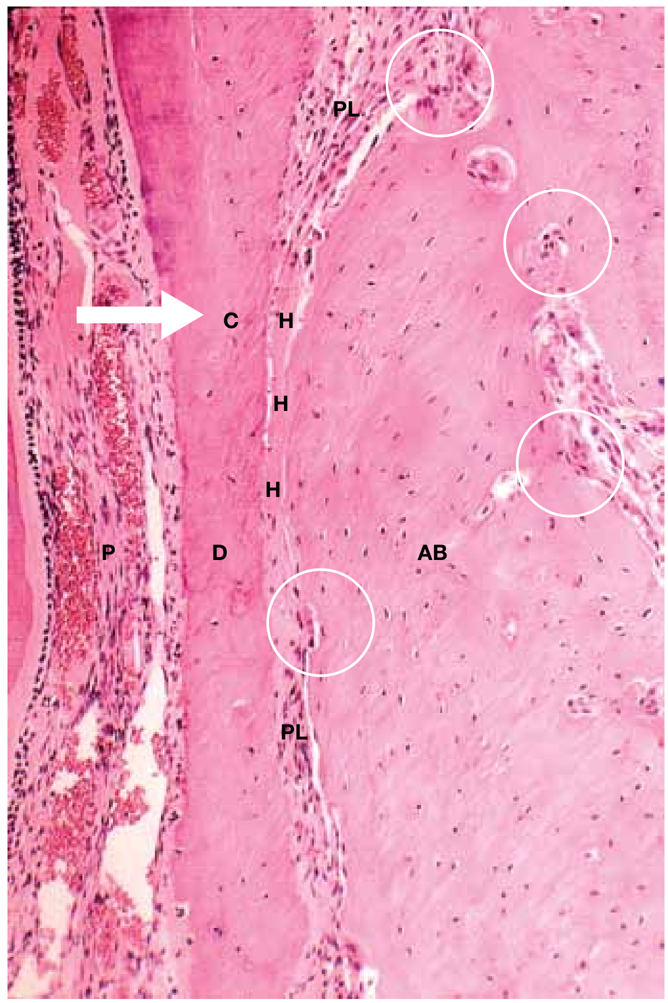

Figure 6. Classic *bone resorption at distance* in the area of compression of the periodontal ligament (PL) of the rat's molar 3 days after been moved. The arrow indicates the direction of applied force and the narrowing of the periodontal space. The area of compression of the periodontal ligament was hyalinized (H) without osteoblasts and cementoblasts. The clasts (circles) act at a distance from the compression area of the periodontal ligament (PL). The morphological pattern of normal dental pulp is highlighted (P). D = dentin, C = cementum; AB = alveolar bone. (HE, 25X).

not move because the clasts are not in metabolic conditions of nutrition and with no metabolism to act in the periodontal surface of the alveolar bone. The remodeling process of bone and hyaline area will be done from the periphery to the center, including the underlying part of the alveolar bone plate (Fig 6).

The bone resorption process and reorganization that should take place in front of the compression of the periodontal ligament, will take place at distance: *bone resorption at distance*, but it is undesirable (Fig 6). In this case, the tooth did not move neither displaced minimally, thus can not have broken the vascular and nerve bundle.

This explanation helps to understand why the teeth that act as anchoring for palatal expansion appliances, the strongest possible force to be applied to a tooth, do not suffer necrosis neither pulp aging. In short, *the more intense the orthodontic force applied is, the smaller the chance of the tooth to move in its socket; and as consequence, there is no way to infer associated pulp necrosis.* Valadares Neto,[13] in his master research in 2000 with me as advisor, analyzed the effects of rapid maxillary expansion in the dentin-pulp complex in 12 adolescents. Using devices like Hass, he examined microscopically the entire length of the pulp and dentin of 12 premolars right after the removal of the appliance with the expansion of the jaw established and other 12 premolars after 120 days from the removal of appliances. Other 6 premolars of adolescents that did not undergo any orthodontic and/or orthopedic procedure were used as control group. In every analyzed teeth the pulp-dentin complex was fully normal, without any microscopically detectable change.

And when the pulp necrosis is diagnosed in sound teeth during the orthodontic treatment?

Based on the above explanations, it is perfectly possible to understand why orthodontic treatment does not induce pulp necrosis nor accelerates its aging. In all the cases in which pulp necrosis is detected during orthodontic treatment, the history of dental traumatism must be recalled. Patients do not remember those concussions and small dental injuries in children, but they occur daily. Small strokes, bump and home accidents can be seemingly innocent, but by concentrating forces at the apex they may cause sudden and small displacements with rupture in the pulp vascular bundle. In many cases of dental traumatism, no coronary nor gingival damage or bleeding occur, but there may be aseptic pulp necrosis. In some dental traumatism, there may be severe gingival damage and heavy bleeding, but without breaking the pulp vascular bundle.

Dental concussion can also occur in the following situations: teeth that act as levers to support the extraction of adjacent teeth, small forceps beats in the extraction of third molars, unerupted and pulled canines luxation, laryngoscope trans-operative beats during general anesthesia, or even accidental bites in cutlery, seeds or strange materials during feeding.

There is no clinical, laboratorial or experimental evidence to assign, although theoretically, the pulpal necrosis as a result of orthodontic movement.[5-8,10,11,12] When facing a situation like this, try to recall the history of dental traumatism and do not assign pulpal necrosis to the orthodontic movement.

Final Considerations

1. The aseptic pulp necrosis cannot be attributed clinically and experimentally to orthodontic movement.
2. In cases of pulpal necrosis during orthodontic treatment, the history of dental traumatism should be researched, especially the lighter types, such as concussion.
3. In cases of very strong forces used in orthodontic and orthopedic treatment, tooth movement does not occur and displacement with rupture of the pulp vascular bundle has even less chance of happening.
4. Dental traumatism, orthodontic tooth movement and occlusal trauma situations are totally different from each other, although they are physical events on the tissues. The biological effects in each of these three situations are different and specific and therefore not comparable.

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A NiTi rotary instrument manufactured by twisting: morphology and mechanical properties

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ABSTRACT

Objectives: The surface morphology of TF® endodontic instruments was studied using stereomicroscopy and scanning electron microscopy (SEM). Mechanical tests were done for flexibility and microhardness. **Methods:** Four tapers of TF® files were used (0.04; 0.06; 0.08 and 0.10 mm/mm). The stereomicroscopy associated with the AxioVision® program was used to measure the tip angle, the helical angle, the taper and the tip diameter of the instruments. SEM was used to identify surface defects due to machining and finishing. The flexibility and the microhardness were measured with bending and microhardness Vickers tests, respectively. **Results and Conclusion:** The analysis showed that the manufacturer complied with the values recommended by the ANSI/ADA standard number 28. The SEM results showed many surface defects and a distortion of the instrument helix. It was observed that the instrument flexibility changes with its taper. The forces to induce the phase transformation by stress on instruments with taper 0.04; 0.06 and 0.08 mm/mm were 100 gf, 150 gf and 250 gf, respectively. The values of Vickers microhardness of the instruments are compatible with rotary instruments manufactured by the machining process.

Keywords: Endodontic instruments. NiTi alloy. R-phase. Materials characterization. Mechanical tests. NiTi manufacturing methods.

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Introduction

In 1988, Walia et al[1] used a new metal alloy to manufacture endodontic instruments, the NiTi alloy. The instruments produced with this alloy had a lower Young modulus than the instruments made with stainless steel, thus allowing the endodontic treatment of cases with large root curvatures. The use of instruments made with stainless steel could make the treatment more difficult.

The first endodontic instruments of NiTi where manufactured by a machining process using burs. With the development of new NiTi alloys, the study of the mechanisms involved in the phase transformation and better control of the microstructure, it was possible to develop a new manufacturing method based on twisting. The TF® instruments (Twisted Files, California - USA) are manufactured by twisting. This new generation of instruments has better clinical properties.

In the present work the surface morphology of endodontic instruments manufactured by twisting was investigated and microhardness and flexibility measurements were performed. These properties are important to understand the clinical behavior and to develop new instruments.

Materials and methods

Morphology

The TF® endodontic instruments (Twisted Files, California) used in this study has a length of 27 mm, a tip diameter (D_o) of 25 mm. Three different tapers were used (0.04, 0.06 and 0.08 mm/mm).

The tip angle, the tip length and the taper were determined with an optic microscope Zeiss with a pixeLINK camera model PL- a662 and a light source Zeiss 1500 LCD. The taper was determined with an amplification of 1.6X. The other dimensions were quantified with an amplification of 5X. All dimensions of the instruments were determined with the program AxioVison 4.4®. Five instruments with each taper were investigated.

Bending tests (at 45º)

The bending tests were performed with an apparatus connected to a universal material testing system EMIC DL1000 (EMIC Equipment, Brazil). A 20 N load cell was used to measure the force necessary to bend the tip of the instruments by 10°, 20°, 30° and 45°. The tests were performed according to ADA standard 28, with the force applied 3 mm from the tip of the instrument.

Vickers Microhardness

For microhardness testing, the instruments were embedded in epoxy resin. The fixation cable was parallel to the recipient base with the purpose of keeping the central longitudinal surface outside of the resin after polishing. The instruments were prepared with sandpaper 200, 300, 400, 600 and 1200 and polished with alumina particles of 0.5 μm.

The Vickers indentations were made with 100 gf during 15 s using a microdurometer Bhueler model 1600-5300. Five indentations were made in the working part and five in the neck of each specimen.

Scanning electron microscopy (SEM)

Two instruments of each taper were submitted to SEM (JEOL, LSM 5800LV) to evaluate the morphologies of the cutting edge, the tip and interface of the neck region with the fixation cable.

Statistical analysis

The data of the bending tests and the Vickers microhardness were analyzed statistically by the Kruskal-Wallis method and complemented with the Student-Newman-Keuls multiple comparison test to compare the tapers. The microhardness at the neck region was compared applying the Mann-Whitney test. The level of significance of all analyses was 5%.

Results

The results of the statistical analysis are shown in Tables 1 and 2. The bending testing results are shown in Table 3. Figure 1 shows a mean curve obtained from 10 bending tests performed in instruments with taper 0.06. The tests for other tapers showed similar curves.

The curves show a slope change that is attributed to a phase transformation. The values of the forces necessary to bend the instruments by 10°, 20°, 30° and 45° are shown in Table 2 and the forces necessary to induce phase transformation by stress are shown in Table 3.

Statistical analysis (Kruskal-Wallis test) demonstrated that there was a significant difference between instruments with different tapers ($P < 0.00001$). Then, the Student-Newman-Keuls multiple comparison test revealed that the instrument of taper 0.04mm/mm is more flexible than instruments of tapers 0.06 and 0.08 mm/mm. Moreover, the instrument of taper 0.06 mm/mm proved to be more flexible than the instrument of taper 0.08 mm/mm.

The Vickers microhardness average values at the neck region and at the working region of the instruments are shown in Table 4.

The Vickers microhardness results for each taper were submitted to the Mann-Whitney test and there was no significant difference between the values in the neck region and in the working region for all instruments ($p > 0.05$).

Table 1. Tip angle, Tip length (L) and taper of the instruments.

Instrument	Taper 0.04	Taper 0.06	Taper 0.08	Taper 0.10
Tip angle	26.56 ± 4.39	32.41 ± 7.59	32.39 ± 13.89	25.48 ± 4.92
L (mm)	0.24 ± 0.011	0.25 ± 0.013	0.24 ± 0.007	0.26 ± 0.012
Taper	0.039 ± 0.0029	0.061± 0.0016	0.077 ± 0.001	0.099 ± 0.0022

Table 2. Average values of the maximum forces to bend at 45° (gf) and respective standard deviations.

Instrument	Taper 0.04	Taper 0.06	Taper 0.08
10°	67.82 ± 7.02	130.7 ± 17.21	179.7 ± 20.62
20°	92.26 ± 4.36	183.9 ± 16.17	295.2 ± 26.27
30°	120.3 ± 7.27	247.5 ± 20.61	390.3 ± 23.15
45°	131.7 ± 9.43	263.6 ± 23.18	400.7 ± 23.88

Table 3. Average forces for phase transformation by stress.

Instrument	TF 0.04 mm/mm	TF 0.06 mm/mm	TF 0.08 mm/mm
Average force	100 gf	150 gf	250 gf

Table 4. Vickers microhardness of the instruments.

Instrument	HV neck region	HV working region
0.06	272.4 ± 31.6	291.2 ± 24
0.08	292.8 ± 33.8	293 ± 17
0.10	315.5 ± 33.7	279 ± 10.7

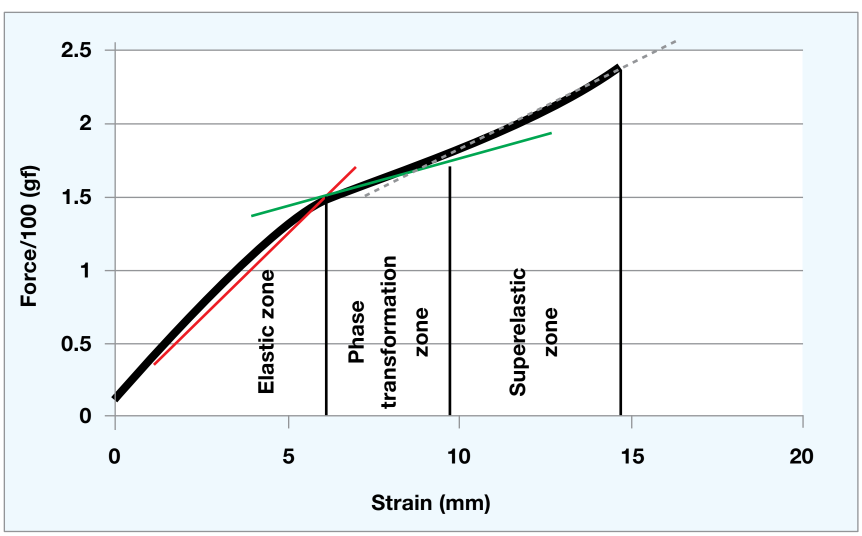
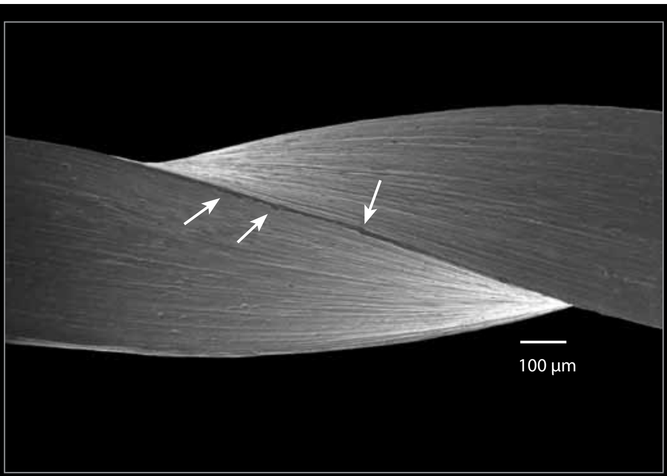


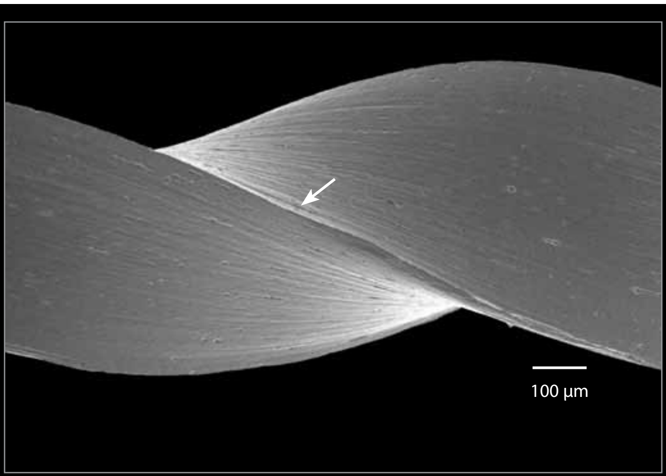
Figure 1. Mean curve for taper 0.06 mm/mm TF® files. The red line represents the elastic region, the green line phase represents the transformation region and the dashed line the superelastic region.

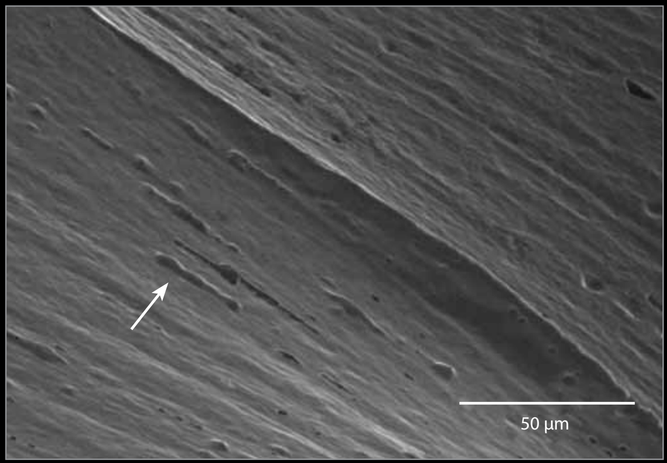
The microhardness values were also analyzed by the Kruskal-Wallis test. The statistical analysis confirmed that there was no significant difference among the groups (p=0.658). It is possible to conclude that the Vickers microhardness is independent of the taper and instrument region tested.

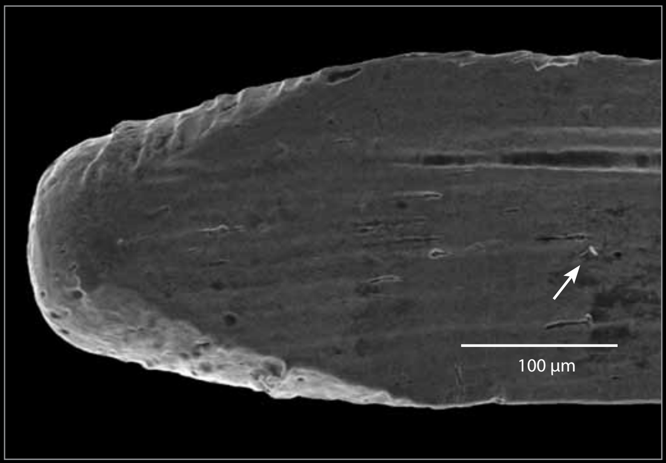
Surface analysis showed manufacturing defects in all instruments analyzed (Fig 2).

Figure 2 shows grooves produced in manufacturing process. It is possible to see the drawing tool marks along the longitudinal direction. All the samples had microcavities.









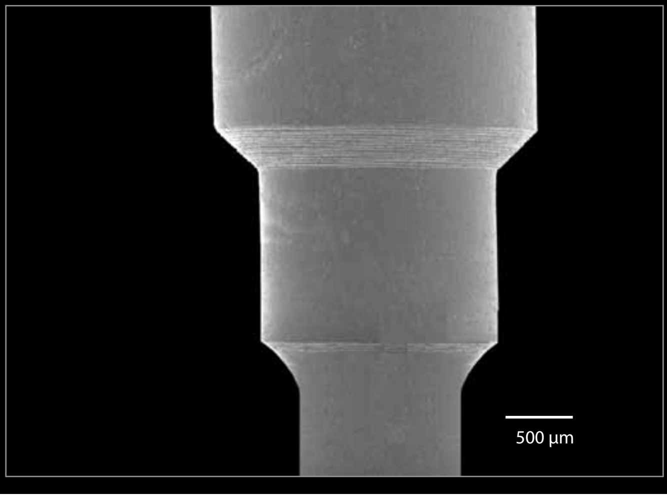


Figure 2. TF® instrument taper 0.04mm/mm images. **A**) Lateral cutting edge showing shavings (magnification of 60×). **B**) Curvature at the edge inherent to the manufacturing process (magnification of 100×). **C**) Pores present at the instrument magnification of 500×). **D**) Presence of burs (magnification of 300×). **E**) Junction of neck region to cable (magnification of 27×).

Discussion

Is important that the instrument tip has a good finishing and a transition angle that permits the introduction in the root canal. Small angles (less than 33°) can generate steps or deviations. The TF® instruments have a progressive tip angle that allows introduction in regions with a substantial curvature without deforming the canal, thus promoting a safe enlargement. The round tip can be classified as smooth.[2] Due this configuration, it is likely that the instrument will not cause damage to the root.

The instrument dimension D_o is determined by the diameter at the base of the tip basis, which serves as a reference during introduction. The value of D_o and the taper permits to determine the work diameter in a given canal region. A simple calculation can be used to determine what instruments can be used in sequence to perform an effective work. The diameter values of the tip bases (D_o) found on this study meet the ANSI/ADA standard number 28 recommendation. In the present work, we observed that the tip angles conform to the standard recommendations.

According to Thompson[3] the phase transformation of NiTi alloys does not show macroscopic changes when the application of an external force changes the microstructure. The bending tests showed a change in slope after an initial linear increase that resembled the Hooke law. This change is attributed to an austenite-to-martensite phase transformation. This slope change is in agreement with the results reported by Thompson[3]. In the beginning the material is in the elastic region, at the end the material is in the superelastic region and between the two regions the material in the phase transformation region.

In this study, we plotted the relation between the force and the strain. Thompson[3] probably used a wire in his experiments, so it was easy to calculate the stress (σ), using the area of the specimen. In our case, since the shape of the file is very complex, it is impossible to calculate the stress with any accuracy. However, since the stress is proportional to the force ($\sigma = F/A$), the shape of the curve is the same.

Thompson[3] mentioned that the preparation of the root canal promotes the martensite transformation by stress of NiTi alloy instruments. The stress level at which the phase transformation happens is not mentioned by the authors, and was found by us to be different for each instrument taper. This is an important information for future studies and for clinical practice.

According to Schäfer et al,[4] the cross section is the main factor that affects the bending tests. This is reasonable, since that a larger area implies a larger volume of metal at the core of the instrument. In the present study, a factor that influenced the maximum force to bend the instruments to 45° was the taper. The taper has the same influence as the cross section, for the same reason. If the tip diameter is kept constant, a larger taper will promote less flexibility, as was observed in the tests.

According to Miyai et al[5] and Hayashi et al,[6] the instrument flexibility is influenced by a phase transformation. The R-phase or rhombohedral has a large memory form effect and the Young modulus is lower than that of austenite, so an instrument that goes through a martensitic transformation will be more flexible.

Yahata et al[7] used the same scheme proposed by Miyai et al[5] to study the flexibility of annealed samples. However, differently from this study, the authors did not measure the average force for phase transformation when the instrument is submitted to stress.

Other values found in this study were compatible with NiTi alloys. Lopes et al[8] found average values of 345 HV in NiTi instruments (Files NiTi-flex). Serene et al[9] found values between 303 and 362 HV for the microhardness of NiTi alloys used in the manufacture of endodontic instruments. The average value found in the the present work was 289 HV. This value is consistent with others from the literature.

In this work it was observed that the manufacturing process did not change the Vickers microhardness, probably because of the thermal treatment, that could be lower than the temperature of recrystalization proposed by Kuhn and Jordan.[10]

According to the manufacturer,[11] the absence of other metal at the fixation cables avoids galvanic corrosion. The instrument is really formed by only one piece. However, galvanic corrosion should not be an important problem because of the low life in cycle at the clinic. It will be important only if the instrument remains in stock for a long period of time in adverse conditions.

According to Kim et al,[12] the TF® instruments present a significant resistance to fracture by rotating-bending fatigue when compared with others instruments manufactured by the machining process, corroborating the results obtained by Gambarini et al[13] and Larsen et al.[14] This can be explained by the fact that machining produces perpendicular defects that favor nucleation and propagation of cracks.

Even presenting good results in flexion-bending fatigue tests, the TF® files should have a better surface finishing, that would improve the clinical performance concerning durability in relation to the fracture. The surface morphology found at this work was very similar to that found by Kim et al.[12] Despite the eletropolishing, the surface is not completely flat and has machining marks from the manufacturing process. This observation corroborates the results of that study.

Conclusions

Based on the results we concluded that:

a) the dimensions of the TF® files meet the ANSI/ADA standard number 28 recommendations;
b) the files present many defects from the manufacturing process;
c) the instrument flexibility decreased with increasing taper;
d) the phase transformation induced by stress average forces to the TF® files of taper 0.04; 0.06 and 0.08 mm/mm where 100 gf, 150 gf and 250 gf, respectively, and
e) the TF® Vickers microhardness values were similar to those of NiTi rotary instruments manufactured by the machining process.

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Effect of intracanal posts on dimensions of cone beam computed tomography images of endodontically treated teeth

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ABSTRACT

Objectives: This study evaluated the effect caused by intracanal posts (ICP) on the dimensions of cone beam computed tomography (CBCT) images of endodontically treated teeth. **Methods:** Forty-five human maxillary anterior teeth were divided into 5 groups: Glass-Fiber Post®, Carbon Fiber Root Canal®, Pre-fabricated Post – Metal Screws®, Silver Alloy Post® and Gold Alloy Post®. The root canals were prepared and filled; after that, the gutta-percha filling was removed, and the ICP space was prepared. The post cementation material was resin cement. CBCT scans were acquired, and the specimens were sectioned in axial, sagittal and coronal planes. The measures of ICP were obtained using different 3D planes and thicknesses to determine the discrepancy between the original ICP measurements and the CBCT scan measurements. **Results:** One-way analysis of variance, Tukey and Kruskall-Wallis tests were used for statistical analyses. The significance level was set at $\alpha = 5\%$. CBCT scan ICP measurements were from 7.7% to 100% different from corresponding actual dimensions. **Conclusion:** Gold alloy and silver alloy posts had greater variations ($p>0.05$) than glass fiber, carbon fiber and metal posts ($p<0.05$). Gold alloy and silver alloy post dimensions were greater on CBCT scans than on original specimens.

Keywords: Cone beam computed tomography. Artifact. Intracanal post. Post.

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Introduction

Root canal obturation is a major step in the last phase of endodontic treatment, which is completed with coronal restoration. However, endodontically treated teeth often have a substantial loss of dental structure and need an intracanal post.[11]

Several types of intracanal posts (ICP) have been recommended for dental reconstructions according to the analysis of important restoratives aspects: the possibility of endodontic post failure, which may result in loss of retention; the risk of root canal reinfection due to bacterial microleakage; the effect of intracanal post length on apical periodontitis; the retentive effect of adhesive systems for the different types of posts; the possibility of stress concentration; and the difference in modulus of elasticity between post and dentin.[6,25]

Pathological and clinical findings, often supported by radiographs, provide the basis for endodontic therapy protocols and treatments. Images, however, are necessary in all phases of endodontic treatment.[11]

Since the discovery of X-rays by Roentgen in 1895, radiology has witnessed the constant development of new technologies. The angle variations proposed by Clark and the development of panoramic radiography produced novel applications in endodontics. Cone beam computed tomography (CBCT) has recently introduced three-dimensional (3D) imaging into dentistry[2,24] and brought benefits to specialties that had not yet enjoyed the advantages of medical CT due to its lack of specificity. Computerized tomography (CT) is an important, nondestructive and noninvasive diagnostic imaging tool.[2,5,24,29]

CBCT produces 3D images of a structure because it adds a new plane: depth. Its clinical application ensures high accuracy and is useful in nearly all areas of dentistry.[2,5,8,9,10,24,29] However, dimensions misdiagnoses may result from imaging artifacts. Metal or solid structures (higher density materials) may produce nonhomogeneous artifacts and affect image contrast. Concerns about diagnostic errors have motivated authors to study alternatives to correct for beam-hardening artifacts during image acquisition, image reconstruction, or under other conditions.[11-25]

Jian and Hongnian[16] found that beam hardening is caused by the polychromatic spectrum of the X-ray beam and that artifacts decrease image quality. Katsumata et al[18,19] reported that artifacts caused by halation or saturation from an imaging sensor decrease CT values on the buccal side of the jaws. In dental CBCT imaging, artifacts may change CT values of the soft tissues adjacent to the lingual and buccal sides of the jaws. The CT values of hard tissue structures may also be similarly affected.

CBCT images showing teeth with solid plastic or metal ICP may project ghost images over the areas surrounding it and mask the actual root canal structures, which increases the risk of clinical misdiagnosis. Few studies investigated misdiagnosis in association with CBCT images and ICP. This study evaluated the effect of original ICP on dimensional of CBCT images of endodontically treated teeth.

Material and Methods

Tooth preparation

This study is the continuation of a preliminary evaluation of the effect of CBCT slice on the visualization of endodontic sealers. The study sample comprised 45 maxillary anterior teeth extracted for different clinical reasons at the Dental Urgency Service of the Federal University of Goiás, School of Dentistry, Goiânia, Brazil. This study was approved by the Ethics Committee of the Federal University of Goiás, Brazil. Preoperative radiographs of each tooth were obtained to confirm the absence of calcified root canals and internal or external resorption, and the presence of a fully formed apex.

The teeth were removed from storage in 0.2% thymol solution and were immersed in 5% sodium hypochlorite (Fitofarma, Lt. 20442, Goiânia, GO, Brazil) for 30 min to remove external organic tissues. The crowns were removed to set the remaining tooth length to a standardized length of 13 mm from the root apex. After initial radiographs, standard access cavities were prepared and the cervical third of each root canal was enlarged with ISO # 50 to # 90 Gates-Glidden drills (Dentsply/Maillefer, Ballaigues, Switzerland). Teeth were prepared up to an ISO # 50 K-File (Dentsply/Maillefer) 1 mm short of the apical foramen. During instrumentation, the root canals were irrigated with 3 ml of 1% NaOCl (Fitofarma) at each change of file. Root canals were dried and filled with 17% EDTA (pH 7.2) (Biodinâmica, Ibiporã, PR, Brazil) for 3 min to remove the smear layer.

The teeth were randomly allocated into 5 groups according to the intracanal post material: Group 1 (n = 9) - Pre-fabricated Glass-Fiber Post® (White post DC®, FGM, Joinville, SC, Brazil); Group 2 (n = 9) - Pre-fabricated Carbon Fiber Root Canal® (Reforpost Carbon Fiber RX, Angelus, Londrina, PR, Brazil); Group 3 (n = 9) - Pre-fabricated Post – Metal Screws® (Obturation Screws®, FKG, Dentaire, La Chaux-de-Founds, Swiss); Group 4 (n = 9) – Silver Alloy Post® (Silver Alloy la Croix®, Rio de Janeiro, RJ, Brazil); Group 5 (n = 9) – Gold Alloy Post® (Gold Alloy Stabilor G®, Au-58.0, Pd-5.5, Ag-23.3, Cu-12.0, Zn trace, Ir trace; DeguDent Benelux BV, Hoorn, Netherlands). It was considered as control the original specimen of each group.

After root canal preparation was completed, all teeth were filled with AH PlusTM (Dentsply/Maillefer) and gutta-percha points, and prepared according to the manufacturer's instructions and using a conventional lateral condensation technique. The diameters of the prefabricated posts used in Groups 1 to 3 were compatible with the diameter of prepared root canals. For Groups 4 and 5, silver and gold metal posts were fabricated after obtaining impressions of the root canals.

The gutta-percha filling was removed and an intracanal post space was prepared using Gates-Glidden drills #2 to #3 (Dentsply/Maillefer) and Largo drill #1 (Dentsply/Maillefer) to achieve a post length of 8 mm and to leave at least 4 mm of filling material in the apical third (Fig 1). The post cementation material used was resin cement (RelyX Unicem, 3M ESPE, Seefeld, Germany) strictly according to manufacturer´s instructions.

Images Analysis

CBCT scans were acquired to obtain 3D images. The teeth were placed on a plastic platform positioned in the center of a bucket filled with water to simulate soft tissue, according to a model described in previous studies.[18,26,28] CBCT images were acquired with a first generation i-CAT Cone Beam 3D imaging system (Imaging Sciences International, Hatfield, PA, USA). The volumes were reconstructed 0.2 mm isometric voxels. The tube voltage was 120 kVp and the tube current, 3.8 mA. Exposure time was 40 seconds. Images were examined with the scanner's proprietary software (Xoran version 3.1.62; Xoran Technologies, Ann Arbor, MI, USA) in a PC workstation running Microsoft Windows XP professional SP-2 (Microsoft Corp, Redmond, WA, USA) with an Intel® Core™ 2 Duo-6300 1.86 Ghz processor (Intel Corporation, USA), NVIDIA GeForce 6200 turbo cache videocard (NVIDIA Corporation, USA) and an EIZO - Flexscan S2000 monitor at 1600x1200 pixels resolution (Eizo Nanao Corporation Hakusan, Japan).

Root sectioning

After obtaining the CBCT scans, each specimen was carefully sectioned in axial, sagittal or coronal planes using an Endo Z bur (Dentsply/Maillefer) at high speed rotation under water-spray cooling. The cross-sectional slices for the axial plane were obtained at 8 mm from the root apex; and for sagittal and coronal planes, the roots were sectioned longitudinally along the center of the root canal (Fig 1).

Measurement of specimens and CBCT slices

The CBCT scans of intracanal posts (ICP) were measured in the axial, sagittal or coronal planes. All measurements were made at 8 mm from the root apex (Fig 1). ICP measurements on axial slices were made in the buccolingual direction; on sagittal slices, in the mesiodistal direction; and on coronal slices, in the buccolingual direction. All teeth were measured by two endodontic specialists using a 0.01-mm resolution digital caliper (Fowler/Sylvac Ultra-cal Mark IV Eletronic Caliper, Crissier, Switzerland).

To determine the discrepancy between original ICP values and CBCT values, all measurements were made on the same axial, sagittal and coronal sites. All the CBCT measurements were acquired by two dental radiology specialists using the measuring tool of the CBCT proprietary software (Xoran version 3.1.62; Xoran Technologies, Ann Arbor, MI, USA). CBCT dimensions were reformatted using 0.2-, 0.6-, 1.0-, 3.0- and 5.0-mm slice thicknesses.

The two calibrated examiners measured all the specimens and CBCT images and evaluated ICP dimensions in the directions previously described. When a consensus was not reached, a third observer made the final decision.

One-way analysis of variance (ANOVA), Tukey and Kruskall-Wallis tests were used for statistical analyses. The level of significance was set at $\alpha = 5\%$.

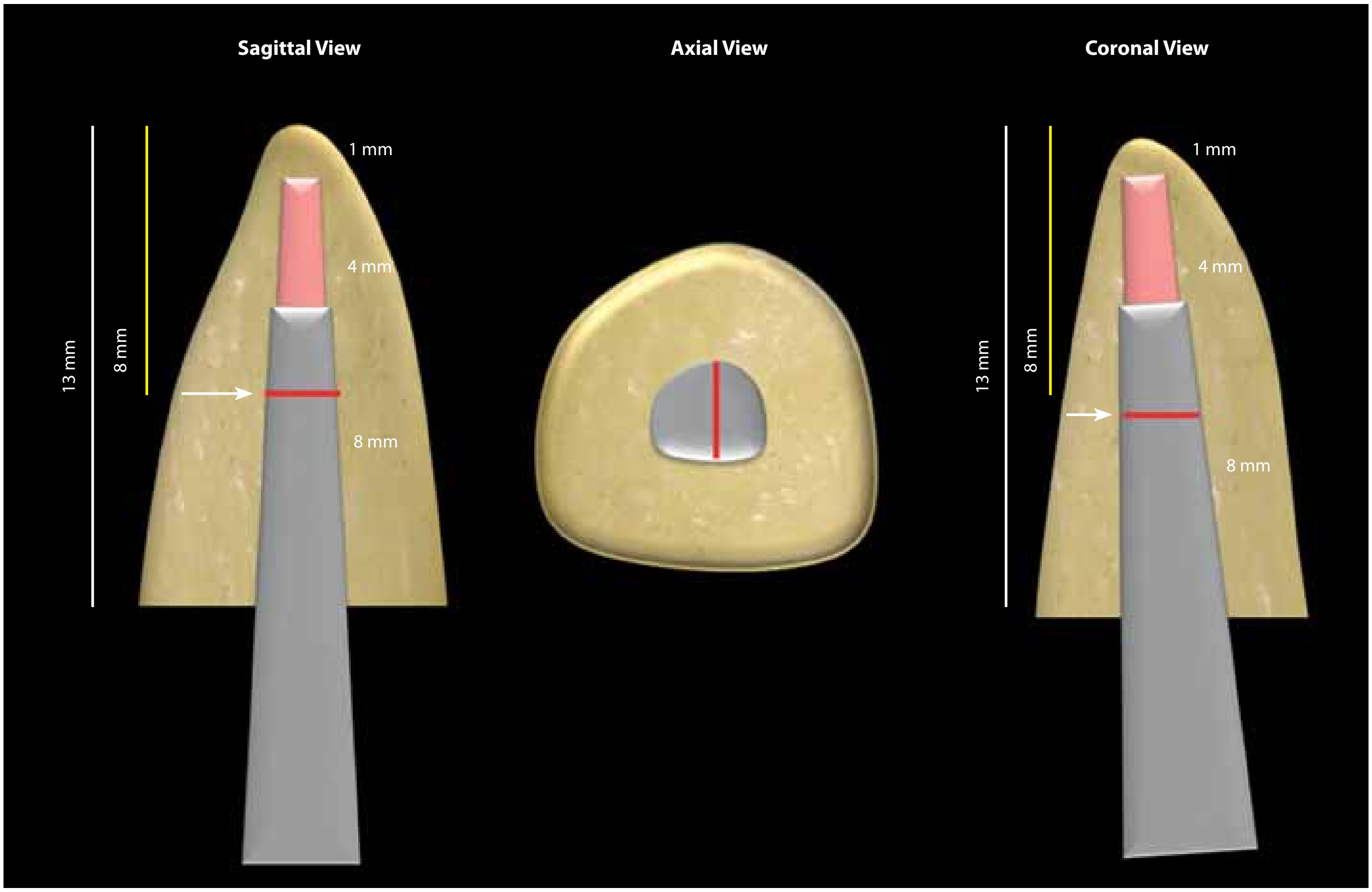

Figure 1. Schematic representation of sectioning root method and posts length, showing the sagittal, axial and coronal views.

Table 1. Percentage (%) of original ICP dimension increase on CBCT scans according to slice thickness and planes for each type of endodontic material (α=5%).

Thickness/Plane	Glass fiber	Carbon fiber	Metallic pre-fabricated	Silver	Gold
0.2 mm/ Axial	16.70	7.70	50.00	100.00	73.30
0.2 mm/ Coronal	16.70	38.50	66.70	85.70	100.00
0.2 mm/ Sagittal	0.00	33.30	53.80	57.10	57.10
0.6 mm/ Axial	16.70	7.70	50.00	100.00	73.30
0.6 mm/ Coronal	16.70	38.50	66.70	85.70	100.00
0.6 mm/ Sagittal	0.00	33.30	53.80	57.10	57.10
1 mm/ Axial	16.70	-7.70	50.00	100.00	73.30
1 mm/ Coronal	16.70	38.50	66.70	85.70	100.00
1 mm/ Sagittal	0.00	33.30	53.80	57.10	57.10
3 mm/ Axial	16.70	-7.70	50.00	100.00	73.30
3 mm/ Coronal	16.70	38.50	50.00	85.70	84.60
3 mm/ Sagittal	0.00	16.70	38.50	57.10	42.90
5 mm/ Axial	16.70	-7.70	50.00	100.00	73.30
5 mm/ Coronal	16.70	38.50	50.00	71.40	84.60
5 mm/ Sagittal	0.00	16.70	23.10	57.10	42.90
P value	**0.001***	**0.001***	**0.001***	**0.001***	**0.001***

*Interaction between type of cut and slice thickness and type of post significantly by Kruskall Wallis test.

Results

The increase of ICP dimensions in CBCT images ranged from 7.7% to 100% (Table 1). Differences were significant between glass fiber post, carbon fiber post and metal posts (Table 2). Figures 2-7 show the CBCT sagittal, axial and coronal views of the ICP. No significant differences were found when different slice thicknesses were used.

Table 2. Percentage (%) of original ICP dimension increase on CBCT scans in each group according to study variables (post, slices thickness and planes) and statistic analysis (α=5%).

Factor	Groups				
Posts*	Glass Fiber	Carbon Fiber	Metallic pre-fabricated	Gold	Silver
	11.13^{D}	21.20^{C}	51.54^{B}	72.85^{A}	79.98^{A}
Thickness**	0.2 mm	0.6 mm	1 mm	3 mm	5 mm
	50.44^{A}	50.44^{A}	49.41^{A}	44.20^{A}	42.22^{A}
Planes***	Axial		Coronal	Sagittal	
	47.69^{A}		58.38^{A}	35.95^{B}	

Different letters in horizontal demonstrate statistically significant difference with p <0.05.
*p=0.0001 by ANOVA and Tukey test.
**p=0.607 by ANOVA.
***p=0.0001 by ANOVA and Tukey test.

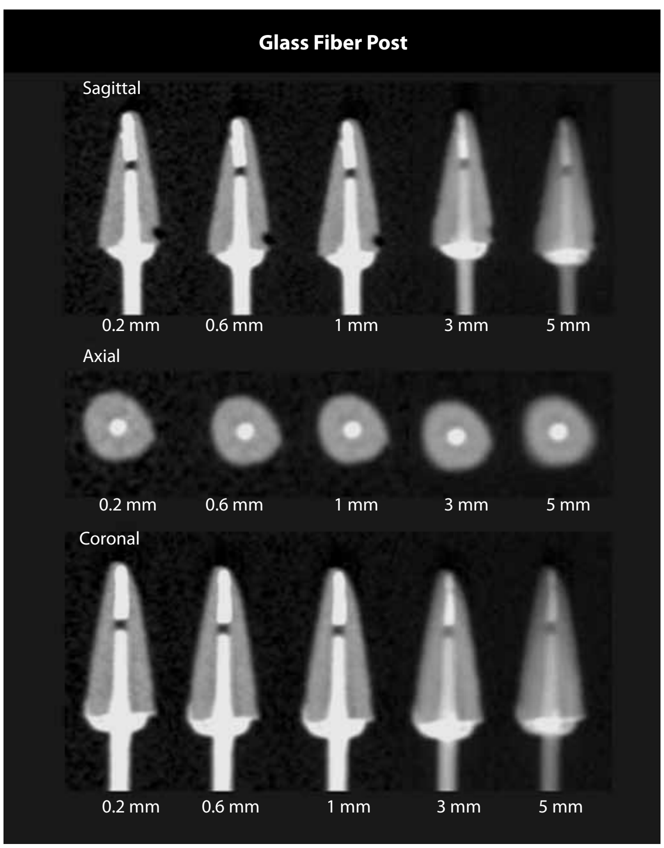

Figure 2. CBCT images of root canal filling with Glass Fiber Post in different slice thickness (0.2 mm, 0.6 mm, 1 mm, 3 mm and 5 mm) and planes (sagittal, axial and coronal).

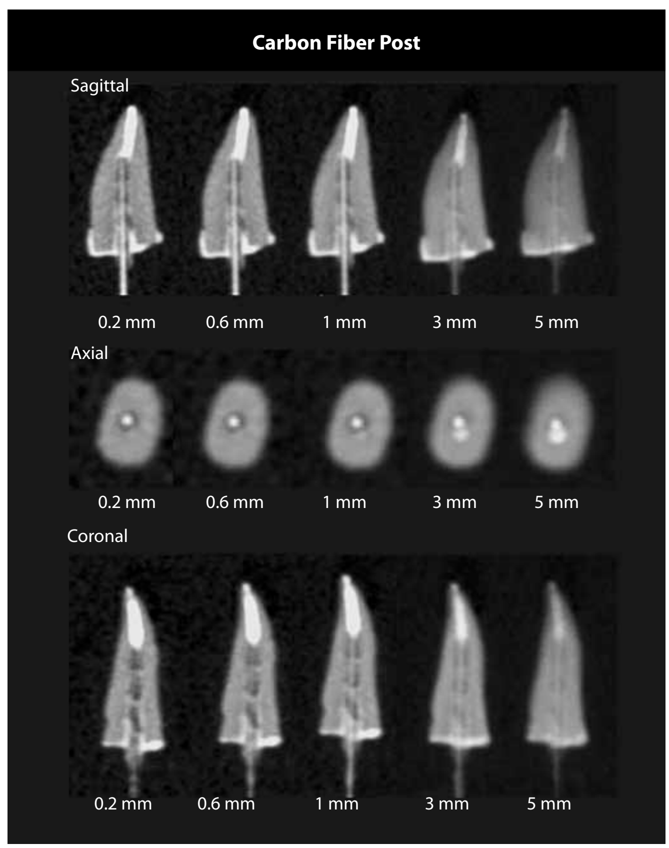

Figure 3. CBCT images of root canal filling with Carbon Fiber Post in different slice thickness (0.2 mm, 0.6 mm, 1 mm, 3 mm and 5 mm) and planes (sagittal, axial and coronal).

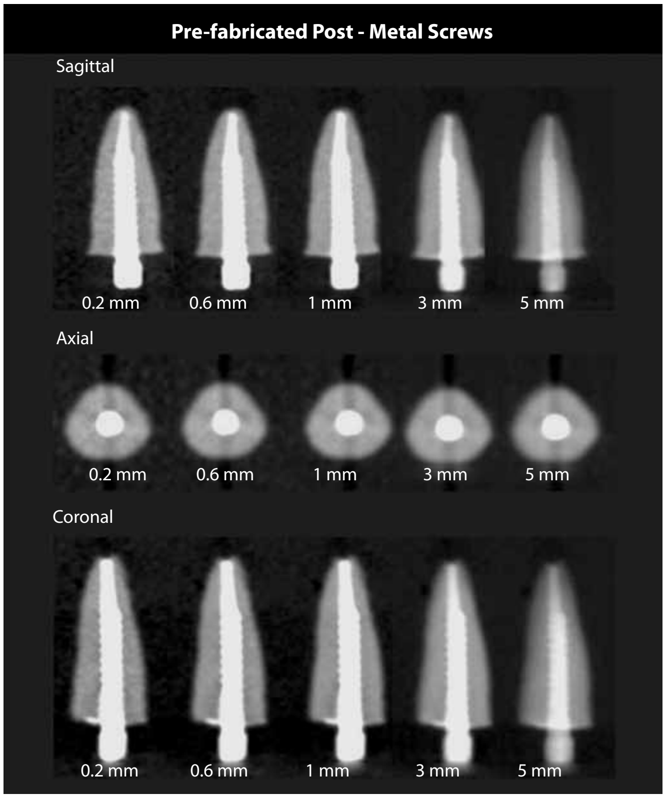

Figure 4. CBCT images of root canal filling with Pre-fabricated Post – Metal Screws in different slice thickness (0.2 mm, 0.6 mm, 1 mm, 3 mm and 5 mm) and planes (sagittal, axial and coronal).

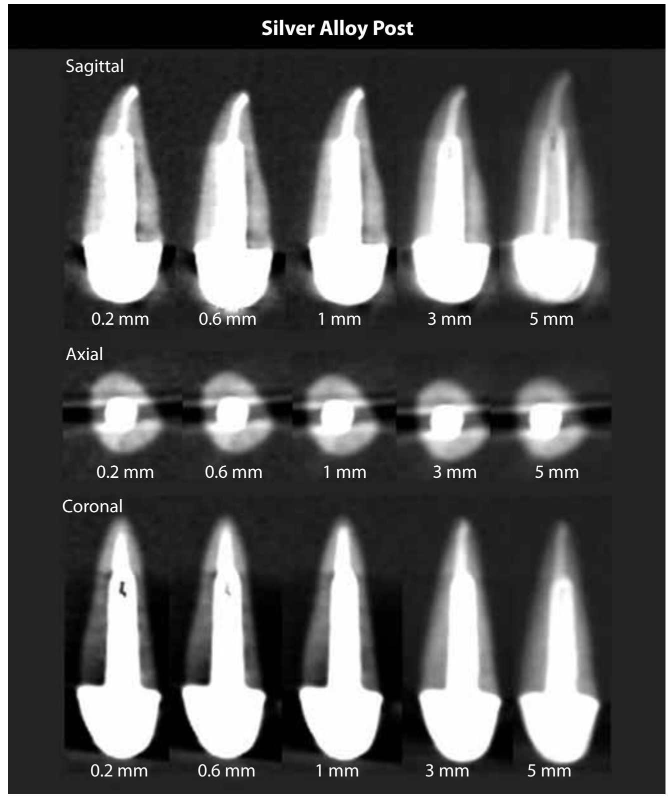

Figure 5. CBCT images of root canal filling with Silver Alloy Post in different slice thickness (0.2 mm, 0.6 mm, 1 mm, 3 mm and 5 mm) and planes (sagittal, axial and coronal).

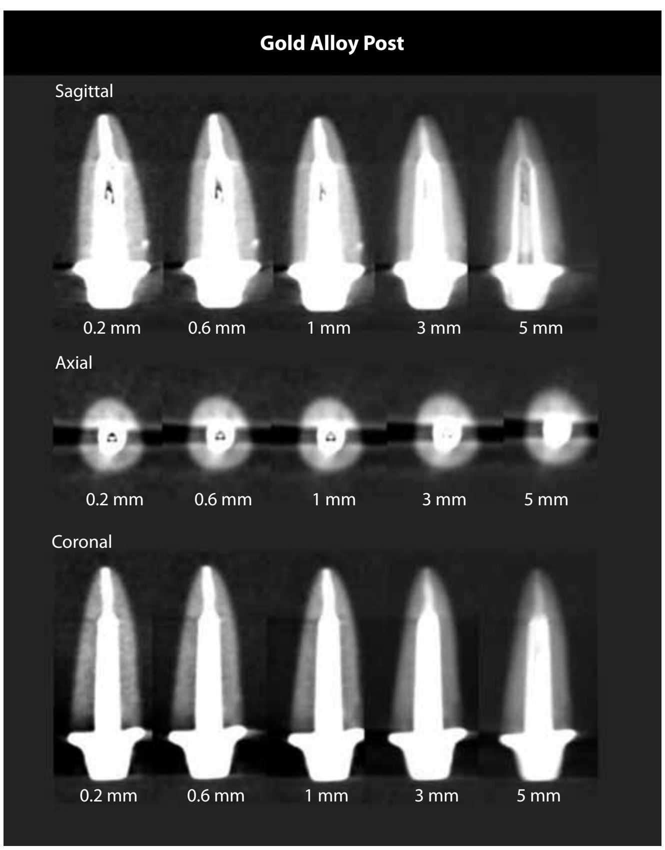

Figure 6. CBCT images of root canal filling with Gold Alloy Post in different slice thickness (0.2 mm, 0.6 mm, 1 mm, 3 mm and 5 mm) and planes (sagittal, axial and coronal).

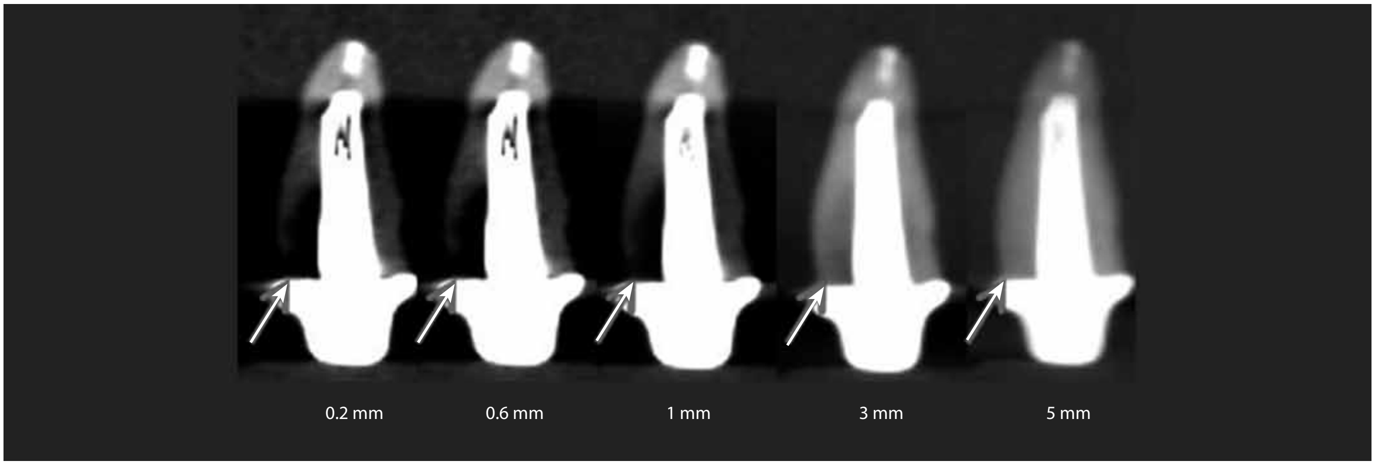

Figure 7. CBCT images of root canal filling with Gold Alloy Post, in different slice thickness (0.2 mm, 0.6 mm, 1 mm, 3 mm, and 5 mm) in coronal view showing metallic artifact in some slices.

Discussion

The 3D visualization of a tooth and oral structures using CBCT imaging represents an impressive advance in dentistry. In the past, 3D structures were superimposed on periapical radiographs; today, they may be perfectly assessed using CBCT scans.[2,5,8,9,10,24,29] Periapical radiographs are the standard method to evaluate root canal filling and ICP. However, several authors have described their limitations.[8,9,10] At the same time, high density materials may produce image artifacts, which may limit interpretation, reduce image quality, and induce diagnostic errors conditions.[3,4,7,12,13,14,16-21,26,27,28]

Few studies have evaluated imaging artifacts associated with ICP. Our findings showed that dimensional values measured on CBCT scans of gold and silver alloy posts are greater than the original specimen measurements (Tables 1 and 2). Beam hardening effects may be seen depending of the type of ICP. These results have important clinical implications, particularly when artifacts cover parts of the root and simulate or mask root pathologies. Therefore, the interpretation of CBCT scans of teeth reconstructed with ICP must be cautiously made, which justifies the use of periapical radiographs as a reference for endodontic diagnoses. Clinical examinations should always be used as a support to imaging diagnoses.

CBCT measurement tools provide satisfactory information about linear distances within an anatomic volume.[1,8,9,10,15,23,30] However, metal ICP may generate artifacts on reconstructed images, which may affect CBCT measurements. Our results did not show any significant differences between gold alloy and silver alloy posts; however, differences between metal, glass fiber and carbon fiber posts were significant (Table 2). The occurrence of imaging artifacts on CBCT scans of metal ICP should always be suspected because artifacts may limit image interpretation and characterize potential risks of misdiagnosis. No significant dimensional differences were found in this study when different slice thicknesses were used (Table 2).

CBCT reconstructions may have greater image dimensional values, as well as lack of image homogeneity and definition. Other studies have already discussed similar findings.[1,4,7,15,17-20,30]

CBCT scans of endodontically treated teeth and ICP should be carefully examined because of the higher density of metal posts and their capacity to generate image artifacts. Density artifacts affect diagnostic procedures,[28] and beam hardening correction methods have already been evaluated. Artifacts appear as cupping, streaks, dark bands, or flare artifacts, and are associated with special absorption of low-energy photons.[4,7,16-21,26,27,28] A recent study[3] suggested that the use of a harder energy beam during scanning may result in less artifact formation. The effects of beam hardening-induced cupping artifacts may also be reduced by using beam filtration.[22]

Further studies should evaluate the clinical implications of metallic artifacts and the strategies to minimize them. Our results revealed that the dimensions of gold-alloy and silver-alloy ICPs were greater on CBCT scan measurements than on the actual specimen.

Acknowledgments

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Efficacy of chemo-mechanical preparation with different substances and the use of a root canal medication in dog's teeth with induced periapical lesion

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ABSTRACT

Objectives: to evaluate the effect of instrumentation, irrigation with different substances and the use of calcium hydroxide on bacterial load and microbiota profile in dog's teeth with pulp necrosis and periapical lesion. **Methods:** Fifty five root canals were divided into groups: I) Saline (SSL) (n=11); II) natrosol gel (n=11); III) 2.5% NaOCl (n=11); IV) 2% CHX-gel (n=11); V) 2% CHX-solution (n=11). Endodontic samples were cultured, microorganisms counted and the microbiota analyzed at different sampling times — s1, s2 and s3. **Results:** At s1, the mean CFU counts ranged from 5.5 $x10^5$ to 1.5 x 10^6. These values dropped significantly at s2 ($p<0.05$). No statistical significant difference was found between s2 and s3. Changes in root canal microbiota were found at s2 and s3. **Conclusion:** Regardless the use of calcium hydroxide as a root canal medication, 2.5% NaOCl and 2% CHX-gel demonstrated a potent antimicrobial activity against endododontic pathogens *in vivo*.

Keywords: Sodium hypochlorite. Chlorhexidine. Calcium hydroxide. Endodontic infection. Root canal medication.

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Introduction

Apical periodontitis is an infectious disease caused by microorganisms colonizing the root canal system.[1] One of the main goal in endodontic treatment is to eliminate or at least reduce the bacterial population within root canal to levels that are compatible with the healing process of periapical tissues.[2]

The antimicrobial efficacy of endodontic procedures has been evaluated over a known numbers of bacteria in root canals by culture[3,4,5,6,7] and molecular techniques.[8,9]

To an optimally disinfection of root canal system, endodontic treatment comprises both mechanical and chemical phases. The first involves the action of the instruments in dentin walls combined to the flow and backflow of the irrigant solution. It acts primarily on the main canal which harbors the largest number of bacterial cells, assuming a prior role in the root canal disinfection.[3]

However, due to the anatomical complexities in root canal system[3,4,10] and the restricted action of the instruments in the main root canal, the mechanical phase does not eradicate bacteria from the entire root canal system[3,11,12] requiring a chemical phase, which involves the use of potent antimicrobial agents to act deeply in dentin tubules.[3,4]

Several auxiliary chemical substances have been proposed over the years to be used during chemo-mechanical preparation, but sodium hypochlorite (NaOCl) remains the most widely used one. Recently, chlorhexidine has been tested as a potential substance.[5,9,13,14] Most antimicrobial comparisons between the two auxiliary chemical substances are demonstrated by in vitro studies[13,14,16-19] over a selected microorganism. Indeed, not only controversy exists among these studies but also limitation in the reproducing models of the infection (mono-infection) must be considered.

As a matter of fact, in vivo studies have also been inconsistent in their findings when comparing NaOCl and CHX; with NaOCl being more effective[9,20] or with no significant difference existing among them.[14,15]

The use of an inter-appointment root canal medication — calcium hydroxide [$Ca(OH)_2$] — has been recommended to help eliminate remaining bacteria strategically located in the root canal system after chemo-mechanical procedures.[4,10,21,22,23]

While some studies[4,11,12,24] had reported a further bacterial load reduction after the placement of $Ca(OH)_2$, others[10,21,22] demonstrated an increase in the proportion of positive cultures and bacterial counts. Indeed, its effectiveness in significantly increasing bacterial load reduction and the number of negative culture after chemo-mechanical procedures in clinical practice has been doubtful.[11,21,23,25]

Moreover, most in vivo studies[7,9,13,23] investigating the antibacterial effects of root canal procedures had provided only quantitative data, not determining its effect on the microbiota involved, which assumes special relevance to the establishment of therapeutic strategies.

This clinical study was conducted to evaluate the effect of instrumentation, irrigation with different substances and the use of calcium hydroxide on bacterial load and microbiota profile in dog's teeth with pulp necrosis and periapical lesion.

Materials and Methods

Root canal selection

Fifty five root canals (5 single root-canal premolars and 25 multiple root-canal premolars) from adult mongrel dogs were selected. Tooth shorter than 12 mm length and/or incompletely formed apices was excluded. The animals were first anesthetized with intravenous injection of 5% sodium thionembutal (10 mg/Kg body weight). An access opening was made with a high-speed diamond bur under irrigation, the pulp tissues were removed, and apical foramen was standardizing in 0.20 K-file diameter. Afterwards, the root canals remained open and exposed to the oral environment for 6 months to allow microbial contamination. An approval for the study protocol was obtained from the Ethics Committee of the Dental School of Piracicaba.

Microbial sampling

After isolating the tooth with a rubber dam, the crown and the surrounding structures were disinfected with 30% H_2O_2 (v/v) for 30 s, followed by 2.5% NaOCl for an additional 30 s. The disinfectant solutions were inactivated with 5% sodium thiosulphate in order to avoid interference with bacteriologic sampling.[9] Then the sterility control samples were taken from the toot surface with sterile paper points. An access cavity was prepared with sterile high-speed diamond burs under irrigation with sterile saline. Before entering the pulp chamber, the access cavity was disinfected by the same protocol as above and new sterility control samples were taken of the cavity surface and streaking it on blood agar plates. For the inclusion of the tooth in the study, these control samples had to be negative. All subsequent procedures were performed aseptically. The pulp chamber were accessed with burs and rinsed with sterile saline, which was aspirated with suction tips. The first root canal sample (s1) was taken as follows: five sterile paper points were placed for 1 minute period into each canal to the total length calculated from the pre-operative radiograph and then pooled in a sterile tube containing 1 ml Viability Medium Göteborg Agar (VMGA III). Afterwards, the baseline samples (s1) were transported to the laboratory within 15 minutes for microbiological procedures.

Clinical procedures

After accessing the pulp chamber and subsequent microbial sampling (s1), teeth were randomly divided into groups according to the substances applied, as follows: I) saline solution (SSL) (n=11); II) natrosol gel (n=11); III) 2.5% NaOCl (n=11); IV) 2% CHX-gel (Endogel, Itapetininga, SP, Brazil) (n=11) and V) 2% CHX-solution (n=11). The pulp chamber was thoroughly cleaned with substances from each group. A K-file size 10 or 15 (Dentsply Maillefer, Ballaigues, Switzerland) was placed to the full length of the root canal calculated from the pre-operative radiographs. The coronal two-thirds of each canal was initially prepared using rotary files (GT® rotary files size 20, 0.06 taper - Dentsply Maillefer, Ballaigues, Switzerland) at 350 rpm, 4 mm shorter than the estimated length. Gates-Glidden burs sizes 5, 4, 3 and 2 (DYNA-FFDM, Bourges, France) were used in a crown-down technique reaching 6 mm shorter than the working length (1 mm from the radiographic apex). Afterwards, the working length was checked with a radiograph after inserting a file in the canal to the estimated working length, confirmed by the apex locator (Novapex, Forum Technologies, Rishon le-Zion, Israel). The apical preparation was performed using K-files ranging from size 35-45, followed by a step back instrumentation, which ended after the use of three files larger than the last filed used for the apical preparation. The working time of the chemo-mechanical procedure was established at 20 minutes for all cases.

In the CHX and natrosol gel groups, root canals were irrigated with a syringe (27-gauge needle) containing 1 ml of each substance before the use of each instrument, being immediately rinsed afterwards with 4 ml of saline solution. Particulary, in NaOCl-group, the use of each instrument was followed by an irrigation of the canal with 5 ml of 2.5% NaOCl solution. CHX activity was inactivated with 5 ml solution containing 5% Tween 80% and 0.07% (w/v) lecithin over a 1 min period. NaOCl was inactivated with 5 ml of sterile 5% sodium thiosulphate over a 1 min period. A second bacteriological sample was taken (s2), as previously described.

After drying the canal with sterile paper points, all teeth were dressed with a thick mix of a paste of calcium hydroxide (Merck, Darmstad, Germany) with sterile saline. The calcium hydroxide slurry was plugged in the canals with a lentulo spiral. Radiographs were taken to ensure proper placement of the calcium hydroxide in the canal. The access cavity was restored with 2 mm of Cavit™ (3M Dental Products, St Paul, MN, USA) and Filtek™ Z250 (3M Dental Products), in order to prevent coronal microleakage. After 14 days, teeth were aseptically accessed under rubber dam isolation and the calcium hydroxide was removed by the use of the master apical file and with sterile saline and careful filling the canal with the master apical file. A third bacteriological sample (s3) was taken, as previously described.

Culture technique

The transport medium containing the root canal samplings was shaken thoroughly in a mixer inside an anaerobic chamber for 60 s (Vortex, Marconi, São Paulo, SP, Brazil). The transport medium contained glass beads of 3 mm in diameter in order to facilitate mixing and homogenization of the sample prior to cultivation. Serial 10-fold dilutions were made up to 1:104 in tubes containing Fastidious Anaerobe Broth (FAB, Lab M, Bury, UK). Fifty µL of the serial dilutions 1:10^2 and 10:10^4 were plated, using sterile plastic spreaders, into 5% defibrinated sheep blood Fastidious Anaerobe Agar (FAA, Lab M), in which 1ml/l of hemin and 1ml/l of vitamin K1 were added, so as to culture non-selectively obligate anaerobes. Plates were incubated anaerobically (80% N_2, 10% H_2, 10% CO_2) at 37° C for 7 days (Peters LB 2002). Subsequently, 50 µL of each dilution were inoculated on BHI agar plates (Brain Heart Infusion agar, Oxoid, Basingstoke, UK), supplemented with 5% sheep blood, and incubated aerobically (37° C, air) for 24 and 48 h. After incubation, the total CFU value was counted using a stereomicroscope at 16 x magnifications (Zeiss, Oberkoren, Germany).

Microbial characterization

Preliminary characterization of microbial species were based on colony features (i.e. size, color, shape, height, lip, surface, texture, consistency, brightness and hemolysis) visualized under a stereoscopic lens (Lambda Let 2, Atto instruments Co., Hong Kong). Isolates were purified by subculture. Gram-stained and gaseous requirements were established by incubation for 2 days under aerobic and anaerobic environments.

Based on microbial colony features, Gram-stain and gaseous requirements, it was possible to determine the microbiota profile from root canals at different samplings moments (s1, s2, s3).

Statistics

Statistical comparisons were made between all groups (I-V) at the same samplings moments (s1, s2 or s3) and between s1, s2 and s3 in each group using the Kruskall-wallis test for non-parametric data (CFU counts, percentages of gram-positive rods and cocci, percentages of facultative and strict anaerobes species). When significant differences were found in the Kruskall-wallis test, Mann-Whitney test was performed to demonstrate where the differences were located. P-values <0.05 were considered statistically significant.

Results

Sterility check samples taken from the rubber dam, the crown and its surrounding structures tested before and after entry into the pulp chamber showed no microbial growth. The mean of the total colony forming unit (CFU) counts in the baseline samples (s1) ranged from 5.5×10^5 to 1.5×10^6 (Table 1). At s1, no statistically significant difference was found between any of the mean CFU values found in all groups: GI) 9.3×10^5, GII) 5.5×10^5, GIII) 6.7×10^5, GIV) 6.4×10^5 and GV) 1.5×10^6 (all P>0.05) (Table 1). These values dropped significantly as a result of root canal instrumentation (s2): GI) 1.6×10^4, GII) 1.4×10^4, GIII) 7.6×10^2, GIV) 3.2×10^2 and GV) 2.6×10^3 (Table 1). At s2, statistically significant differences were found between all the mean CFU values (p<0.05), except when comparing GIII (NaOCl-group) with GIV (CHX-gel-group) (p>0.05) (Table 1), as both substances reduced almost 100% of the bacterial load (Fig 1).

After application of $Ca(OH)_2$ for 2 weeks (s3) bacterial mean CFU values dropped even lower than those at the end of the first visit (s2): GI) 6.7×10^3, GII) 5.3×10^3, GIII) 1.4×10^2, GIV) 1.8×10^2 and GV) 1.2×10^3 (Table 1). Higher and significant percentage levels of bacterial load reduction were found between s2 and s3 in group I (SSL), II (Natrosol-gel) and V (CHX-solution) (p<0.05) (Fig 1).

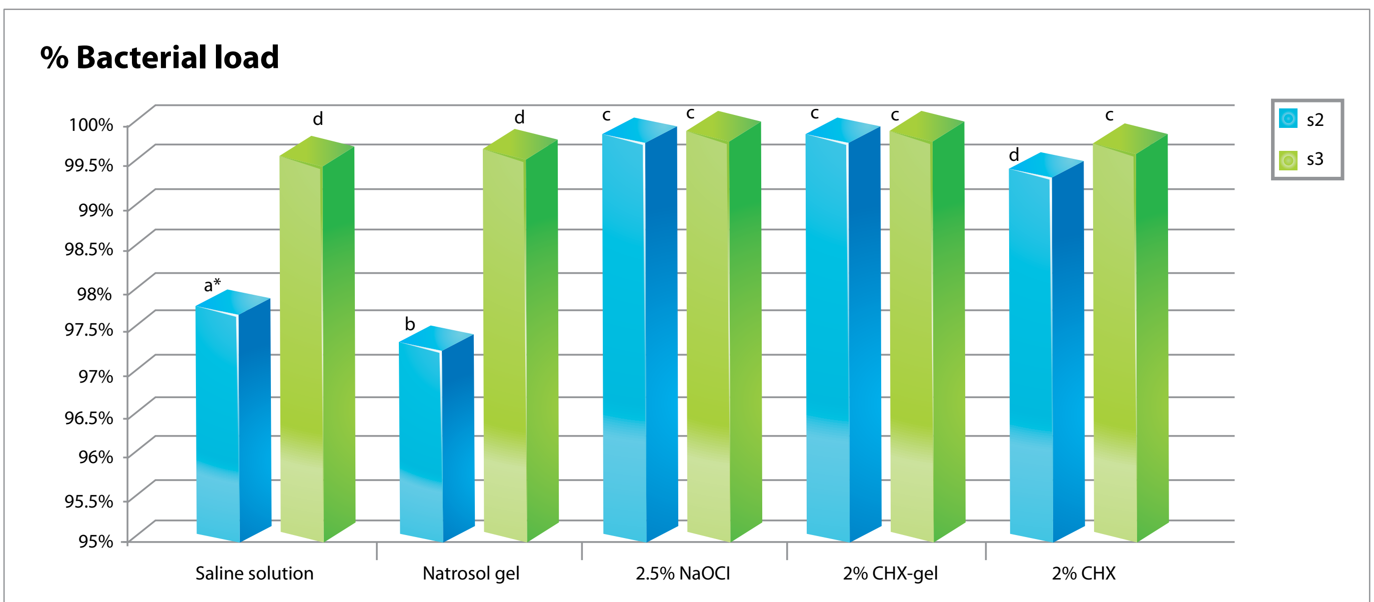

Figure 1. Mean percentage values of reduction in bacterial counts (CFU) from root canal samples obtained after root canal instrumentation (s2) and root canal medication (s3). *Same letters indicate no statistical difference among the groups (P > 0.05).

Nevertheless, no statistically significant difference in percentage levels of bacterial load reduction was found in groups III (NaOCl) and IV (CHX-gel) (Fig. 1) comparing s2 and s3.

In contrast to s2, at s3 no statistically significant difference was found in bacterial load between CHX-solution (GV) and NaOCl (GIII) or CHX-gel (GIV) ($p>0.05$) (Table 1). Distribution in mean percentage values of bacterial load reduction after root canal instrumentation (s2) and after root canal medication (s3) are shown in Figure 1.

A mixed microbiota, comprised predominantly by

Table 1. Quantity bacterial of UFC in 55 root canals with necrotic pulp and periapical lesions induced in the initial samples (S1) after root canal instrumentation (s2) and after intracanal medication (S3).

	Saline solution (GI)			Natrosol gel (GII)			2.5% NaOCl (GIII)			2% CHX-gel (GIV)			2% CHX-solution (GV)		
Samples	s1	s2	s3	s1	s2	s3	s1	s2	s3	s1	s2	s3	s1	s2	s3
H1	2.2^D	2.46^C	8.0^A	3.0^D	1.26^C	6.0^A	6.8^D	4.0^A	2.0^A	4.2^D	4.0^A	2.0^A	3.6^D	5.8^B	8.0^A
H2	8.6^D	1.96^C	3.94^C	2.6^D	1.88^C	8.0^A	3.4^D	1.0^B	2.0^A	5.4^D	4.0^A	2.0^A	2.4^D	3.6^B	1.2^B
H3	4.2^D	9.0^B	1.66^C	3.2^D	2.88^C	1.0^B	3.8^D	2.0^A	2.0^A	5.4^D	2.0^A	2.0^A	6.0^D	2.4^C	3.92^B
H4	4.0^D	2.9^C	1.0^B	3.6^D	1.08^C	4.0^A	6.8^D	8.0^A	2.0^A	3.0^D	2.0^A	2.0^A	5.8^D	3.6^B	2.14^B
H5	5.2^D	2.08^C	1.0^B	3.8^D	8.6^B	1.0^B	4.2^D	6.0^A	0	9.4^D	2.0^A	2.0^A	6.2^D	2.6^B	6.0^A
H6	2.12^E	1.36^C	2.0^B	5.6^D	1.44^C	3.08^C	1.22^E	4.0^A	2.0^A	6.4^E	2.0^A	0	4.4^E	2.2^B	1.0^B
H7	1.78^E	1.7^C	1.6^B	2.8^D	1.16^C	1.9^C	8.4^D	2.0^B	2.0^A	3.8^D	4.0^B	2.0^A	6.2^D	2.4B	1.0^B
H8	3.0^D	1.84^C	2.4^B	1.52^E	2.04^C	2.0^B	4.8^E	1.2^B	2.0^A	4.6^C	2.0^B	2.0^A	5.0^D	1.8B	1.0^B
H9	5.6^D	1.3^C	1.8^B	1.08^E	1.64^C	1.6^B	6.2^E	1.0^B	0	1.66^E	2.0^B	0	6.8^E	1.4B	4.0^A
H10	1.64^E	9.0^B	5.6^B	4.2^D	4.6^B	1.8^B	1.22^E	4.0^A	2.0^A	1.28^E	8.0^A	6.0^A	6.2^E	2.4B	8.0^A
H11	1.48^E	1.12^C	2.4^B	6.4^D	7.4^B	4.0^A	9.0^D	4.0^A	2.0^A	3.0^D	4.0^A	0	3.6^D	1.8B	4.0^B
Mean	9.3^D**a***	1.6^C**b**	6.7^B**e**	5.5^D**a**	1.4^C**c**	5.3^B**e**	6.7^D**a**	7.6^A**d**	1.4^A**d**	6.4^D**a**	3.2^A**d**	1.8^A**d**	1.5^E**a**	2.6^B**e**	1.2^B**d**

Different lowercase letters, in bold, represent differences in the statistical viewpoint ($p < 0.05$). A $=10^2$, B $=10^3$, C $= 10^4$, D $= 10^5$, E $= 10^6$.

Table 2. Frequency (on percentage mean values) of the profile of the microbiota of root canals with necrotic pulp and periapical lesion in the initial samples (S1) after root canal instrumentation (s2) and after root canal medication (s3) according to the tested groups (GI, GII, GIII, GIV, GV).

	s1						s2						s3					
	GI	GII	GIII	GIV	GV	Mean	GI	GII	GIII	GIV	GV	Mean	GI	GII	GIII	GIV	GV	Mean
Gram-positive cocci	100	81.8	90.9	90.9	100	92.7	72.7	81.8	81.8	81.8	81.8	79.98	79.98	72.7	72.7	45.5	100	76.4
Gram-negative cocci	27.3	72.7	63.6	36.4	72.7	54.54	54.5	18.2	27.3	0	45.5	29.1	29.1	27.3	0	0	0	7.28
Gram-positive rods	27.3	72.7	36.4	27.3	72.7	47.28	27.3	45.5	27.3	18.2	9.1	25.48	25.48	36.4	9.1	27.3	18.2	21.8
Gram-negative rods	36.4	18.2	9.1	45.5	81.8	38.2	100	90.9	54.5	45.5	100	78.18	78.18	27.3	0	18.2	0	21.8
Strict anaerobes	55.5	58.2	69.4	62.3	37.7	56.62	5.8	18.7	100	100	80	60.9	60.9	16.6	0	100	80	36.4
Facultative anaerobes	44.5	41.8	30.6	36.8	62.3	43.2	94.2	81.3	0	0	20	39.1	39.1	83.4	100	0	20	72.7

strict anaerobe bacteria, was found in the baseline samples (s1) (Table 2).

At s1, Gram-positive cocci bacteria predominated in all groups (GI, GII, GIII, GIV and GV). After chemo-mechanical preparation (s2), a high frequency of Gram-positive cocci and Gram-negative rods bacteria were found. At s3, regardless the auxiliary chemical substance applied during chemo-mechanical preparation, Gram-positive cocci bacteria predominated in all root canal samples (Table 2).

The microbiota profile at different sampling times (s1, s2 and s3), according to the groups tested (GI, GII, GIII, GVI and GV) are shown in Table 2.

Discussion

Culture procedure, used in this study, rather than contemporary techniques (molecular methods)[8,9] is a reliable method to evaluate the antimicrobial efficacy of root canal procedures, due to its capacity to detect viable bacteria afterwards. Additionally, correlation between non-cultivable bacteria and a favorable treatment outcome had been developed over the years.[22,25,26]

Most infecting bacteria (more than 97%) were removed only by the mechanical instrumentation and the flow/back-flow of the irrigant solution (saline solution). However, the addition of an auxiliary chemical substance exhibiting a potent antimicrobial activity is required in order to promote a deeper disinfection in dentin tubules.[3,4] Increased mean values in bacterial load reduction (almost achieving 100%) were found in teeth irrigated with 2.5% NaOCl or 2.0% CHX, demonstrating their potent antimicrobial activity against microorganisms involved in primary root canal infections.

Bacterial load in infected root canals was reduced from 10^5 to 10^2 UFC/ml after chemo-mechanical preparation with either 2.5% NaOCl or 2% CHX-gel. Typical results were shown by Vianna et al[9] detecting a reduction from 10^5 to10^1 UFC/ml in the 2.5% NaOCl-group and from 10^5 to 10^2 UFC/ml in the 2% CHX-gel-group. Alike, Siqueira et al[15] reported a reduction from 10^5 to 10^3 UFC/ml in the 2.5% NaOCl-group and from 10^5 to 10^2 UFC/ml in the 0.12% CHX-gel-group.

Regarding the antimicrobial activity, the present study, in agreement with previous in vivo[14,15] and in vitro studies,[13,16,19] showed no significant difference between the use of NaOCl and CHX-gel as an auxiliary chemical substance, even though a higher mean percent value of bacterial load reduction was found in teeth irrigated with 2.5% NaOCl. In contrast, Vianna et al[9] comparing in vivo the antibacterial efficacy of these two substances by molecular technique (RTQ-PCR) found 2.5% NaOCl to be more effective than 2% CHX-gel. However, the clinical significance in reducing bacterial DNA from infected root canals after chemo-mechanical procedures remains unclear, once dead cells may not implicate in the failure of the endodontic treatment.

Overall, it is reasonable to assume that 2.5% NaOCl and 2% CHX-gel have a potent antimicrobial activity in clinical practice and the choice between them should rely upon their particular and individual properties. CHX-gel seem to posses a residual antimicrobial activity that helps to prevent root canal reinfection.[27,28] In addition, its biocompatibility turns it the choice for teeth with open apices[13] and for patients who are allergic to bleaching solutions as NaOCl.[27] However, its inability to dissolve pulp tissues (an important advantage of NaOCl)[29] is its downside.

The antimicrobial activity of $Ca(OH)_2$ medication applied for 14 days was notable in teeth irrigated with an inert substance (SSL-group and natrosol gel-group). A significant increased reduction in the mean bacterial load was found in comparison with the values after instrumentation — from 1.6 x 10^4 to 6.7 x 10^3 CFU/ml in the SSL-group and 1.4 x 10^4 to 5.3 x 10^3 CFU/ml in the natrosol-group. Nevertheless, its efficacy in reducing bacteria load after chemo-mechanical procedures was consistent but not significant in teeth irrigated with a potent auxiliary chemical substance — from 7.6 x 10^2 to 1.4 x 10^2 UFC/ml in 2.5% NaOCl-group and from 3.2 x 10^2 to 1.8 x 10^2 UFC/ml in 2% CHX-gel.

Even different periods of application of $Ca(OH)_2$ have been found in the literature[4,6,23,25] most findings in the mean bacterial load reduction from "positive-culture" canals (often ≅10^2 UFC/ml) are consistent with our data after its use for 14 days, particularly in teeth irrigated with 2.5% NaOCl and 2% CHX-gel. Thus, the range in percent values of bacterial load reduction found after the placement of $Ca(OH)_2$ medication (97.42% to 99.90%) is also in agreement with the ones previously reported by different authors (91.0-99.9%).[3,11,12]

After the placement of $Ca(OH)_2$ medication for 14 days, the number of root canals yielding negative culture increased, whereas[4] 'positive' samples showed an increase in the number of CFUs values when compared to s2. As a matter of fact, several studies[21,22,23,30] had demonstrated increasing values in bacterial counts after the use of $Ca(OH)_2$ medication. This fact may be explained by the presence of remained bacteria in the dentinal tubules that may escape from the direct action of $Ca(OH)_2$[10] and (re) infect the canal space; and the reduced action of the $Ca(OH)_2$ medication provided by the buffering effect of the dentine.

It is reasonable to assume from the present study that $Ca(OH)_2$ medication has a low ability in vivo to promote a significant bacterial load reduction, particularly in teeth irrigated with 2.5% NaOCl or 2% CHX-gel; and in helping eliminate bacteria in the majority of the infected root canals. Therefore, its is application in clinical practice should not only be to its antimicrobial activity but also to its other properties such as the ability to change the pH of dentin and cementum, the ability to depolymerize bacterial LPS of gram-negative bacteria and its hygroscopic action that eliminates exudates.

Overall, it is important to mention that the efficacy root canal procedures are not due only to the antimicrobial properties of the substances, but also to the susceptibility of root canal flora involved. Therefore, the knowledge of endodontic microbiota and its susceptibility to endodontic therapy is important to help achieving an optimal disinfection of the root canal system.

Regardless the auxiliary substance applied (inert or not) during instrumentation, a predominance of Gram-positive cocci and Gram-negative rods bacteria were found in the root canals, suggesting a non-selective pressure performed by any of the chemical substance tested (NaOCl or CHX). In contrast, after the use of $Ca(OH)_2$ medication, a predominance of Gram-positive cocci species was observed in all "positive" root canal samples. Such a critical finding must be considered in clinical practice, since Gram-positive cocci, particularly E. faecalis, is often implicated in persistent root canal infections, due to its high level of resistance to calcium hydroxide.

Conclusion

In conclusion, regardless the use of calcium hydroxide as a root canal medication, 2.5% NaOCl and 2% CHX-gel demonstrated a potent antimicrobial activity against endododontic pathogens in vivo.

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In vitro determination of direct antimicrobial effect of calcium hydroxide associated with different substances against *Enterococcus faecalis* strains

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ABSTRACT

Objective: To determinate the direct antimicrobial effects of *Casearia sylvestris Swart* (guaçatonga), propylene glycol, and of chlorhexidine associated to calcium hydroxide paste against 40 *Enterococcus faecalis* strains isolated from the oral cavity when direct contact. **Methods:** After activation, the bacterial strains were suspended in sterile saline to 1.0 McFarland standard. The suspension was placed in direct contact with calcium hydroxide paste [$Ca(OH)_2$] + pure propylene glycol, $Ca(OH)_2$ + chlorhexidine 1% in propylene glycol, and $Ca(OH)_2$ + guaçatonga extract in propylene glycol by covering paper points, previously contaminated for 3 minutes, with the different pastes. Antimicrobial activity was evaluated at 6, 24, 48, 72 hours, and at 7 days. After the incubation period, the points were removed from the pastes and incubated in Letheen broth at 37°C for 48 hours. Following that, 0.1ml of the Letheen broth was transferred to tubes containing brain heart infusion (BHI) broth and incubated again at 37°C for 48 hours. Turbidity was observed in the medium. After that, *M-Enterococcus* agar plates were seeded with BHI broth from each tube and colony growth was assessed. **Results:** All the bacterial strains were inhibited by all pastes at the evaluated periods. **Conclusions:** It was concluded that the addition of these substances to calcium hydroxide did not interfere with its direct antimicrobial effect.

Keywords: Environmental Microbiology. *Enterococcus faecalis*. Calcium hydroxide. Products with antimicrobial action.

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Introduction

The Enterococcus genus includes members previously classified as Group D Streptococci due to the presence of Group D cell wall antigen, a glycerol teichoic acid associated with the cytoplasmic membrane. Enterococci are normal inhabitants of the gastrointestinal tract and are found in lesser amounts in the vagina and male urethra.[1]

These have become important pathogenic microorganisms in humans, mainly due to their resistance to antimicrobial agents and to recently studied virulence factors.[2]

These Gram-positive cocci are arranged as pairs or in small chains, and are very hard to differentiate from streptococci. They are facultative anaerobes that thrive at 35°C, typically growing on the surface of blood agar plates as gamma-hemolytic cultures and on M-Enterococcus agar medium as deep-red or purplish colonies. Enterococci are tolerant to bile at 40% and can hydrolyze esculin. Moreover, they are able to grow in the presence of 6.5% sodium chloride, and can be distinguished from bacteria in genus *Staphylococcus* by their inability to produce catalase.[3]

Enterococcus faecalis are frequently found in root canals after failed endodontic therapy.[4,5,6]

Being highly resistant to several medications, they are also among the few microorganisms that display *in vitro* resistance to calcium hydroxide. This resistance is related to a proton pump[7] or to biofilm formation.[8] In an attempt to overcome this resistance, the addition of different substances to calcium hydroxide has been proposed.

One of the additives suggested is chlorhexidine, a biguanide. Calcium hydroxide-chlorhexidine paste has shown better antimicrobial action *in vitro*, compared with calcium hydroxide paste with pure water.[9] Despite its positive antimicrobial effect, this association has shown greater peroxide ion release, resulting in greater tissue irritation.[10]

New alternatives have been proposed in endodontic therapy, including natural substances such as propolis and phytotherapeutic agents. One of these phytotherapeutic agents is *Casearia sylvestris Sw* infusion or alcoholic extract.

This plant is native to Latin America, from Mexico to Argentina. It is found throughout Brazil, being particularly common in the state of São Paulo. It is popularly known as guaçatonga, erva de lagarto ("lizard's herb"), vassitonga, bugre branco, among other names. The word "guaçatonga" originated from the Tupi-Guarani (indigenous language), showing that this species was known by the native populations of Brazil.[11]

Guaçatonga extract has shown antiinflamatory[13] and antimicrobial action.[14] However, no studies showing whether the addition of phytotherapeutic agents to calcium hydroxide paste interferes with its antimicrobial action can be found in the scientific literature.

With this in mind, the objective of the present study was to evaluate the sensitivity of several *Enterococcus faecalis* strains isolated from the oral cavity to direct contact with calcium hydroxide pastes associated with *Casearia sylvestris Sw* (guaçatonga) in propylene glycol, calcium hydroxide with pure propylene glycol, or calcium hydroxide and 1% chlorhexidine in propylene glycol.

Metodology

Preparation of the extract

The *Casearia sylvestris Sw* leaves used in this study were collected at the Lageado farm, School of Agronomical Sciences - Unesp, in Botucatu, state of São Paulo, and identified at the herbarium of the Sagrado Coração University (USC) - Bauru, São Paulo, Brazil.

After harvesting and desiccation, the material was further dehydrated in an air-circulating oven under controlled temperature until constant weight was achieved. Following that, the leaves were triturated in a knife mill and used to prepare the extract. The dehydrated material was macerated in propylene glycol (extracting solution) following a ratio of 25 grams of powder to 200 ml of extracting solution. The plant powder remained in the extracting solution for eight days, with sporadic agitation during that period. The entire extraction process took place in an amber colored container (in order to prevent possible interference by light) and at room temperature (25°C).

Enterococccus Strains

Forty *E. faecalis* strains from the USC Microbiology laboratory bacterial library were used in the present work. These strains were cultured from bacterial samples obtained from the oral cavity of patients seen at the USC School of Dentistry Endodontics clinic in Bauru, Brazil.

All strains had been frozen at -20°C and were isolated in M-Enterococcus agar medium (Difco®). Strains were then identified following a standard identification routine described by Koneman et al.[1]

Activation of the strains was carried out on M-Entorococcus agar plates (Difco®) in an oven set at 36°C for 18-24 hours. Subsequently, colonies were suspended in BHI broth (Oxoid®) until complete turbidity of the medium was observed.

Antimicrobial substances tested

All bacterial substances tested in this study were based on calcium hydroxide P.A. paste (Table 1).

The pastes were prepared by mixing 2 grams of powder to 70 drops of each corresponding vehicle, resulting in a mixture with toothpaste-like consistency after spatulation. For each material tested, approximately 12 grams of calcium hydroxide paste were manipulated.

Table 1. Pastes used in the experiment.

$Ca(OH)_2$ + guaçatonga extract in propylene glycol
$Ca(OH)_2$ + 1% guaçatonga solution in propylene glycol
$Ca(OH)_2$ + pure propylene glycol

Assessment of the antimicrobial activity

The inoculum suspensions in BHI broth (Oxoid®), were diluted in 5 ml sterile saline to reach turbidity corresponding to 1 McFarland standard ($3x10^8$ cells/ml).

For the antimicrobial activity test, 1,200 paper points (Tanari®, Tanariman Ltda), previously sterilized by autoclaving, were immersed in the experimental bacterial suspensions for 3 minutes in order to achieve contamination. Following that, the paper points were aseptically removed from the bacterial suspension and distributed on the surface of sterile Petri dishes. The paper points were then covered by the different pastes being evaluated. The Petri dishes were covered and kept in an oven at 37°C.

At 6, 24, 48, 72 hours, and at 7 days, the paper points were removed from direct contact with the pastes and placed in test tubes containing 4 ml sterile Letheen Broth (Difco®). The broth was incubated at 37°C for 48 hours and visually assessed for macroscopic turbidity.

An inoculum containing 0.1 ml of Letheen broth was transferred to a test tube with 4 ml BHI broth that had been incubated under the same conditions. The BHI broth test tubes with no evidence of turbidity were considered as negative, and the ones displaying turbidity of the broth were seeded on M-Enterococcus agar in order to determine whether the bacterial strains remained viable.

All the experimental procedures were conducted under aseptic conditions with the aid of a laminar flow hood, and assays were performed in duplicate. One experiment was carried out with a standard *Enterococcus faecalis* ATCC 29212 strain.

The pH of each paste was measured after manipulation and placement in deionized water, with the aid of a pH meter.

Results

The pH values for the pastes were: 12.67 for the calcium hydroxide + 1% chlorhexidine, 12.62 for the calcium hydroxide + propylene glycol, and 12.60 for the calcium hydroxide + *Casearia sylvestris Sw* extract.

The assessment of antimicrobial activity for the three different pastes at 6, 24, 48, 72 hours, and at 7 days post-incubation showed that all strains were inhibited in all periods of evaluation (Table 2).

Discusson

The efficacy of $Ca(OH)_2$ paste against *E. faecalis* and other microorganisms has been extensively discussed in the scientific literature.[15-19]

The addition of chlorhexidine has conferred greater antimicrobial efficacy to calcium hydroxide pastes used for disinfection of the dentin tubules.[7] However, Schäfer et al[17] observed no increase in efficacy against *E. faecalis* by associating $Ca(OH)_2$ with chlorexidine.

Ercan et al,[18] in an *in vitro* experiment involving extracted teeth, revealed that 2% chlorhexidine gel was more efficient against *E. faecalis* and Candida albicans compared to plain $Ca(OH)_2$ or to $Ca(OH)_2$ with 2% chlorexidine.

Enterococcus faecalis needs to be maintained in direct contact with calcium hydroxide in order to be killed[20,21]. In the present work, the least amount of time *Enterococcus faecalis* was kept in contact with the pastes was 6 hours, and none of the strains survived.

The results reported in this study for calcium hydroxide paste with chlorhexidine are in agreement with Estrela et al,[21] who used similar methodology.

Table 2. Antimicrobial action of the calcium hydroxide pastes against the different bacterial strains.

	$Ca(OH)_2$ + Propylene glycol					$Ca(OH)_2$ + 1% Chlorexidine					$Ca(OH)_2$ + Guaçatonga extract				
	6h	24h	48h	72h	7d	6h	24h	48h	72h	7d	6h	24h	48h	72h	7d
1	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
2	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
3	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
4	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
5	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
6	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
7	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
8	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
9	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
10	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
11	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
12	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
13	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
14	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
15	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
16	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
17	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
18	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
19	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
20	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
21	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
22	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
23	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
24	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
25	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
26	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
27	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
28	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
29	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
30	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
31	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
32	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
33	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
34	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
35	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
36	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
37	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
38	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
39	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
40	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -

The antimicrobial action of calcium hydroxide arises from the release of hydroxyl ions with consequent pH increase, reaching 11 to 12.5.[22] According to Siqueira-Júnior et al[23] the lethal effect of hydroxyl ions against bacterial cells is mainly due to the damage inflicted on their cytoplasmic membrane, protein denaturation, and direct damage to DNA, although it is now clear that one of the crucial factors for *E. faecalis* survival in high pH is the presence of a proton pump that enables cytoplasmic homeostasis, even in extremely alkaline environments.[7] *Enterococcus faecalis* strains have been found to survive in environments with pH as high as 10.5 to 11.0; pH values have to be greater than 11.5 in order to kill these strains.[24] In the present paper, all the pastes had pH greater than 12.5 and were able to kill all strains.

It is important to emphasize that the pH of calcium hydroxide pastes is generally higher than 11, and that the addition of several substances does not alter these values.[25,26] However, within the dentin tubules, the pH might not reach such high levels,[27,28] hence the suggested association of different substances to the pastes with the goal of enhancing the antimicrobial action, with positive results.[9]

In this paper, $Ca(OH)_2$ pastes in three different vehicles demonstrated great effectiveness against all *E. faecalis* strains after direct *in vitro* contact of the microorganism with the paste. The addition of guaçatonga did not interfere with the antimicrobial action of calcium hydroxide, confirming that the presence of this substance did not alter the pH of the paste. Further experiments should be carried out in order to demonstrate, both *in vivo* and *in vitro* (using extracted teeth) whether similar effect is observed. It is important to take into consideration that for calcium hydroxide to maintain its ability to raise the pH within the dentin tubules, the hydroxyl ions should diffuse throughout dentin in high enough concentrations to exert buffer effect and consequently induce a drastic increase in the local pH values.

The guaçatonga essential oil has shown effective action against Gram-positive bacteria such as *Enterococcus*, *Micrococcus*, *Staphylococcus aureus*, *S. epidermidis*[14] and *Bacillus cereus strains*.[29]

Methods in which the pastes are diffused on the agar surface, as described by Gomes et al,[15] or those involving direct contact with the paste, followed in the present work and others,[21] are susceptible to interference from several variables, namely differences in solubility and diffusion of the paste in the medium, the inoculum, pH of the agar components, agar viscosity, incubation times and temperature, and metabolic activity of the microorganism in the culture medium. All of these factors hinder the extrapolation of the results to a clinical setting, where other different factors may interfere with the antimicrobial action of the paste against microorganisms in the dentin tubules.

Therefore, it is unquestionable that future studies are needed in order to determine whether guaçatonga extract in propylene glycol is able to enhance the efficacy of calcium hydroxide pastes against *E. faecalis* in extracted teeth *in vitro* or *in vivo*, in actual clinical conditions. The discovery of antimicrobial biocomponents derived from this plant, with activity against bacteria found in the oral microbiota, may lead to new therapeutic alternatives in Dentistry.

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Analysis of forces developed during root canal filling by different operators

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ABSTRACT

Objectives: Endodontic procedures might contribute to the development of vertical root fracture as well as other localized defects such as craze lines or incomplete cracks in root dentine. The objective of this study was to evaluate the maximum fracture resistance and the force produced by five different operators in lateral and vertical condensation during root canal filling. **Methods:** 74 human teeth, superior canines (SC) and inferior premolars (IPM) were selected. In order to determine the maximum fracture resistance during condensation, 24 teeth were submitted until failure to an axial compression load in a universal testing machine. Fifty teeth were used in order to measure the axial condensation force by means of a device developed to simulate clinical working conditions. **Results:** Fracture resistance mean values in kg were: SC = 14.96±2.65 and IPM = 7.56±1.05. Mean values of force applied by each of the five operator in Kg were, respectively: 2.49; 3.75; 2.24; 2.08 and 1.18. None of the operators achieved teeth's maximum fracture resistance during procedures. **Conclusions:** Different behaviors among five professionals monitored were observed for the same technique of root canal filling. The increase in strength during condensation had no radiographic improvement of root canal filling. During the root canal filling, lateral and especially vertical condensation, must be performed with reduced apical strength and pressure, avoiding excessive and unnecessary stress to root dentin.

Keywords: Lateral condensation technique. Root canal filling. Condensation force.

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Introduction

Endodontic procedures may contribute to the development of the vertical root fracture as well as other defects such as fissures and incomplete cracks on root dentin.[1] These located defects may have the potential to develop fractures[2] and should, therefore, be prevented. Vertical root fracture is a clinical implication that may be associated with endodontic treatment[3] and, being a longitudinal fracture, it extends throughout the entire thickness of dentine from the root canal to the periodontium.[4] The prognosis is very unfavorable, resulting in tooth extraction or resection of the affected root.[3,4]

Several factors may be responsible for increasing the root fracture risk; some may not be controlled by the dentist such as tooth structure's reduced physical properties caused by physiological and pathological processes.[5] But there are many other factors that may be controlled during and after the endodontic treatment. Amongst others, we can list: access cavity and root canal preparation, irrigation, obturation, post space preparation[1,5] and coronal restoration.[5]

In vitro studies examined the effect of various obturation techniques on endodontically treated teeth's fracture resistance.[1,2,6-11] Greater forces occur when lateral condensation obturation warm vertical compaction or thermomechanical compaction techniques are used, in comparison with the thermoplasticized condensation technique.[6,7] During lateral condensation, the use larger than # 25 spreaders caused a significant reduction on roots fracture resistance.[11] This is due to the fact that the insertion of the spreader during obturation can generate stresses within the root canal.[12] However, even when thin spreaders are used during lateral condensation, root surface craze lines occur.[2] The pressure applied during lateral condensation is no sufficient to cause vertical root fracture,[10] but it can produce a greater number of root dentin defects[12] than noncompaction canal filling was used.[1]

This way, the present study evaluated the fracture resistance and the force produced by five different operators in lateral and vertical condensation during root canal filling.

Material and Methods

Seventy-four freshly extracted single canals human teeth (superior canines and inferior premolars) were stored in 10% aqueous formol solution. Teeth were horizontally sectioned by means of a diamond disc (KG Sorensen, Barueri, SP, Brazil) under water cooling, near their cement-enamel junction. Then, the measured roots were stored individually and their moisture was maintained using a piece of gauze soaked in physiological solution.

Roots were embedded into Adesivo B Flexible Epoxi Resin (Polipox, Interlagos, São Paulo, Brazil), parallel to the walls of 25 mm- height PVC cylinders with an external diameter of 25 mm and an internal diameter of 21 mm. Silicone was used to facilitate the positioning and fixation of the roots inside the cylinder.

Passed 48 hours from the teeth´s inclusion, which corresponded to the period of resin's final polymerization, the samples were randomly divided. Fifty samples were used to measure the axial force applied during lateral and vertical condensation. Five endodontists participated in this study. The professionals were named as A, B, C, D and E. Each professional received 10 samples, being the 2 first used to calibrate the equipment during the monitoring of the lateral and vertical condensation procedures according to each professional. The remaining 8 samples were obturated by the lateral and vertical condensation obturation. Aiming to reproduce dentists working conditions in their offices, a device was specially developed for this study: a 60 cm metallic stem was adapted to the universal testing machine (EMIC DL-2000, São José dos Pinhais, PR, Brazil) with a 20 kg load-cell in a way that the samples' position was similar to that in the oral cavity (Fig 1A). Besides that, over this bar, a metallic support was used so that the professionals could rest their hands on it during the clinical procedures (Fig 1B). During the procedures, the generated forces were recorded by the testing machine M Test software and turned into graphs in order to analyze the applied forces in Kg afterwards.

Root canal instrumentation was carried out with the concern of standardizing its dilatation, following the technique described by Goerig, Michelich and Schultz.[13] After canals drying, the main gutta-percha cone was selected, in way that it presented a locking 1 mm short the radicular apex, matching the work length. A sealer based on zinc oxide and eugenol (Endofill, Dentsply, Petrópolis, Brazil) was inserted into the canal using the main cone and it was applied to the whole canal wall. After the positioning of the main cone, spaces were generated by means of a finger spreader (Maillefer, Ballaigues, Switzerland) compatible to the accessory cones used. During lateral condensation, all the accessory cones were embedded in sealer and inserted in each space, followed by a new condensation successively, until obturation was completed. Excess filling material was removed by Paiva´s pluggers (Golgran, São Paulo, Brazil) heated and held vertical condensation.

During the lateral and vertical condensation experimental tests, the efforts made by the five operators were captured by the load-cell, transferred and saved (Fig 1B). From each condensation procedure, a graph was obtained demonstrating the value and the behavior of the load applied by the professional, as well as the maximum load. All the tests were carried-out at a crosshead speed of 2 mm/min, with a working time of approximately 4 minutes. Data was analyzed allowing the working profile of each operator to be established.

Ten obturated teeth were radiographically evaluated (Agfa Dentus M2 Comfort Dental Film - Speed Group D - Agfa Gevaert N. V., Belgium). For the radiographic examination, all teeth were removed from their PVC cylinders and epoxy resin. Radiographs were taken from each tooth in the buccolingual and mesiodistal positions by means of an X-Ray machine calibrated with an exposure time of 0.3 sec and a focal distance of 8 cm from the roots.

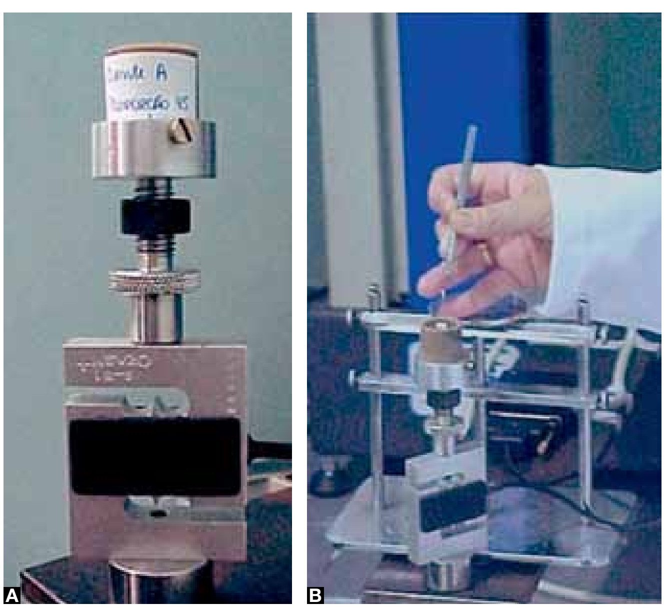

Figure 1. Sample couple with the cylindrical device attached to the load-cell of the universal testing machine (**A**) and monitoring of the long-axis loading force applied during obturation (**B**).

The 24 remaining samples were used to measure the maximum fracture resistance of the roots during lateral condensation. Samples were submitted to a fracture resistance test using a finger spreader compatible to the canal's diameter as a load applying device coupled to the universal testing machine (EMIC DL-2000) at a crosshead speed of 2 mm/min until failure. Data was analyzed and displayed in graphs.

Results

During the mechanical tests, the applied forces were monitored as the lateral and vertical condensation was performed, generating graphs that represent the behavior and magnitude of the maximum force applied during tests. The mean fracture resistance values were: Superior canines = 14.96±2.65 and inferior premolars = 7.56±1.05 Kg. Mean values of the loading forces applied by each operator were, respectively: 2.49 Kg; 3.75 Kg; 2.24 Kg; 2.08 Kg and 1.18 Kg (Table 1).

The difference between the five operators graphs could be verified, demonstrating the individual characteristics of each professional (Fig 2).

The radiographic image of the obturations performed by the all five professionals showed a satisfactory quality, as a compact obturing mass, without voids could be seen inside the root canals in all samples.

Discussion

The comparative evaluation of the axial loading force applied during lateral and vertical condensation of this research aimed to know the magnitude of the force and the load applying behavior of five endodontists, which used the same obturation technique. Using standard mechanical tests, similar to clinical conditions and with samples coupled to the load cell, it was possible in this study and former others to register the behavior of each professional, as in previous studies.[6,7,8]

The use of an electronic monitoring device fitted with the mechanical testing machine, such as that developed in this study, in which the forces generated during the filling steps are recorded in real time and transformed into graphs is of great value for teaching and enhancement of endodontics. For this device was able to verify the pressure at the time of root canal filling, during insertion of the finger spreader in the lateral condensation and the plugger in the vertical condensation. Graduate and undergraduate students learn with their use,

Table 1. Maximum loading forces applied by the professionals during root canal filling (kg) and their mean values (kg).

Professional	Maximum loading force								Mean Values
A	2.31	2.62	2.43	2.64	2.91	2.95	2.22	1.83	2.49
B	4.09	4.20	3.98	4.10	3.95	4.10	2.75	2.85	3.75
C	2.44	1.84	2.36	1.91	2.22	2.22	2.25	2.69	2.24
D	1.60	1.68	1.85	2.88	2.19	1.95	2.36	2.17	2.08
E	1.28	1.34	1.10	1.12	1.10	1.07	1.26	1.20	1.18

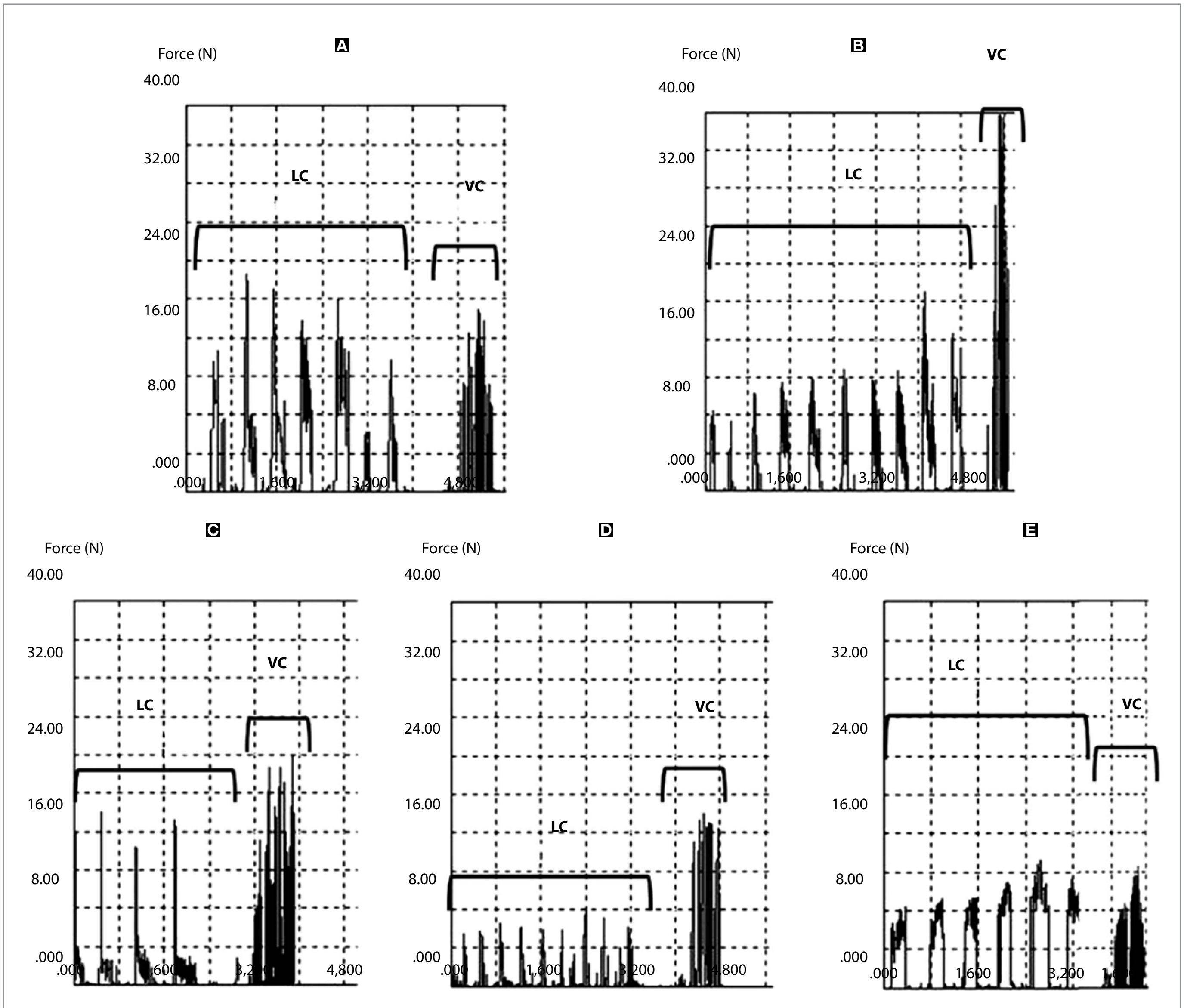

Figure 2. Registering of the behavior and the maximum load force applied by the professionals during lateral (**LC**) and vertical condensation (**VC**).

adequate force of condensation in different techniques of root canal filling, without generating excessive and unnecessary stress to root dentin.

The results of this study demonstrated that there was a variation in the load forces in magnitude as well as in constancy applied by each of the five endodontists. The loads averaged professionals A, C, D and E, are consistent with the loads found in previous studies.[6,7,8]

The behaviors registered from professionals A and E, as shown in graphs were similar. A practically constant load force was maintained from the beginning of obturation, from the insertion of the first cones to its end, with vertical condensation of gutta-percha and their graphs presented a constant curve from the beginning to the end of the procedures. The professionals differed from each other regarding the magnitude of the force applied during the whole procedure. The mean value of the loading forces applied by professional A (2.49 Kg) was different from professional E (1.18 Kg). Regarding the usage of finger spreaders, it could be verified that both professionals applied an apically directed pressure, inserting the spreader from 1 to 2 mm short the working length during lateral condensation.

Professionals B and C also demonstrated similar behavior regarding the distribution of the applied effort and not the magnitude of the loading force. Graphs show an upward curve, revealing that this professionals started obturation using a small amount of force which was increasing until the canals were completely filled. In relation to the magnitude of the applied load, a great difference could be verified amongst theses operators. The mean value of loading forces exercised by professional B was 3.75 Kg and by professional C 2.24 Kg, both distributed in an increasing way.

The graph generated from professional's D behavior during lateral condensation was similar to professionals' A and E graphs, as a constant load was applied in this stage of the procedure. In the moment of vertical condensation, an increase of applied effort was verified, which was demonstrated by a peak in the curve, comparing to the force that had been previously exercised. This way, operator D ranged from a constant low force to a higher one during vertical condensation. This increase of loading force during vertical condensation could be also observed during the tests of operator B, but varying its mean values: 2.88 Kg for professional D and 4.20 Kg for professional B.

Investigating the maximum load force applied by finger spreaders and capable of inducing root fracture, Holcomb, Pitts and Nicholls[9] observed the presence of vertical fracture in teeth tested with a loading force ranging from 1.5 to 3.5 kg. These values are close to the ones registered from the test of operator B. However, the groups of teeth tested this previous study[9] had smaller dimensions when compared to the teeth used this research, which could explain the fracturing of roots submitted to smaller forces.

None of the five endodontists has reached the maximum fracture resistance load fracture because the pressure applied during the lateral and vertical condensation was insufficient. However, studies show that this technique of obturation may cause major defects in the root dentin[12] than noncompaction canal filling was used.[1] The most common defects are the fissure lines and cracks in the root dentin that can result after conclusion of endodontic treatment in vertical root fracture,[6,7,11] because the simply by forces applied to the root during mastication[1] and additional treatments such as post-space preparation.[2]

Each of the five endodontists examined demonstrated a different working profile when performing the same obturation technique and taking in account that the radiographic images revealed a satisfactory and homogenous obturation mass in all specimens. Facing these results, it is recommended that during the process of lateral condensation, professionals apply a constant loading and reduced pressure in the apical direction, always respecting the limit of work and the space provided by the finger spreader. In the vertical condensation, in which we found three endodontists (B, C and D) within the highest values of force applied during the root canal filling, it is recommended using the plugger with a reduced loading in the apical direction. This is because increase of loading did not generate radiographic improvement in the final result of the filling, and can generated, especially in weakened or less dentinal structure roots, the appearance of defects such as fissure lines and/or incomplete cracks.[1] Following these recommendations, the professional will obtain a proper root canal filling, generating little stress on dentin structures. What is an important factor, as the vertical root fracture do not occur instantly, but are, indeed a result of a gradual diminishment of root structure coupled with the use of force and pressure to root dentin.[1]

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Root canal filling with calcium hydroxide paste using Lentullo spiral at different speeds

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ABSTRACT

Objective: This study analyzed the effectiveness of filling the root canal with calcium hydroxide paste using the Lentulo spiral at different speeds. **Methods:** Thirty mandibular premolars after root canal preparation were divided in three groups. Calcium hydroxide paste was inserted in the root canals with a Lentulo spiral at 5,000 rpm (G1), 10,000 rpm (G2) and 15,000 rpm (G3). The optical density was determined by the use of the digital radiography system Kodak Dental RGV-5000. **Results:** The highest optical density obtained in the apical third was in G3 and in the middle and cervical third in G1. Statistical difference (Kruskal-Wallis - Anova) was observed ($p<0.05$) between G1 and G3 in the apical third and G1 and G2 in the middle third. No difference was observed in the cervical third ($p>0.05$). **Conclusion:** Different speeds are necessary for the correct filling of the root canal with calcium hydroxide paste. The 15,000 rpm speed was more effective in filling the apical third and 5,000 rpm speed was more effective in filling the cervical and middle thirds.

Keywords: Calcium hydroxide. Intracanal dressing. Root canal filling.

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Introduction

The success of endodontic treatment is related to different factors like correct cleaning and shaping and sanitization of the root canals.[1] Biomechanical preparation using instruments and irrigating solutions offers a way to combat the endodontic microbiota. In this way, intracanal dressing increases the power of the sanitization process.[2,3]

Calcium hydroxide is, currently, the most used intracanal dressing, due to its physical and chemical properties. It has a high pH, antibacterial activity, acts in the degradation of bacterial lipopolysaccharides, induces healing through the formation of hard tissue, and controls radicular resorption.[3,4,5,6]

As ionic calcium hydroxide dissociation occurs, the paste quantity to be placed within the root canal must be enough to supply hydroxyl and calcium ions over a period of time necessary for sanitization of the root canal system.[4,7,8,9] Its effectiveness is dependent on the direct action between the paste and remaining microorganisms in the dentinal tubules.[10,11] To reach this goal the root canal must be homogeneously and completely filled with the paste showing a tri-dimensionally dense x-ray image.[6,8] Many times, ineffectively of calcium hydroxide can be explained by the manner in witch it is placed, ie, the canals are not filled in the middle and apical thirds.[9]

In general, its insertion is performed by using instruments and endodontic materials, such as K-files, reamers, absorbent paper points, gutta-percha cones, amalgam carriers, McSpadden compactors, Lentulo spirals, ultrasonic and sonic files, ML syringe (SS White), and 27G long needles.[3,4,5]

Cvek et al[12] proposed the use of an injection syringe or Lentulo spiral aided by lateral condensation. Webber et al[13] suggested the use of a plastic transporter to drive the paste into the root canal followed by an effective vertical condensation. To Anthony and Senia[14] the ideal way of calcium hydroxide filling is using the Lentulo spiral. Leonardo[15] recommended the use of a special syringe with a long G-27 needle in the Calen system. Sigurdsson et al[16] comparing the Lentulo spiral, endodontic file and syringe, pointed to the first, the best results accompanied by others authors.[12,14] However, these findings have not been unanimous, and empty spaces have been identified in some studies.[3,17,18] Estrela et al[3] analyzing the placement of calcium hydroxide in dog's teeth obtained the lowest number of empty spaces when the paste was inserted using a K-file, absorbent paper points and vertical pluggers, followed by the Lentulo spiral. Torres et al[19] concluded that the radiodensity of the paste in curved plastic resin block canals was significantly greater using a Lentulo spiral only technique.

But, there are two critical points with the Lentulo spiral filling: the speed and the paste quantity inserted at each time. Different speeds have not yet been studied. In the methodology used by Deveaux et al[18] the speed of 500 rpm was referred to. However, Rahde et al[20] and Caliskan et al[21] only refer to low and moderate speeds, without specifying it.

The aim of this study was to verify the efficacy of calcium hydroxide filling with the Lentulo spiral at different speeds.

Material and Methods

Thirty lower premolars from the tooth bank of the Federal University of Paraná Dentistry School — by authorization of the Research Ethics Committee of the Health Sciences Sector – CEP/SD registration number 584.121.08.07; CAAE research protocol: 2407.0.000.091-08 — were selected for this study. The teeth were classified by an average length of 20 mm and the presence of a single root canal, confirmed through a mesiodistal and buccolingual radiography. The crown was maintained in order to reproduce the clinical conditions.

Access was performed using a spherical diamond-tipped high-speed drill nº. 1014 (KG Sorensen) and completed with nº 3205 (KG Sorensen). The working length was determined 1 mm from the anatomic apex, maintaining patency with a #10 K-file

Root canal preparation was performed in a crown-down technique aided by a reciprocating angle TEP 4R-NSK. Teeth were instrumented to a # 50 master apical file 1 mm from the anatomic apex. The canals were irrigated with 1% sodium hypochlorite followed by 17% EDTA-T witch was left in place for 3 minutes to remove smear layer, followed by a final flush with sodium hypochlorite. The foramen was coated with a small piece of wax to prevent calcium hydroxide extrusion.

The specimens were randomly divided into 3 experimental groups. Calcium hydroxide paste was prepared for each tooth by mixing 1 g of calcium hydroxide P.A. (Merck Kgaa) lot 1020471000 and 0,015 g of barium sulphate P.A. (Alphatec Química Fina: analytic reagent) lot 15559, in two drops of distilled water until a toothpaste consistency.

Lentulo # 40 spiral in a clockwise rotation was inserted in the root canal always with a small paste quantity, at different speeds: G1 = 5,000 rpm; G2 = 10,000 rpm; and G3 = 15,000 rpm, coupled to a 1:1 angle in an Endo Plus electric motor (VK Driller Ltda, Jaguaré, São Paulo, Brazil). The Lentulo spiral was inserted up to 3 mm short of the working length for filling of the apical third. This procedure was repeated 3 times, followed by condensation with an apical plugger which diameter was compatible with that of the root canal diameter[5]. For filling the middle and cervical thirds, the spiral was 5 mm short, and used as described above. The extrusion of the calcium hydroxide paste through access cavity, clinically determined the complete filling.

To analyze the quality of root canal filling, the Kodak Digital Dental Systems (RVG 5000- Eastman Kodak Company, Rochester, NY, USA), was used. It has an electrical and optical sensor of 3 justaposed slides: a scintillation crystal, fiber optics, and a CCD (charge coupled device), producing an electrical signal that generates an image with a real image resolution of 14 px/mm and resolution of 27.03 px/mm.

A millimeter screen (Plexus odonto-technology, Gloucester, UK) was connected to a shield made of light cardboard (2.0 cm by 1.5 cm) and fixed to the sensor in the digital system. It was kept connected to the Rx device by means of a positioner in the digital system (Rinn XCP - DS).

The crown of each specimen was fixed to an Ependorf tube with ethyl cyanoacrylate. The tube was cut at by using a carborundum disk, leaving it 20 mm in length. Transversal grooves were made to obtain an insertion pathway in the casting material made of silicone Speedex putty (Coltène Swiss AG), used as a connection between the positioner and the Rx tube.

The radiographic apparatus (Spectro 70 X, Dabi-Atlante) was used with an electrical stabilizer (Gnatus T-1. 200S 110 V.), 70 kVp and 7mA. The cylinder was positioned perpendicularly at a distance of 5.0 cm and with an exposure time of 0.32 seconds. Optical density values in pixels were obtained from the digital image capture using filter tools for clarity and densitometry analysis from the digital system, following the millimeter ruler lines from the apical to the cervical subdivided into thirds at equidistant points. The data obtained from the images of each of the specimens, before and after filling with calcium hydroxide paste were registered. The pixels difference before and after filling were statistically analyzed by means of the Kruskal-Wallis test ($p<0.05$).

Results

Statistical tests (Levene, K-S, and Lilliefors) were used to verify the normality and homogeneity of the data. The absence of this in all groups analyzed directed to the Kruskal-Wallis ANOVA median test ($p<0.05$), which showed a statistical difference ($p=0.0318$).

The optical density means and standard deviation for each group in the cervical, middle and apical third were: 43.25 (±20.90), 38.70 (±24.40) and 16.71 (±19.85) for G1; 36.98 (±15.97), 23.16 (±15.16) and 20.28 (±22.48) for G2 and 35.75 (±22.21), 21.21(±10.16), 25.17 (±15.26), respectively.

Once the minimum significant difference was calculated, multiple comparisons were performed, demonstrating a difference between G1, G2 and G3 and from G1 to G3 in the apical third ($p<0.05$). In the cervical third, independently of speed there was no significant statistical difference ($p>0.05$).

Figure 1 shows the results for different speeds in all thirds.

Discussion

The effectiveness of intracanal dressing with calcium hydroxide has been observed by various authors.[4,6,11,12,22,23,24] However, its application needs special attention in order to completely fill the root canal space. It needs a direct contact with dentine walls in order to act in a direct and indirect mode.[4,5,7,9,18] Holland et al[25] states that if the root canal is not well instrumented and thoroughly irrigated, the dressing will not be useful. So, root canal must be enlarged to diameters compatible with its anatomic condition. Simcock and Hicks[23] demonstrated that, independently of the technique used, in canals that were only slightly enlarged, the filling proved ineffective. That is why in this experiment root canals were enlarged to a # 50 K-file.

The calcium hydroxide paste was prepared with a distilled water base, because it is a hydrosoluble vehicle, which increases the effectiveness of calcium hydroxide.[3,24,25] Barium sulphate was used as a radiopaque substance to differentiate the optical density of the calcium hydroxide from the dentine. The ratio of barium sulphate used to calcium hydroxide was 1:2.[13,17]

The insertion of the paste was performed using small quantities at a time. When activated, the Lentulo spiral launched the paste against the canal walls, and the use of a plugger allowed its condensation in all thirds.

The speeds used were determined based on the maximum speeds possible in dental equipment (around 20,000 rpm). The higher the speed and the quantity of paste in the Lentulo, the greater the quantity of air that ends up being retained inside of the root canal, generating air bubbles formation that do not allow the complete filling and, consequently, the desired action. Thus, the speeds used in the study were 15,000 rpm, 10,000 rpm, and 5,000 rpm, which were maintained constant through an electric motor (Driller – São Paulo, Brazil).

Digital radiography today represents one of the great advances in imaging, allowing speed and simplicity in the capture of images with a significant reduction in exposure time and allowing standardization, high-quality analysis, besides becoming a viable and safe alternative for the results interpretation, conferring greater diagnostic precision. The use of digital technology besides being reproducible is a system that allows almost instant images of the structures to be observed, without the need for chemical processing and with a reduced exposure time.[26]

The assessment of areas filled in the cervical, middle,

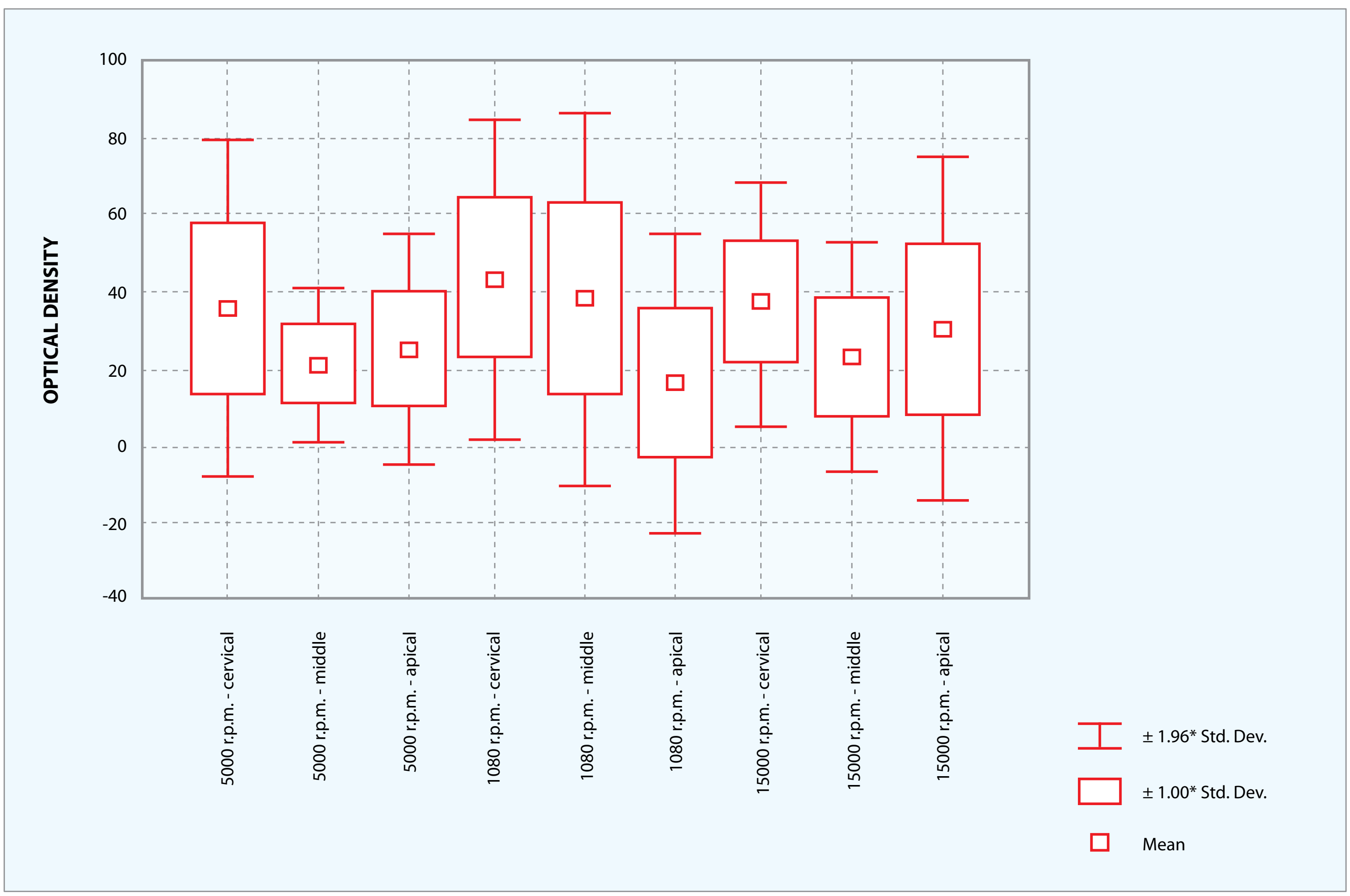

Figure 1. Optical density in the groups and thirds.

and apical thirds was performed based in the number of pixels (optical density) in the captured digital image. The millimeter screen used had the objective of serving as a measurement parameter before and after each of the specimens was filled with paste, at equidistant points, both in the dentine and in the root canal.[4]

The higher the optical density the better the filling of the root canal. The results obtained demonstrate that the middle third in G1 was better filled than G2 and G3, and statistically significant ($p<0.05$). G3 was better filled in the apical third than G1 and G2, statistically significant in relation to G1 ($p<0.05$).

Our results are in agreement with those by Cvek et al,[12] Sigurdsson et al,[16] Deveaux et al,[18] Torres et al[19] and Radhe et al.[20] The greatest difficulty in clinical practice is filling the apical third. According to our results, beginning with 15,000 rpm for the apical third, and then reducing the speed to 5,000 rpm to fill the middle and cervical thirds, can help the three-dimensional filling of the root canal.

More studies are necessary, but our results allow us to infer that different speeds are necessary for complete calcium hydroxide filling with the Lentulo spiral.

Conclusion

1. Different speeds are necessary for the correct filling of the root canal with calcium hydroxide paste.
2. The speed of 15,000 rpm was more effective in filling the apical third.
3. The speed of 5,000 rpm was more effective in filling the cervical and middle thirds.

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Location of the apical foramen and its relationship with foraminal file size

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ABSTRACT

Aim: This article analyzed the location of the apical foramen and its relationship with foraminal file size in maxillary central incisors. **Methods:** Eighty four human maxillary central incisors were used in this study. K-files of progressively increasing diameters were inserted into the root canal until it got snugly fit and the tip was visible at the apical foramen. The files were removed and teeth were cross-sectioned 10 mm from the root apex. The files were then reinserted, fixed with a cyanoacrylate-based adhesive, and sectioned at the same level as the root. The root apices were examined using a scanning electron microscope set at 140x magnification, the images were captured digitally and the results were subjected to Chi-square test. **Results:** It was observed that 63 (75%) of the apical foramen emerged laterally to the root apex and 21 (25%) coincided with the apex. The results presented statistically significant differences ($\chi^2=22.1$; $p=0.00$). **Conclusions:** Lateral emergence of the apical foramen is more common than coincidence of the foramen with the apex in maxillary central incisors. This anatomical characteristic may have influence on determination of the foraminal file size.

Keywords: Apical patency. Apical foramen. Endodontic instruments.

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Introduction

Correlation between the presence of microorganisms in the cementum portion of root canal and the development of periapical lesions[2,7,10,13,14] suggests the need for including instrumentation of this segment of the canal during endodontic therapy.[16]

Apical patency consists of the passive use of a small size file through the apical constriction without enlarging it[3] and it is believed to promote cleaning of cemental canal.[4,6,19]

According to Souza[16], Hülsmann and Schäfer,[5] it seems unlikely that the cementum portion of the canal can be cleaned by this procedure alone as it has been suggested by some authors.[4,6,19] It may be necessary to employ larger instruments, with diameters more compatible with that of the cemental canal, in order to exert some pressure against its walls.[16]

Considering that lateral emergence of the apical foramen in relation to the root apex is a common occurrence,[1,8,9,11,12,17,18] it is possible that the use of larger and less flexible instruments constitutes a challenge for the foraminal file.

The goal of this study was to analyze the lateral opening of the apical foramen and its relationship with the size of the foraminal file in maxillary central incisors.

Material and Methods

Eighty four human maxillary central incisors with complete roots were obtained from the tooth bank at the School of Dentistry of the Bahiana School of Medicine and Public Health. The criteria adopted for selection of the specimens were absence of complex external anatomy, accentuated curvature, incomplete root formation and apical resorption, observed by means of direct examination and periapical radiographs.

After access and preparation of the pulp chamber with a #3 carbide round bur (KG Sorensen, Cotia, Brazil) and Endo-Z bur (Maillefer, Ballaigues, Switzerland), canals were irrigated with 1 ml 2.5% sodium hypochlorite and explored with a #15 K-file (FKG Dentaire, La-Chaux-de-Fonds, Switzerland), inserted until the tip was visible at the apical foramen.

After that, K-files (FKG Dentaire, La-Chaux-de-Fonds, Switzerland) of progressively larger diameters were inserted with gentle watch-winding motion until binding and the tip was visible at the apical foramen. Size was annotated and the file was removed.

Teeth were cross-sectioned 10 mm from the root apex with a double-face diamond disk (KG Sorensen, Cotia, Brazil) and the files were reintroduced up to the foramen and fixed with cyanoacrylate-based adhesive. After the adhesive was set, the files were sectioned at the same level as the root.

Roots were fixed in stubs and gold sputtered and a Scanning Electron Microscope (SEM) Philips XL-30 (Philips, Eindhoven, Netherland) was used at 140x magnification. The images were digitally captured in order to determine the position of the foramen in relation to the root apex and the results were subjected to Chi-square test at 5% significance for comparison of frequencies.

Results

It was observed that 63 (75%) apical foramen presented lateral emergence in relation to the root apex and 21 (25%) coincided with the apex (Figs 1 and 2). The results presented statistically significant differences (χ^2=22.1; p=0.00). Table 1 shows the diameter and frequency of the files that bound at the cemental canal.

Table 1. Distribution, frequency, medium and median values of the files that bound in the cemental canal.

File	Number of canals	$\bar{X}$ ± SD	Median
25	5	37±7.74	35
30	21		
35	21		
40	17		
45	10		
50	7		
55	2		
60	1		

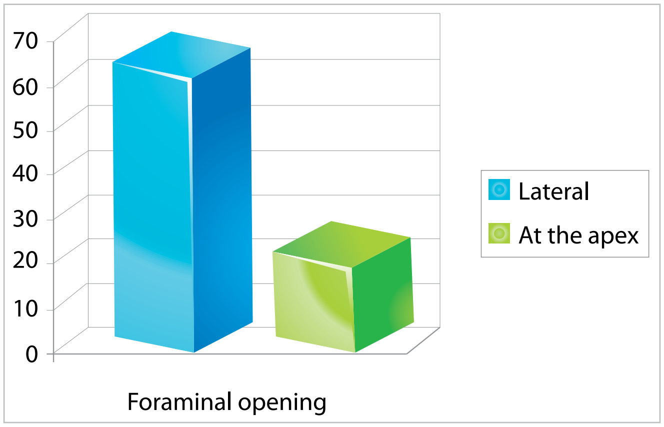

Figure 1. Apical foramen opening in relation to radicular apex.

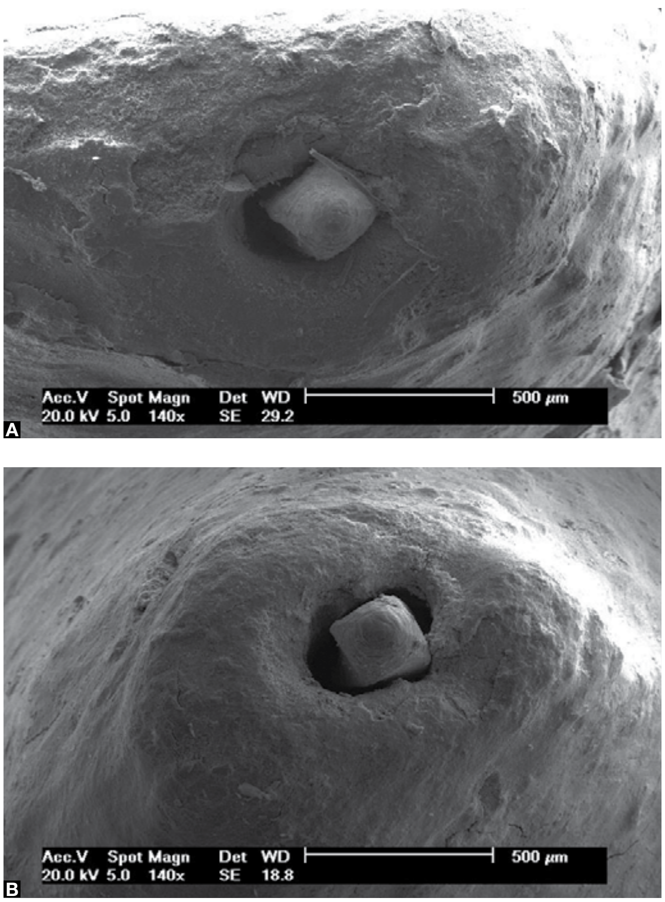

Figure 2. Location of the apical foramen in relation to the root apex. **A**) Apical foramen emerging laterally. **B**) Apical foramen coinciding with the root apex.

Discussion

The endodontic literature has demonstrated the importance of infection control for therapy success.[10] Mechanical action of the instruments against the root canal walls has been shown as fundamental to reach this aim.

Due to the lack of instruments that adequately fit the anatomy of the entire canal, instrumentation is normally carried out using files of sequentially larger diameters. Considering that mechanical action is an important factor to achieve cleanliness of dentin portion of the root canal, instrumentation of the cementum portion should deserve the same considerations. In other words, the instrument should exert pressure against the cemental canal walls in order to effectively achieve cleanliness.

Therefore, when instrumenting the cementum portion of the canal, using at least one file that binds against its walls is more effective than relying on a smaller instrument. Probably, sequential use of larger diameter instruments would contribute towards greater predictability of the results.[15,16]

In order to achieve adequate contact between the endodontic files and the foramen opening, it is possible that instruments 3 to 4 sizes larger than the one that initially bound at the foramen should be employed.

In the present study, 63 (75%) of the apical foramen emerged laterally to the root apex, while only 21 (25%) coincided with the apex (Figs 1 and 2). Analysis of the data by the Chi-square test revealed statistically significant differences (χ^2=22.1; p=0.00).

It should be kept in mind that in order to penetrate foramen emerging laterally, endodontic files have to be pre-curved. In the present study, we encountered no difficulties when exploring and accessing the apical foramen with a #15 K-file (FKG Dentaire). However, as the diameter of the instruments increased, to identify the instrument that better fit the apical foramen, it became progressively more difficult to penetrate into the foramen.

Once files with larger diameter are less flexible, these instruments may present more challenges for penetration into laterally-emerging foramen, a frequent occurrence in the present study. As observed in Table 1, the mean size of the files that bound at the foramen was 37±7, which corresponds approximately to a #35 K-file. It may be challenging to instrument the cementum portion of some canals with instruments 3 to 4 sizes greater than a # 35 file.

Knowing that in necrotic teeth this segment of canal is infected, especially when periapical lesions are present, instrumentation of this portion of the canal seems logical. Therefore, this step of treatment is subjected to the rules of instrumentation, particularly to the recommendation that mechanical action should be ensured by physical contact of the files with the canal walls.

Still, it is important to bear in mind that numbers in endodontics should be considered as references, and should not be viewed as absolute requirements. Regarding instrumentation of the dentinal canal, its anatomy and the characteristics of the instruments employed should guide the principles of root canal instrumentation. Likewise, these same factors should be considered when performing instrumentation of the cementum portion of the canal. In other words, this step of endodontic therapy should not follow rigid pre-established principles, but rather, each clinical situation should be individually examined.

It should be remembered that it was not the aim of this study to analyze other anatomical aspects, such as the diameter of the apical foramen or its distance to the root apex. Our goal was solely to identify the location of the foramen in relation to the root apex.

Conclusion

We concluded that lateral emergence of the apical foramen is more common than foramen emergence at the root apex in maxillary central incisors and that this anatomical characteristic may interfere with foraminal file size determination. Further studies should be carried out in order to analyze the location of the apical foramen and its relationship with foraminal file size in other groups of teeth.

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In vitro evaluation of shape changes in curved artificial root canals prepared with two rotary systems

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ABSTRACT

The aim of this *in vitro* experimental analysis was to compare the changes in canal shape after the use of ProTaper Universal NiTi rotary system, ProDesign system, and a hybrid technique using both systems. A total of seventy-five simulated root canals were prepared and divided into five groups (n = 15). For Group 1, the ProTaper Universal System with apical preparation file F3 was used. For Group 2, ProDesign System with apical preparation using file 30/0.2 was used. For Group 3, ProTaper Universal System with apical preparation with file F2 was applied. For Group 4, ProDesign System and ProTaper Universal System with apical preparation with file F2 were applied. For Group 5, ProDesign System and ProTaper Universal System with apical preparation F1 and F2 were used. All instrumentation was performed with the help of Gates-Glidden drills #5, #4, #3, #2 and #1 according to crow-down preparation. The difference and the quotient the amount of removed resin were analyzed within six millimeters of the canal curvature, measured for both inner and outer walls. The amount of zip and elbow apical formation and mean final shape for each type tested were analyzed. Data were analyzed using parametric tests (ANOVA p<0.05), non-parametric test Kruskal-Wallis (p<0.05) and Chi-square test (p<0.05). When difference, quotient and final mean shape were analyzed, the best preparations were observed in groups 2 and 3. Through qualitative and quantitative analysis, the best preparations were obtained with ProDesign System and ProTaper Universal System with apical preparation file F2.

Keywords: Rotary nickel-titanium instruments. Root canal preparation. Curved artificial root canals.

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Introduction

The main goal of preparing root canals is to provide cleanliness and shape, resulting in a surgically prepared canal with tapered shape, seeking to preserve its original anatomy.[1] This task is considerably difficult to be achieved in curved and narrow root canals, because the stainless steel files tend to straighten the canal curvature, causing aberrations which were described by Weine et al,[2] as zip, elbow and danger zones.

The nickel-titanium (NiTi) rotary systems were designed to prepare root canals with marked curvatures. The ProDesign (Easy®, Belo Horizonte, Brazil) system is composed of rigid preparation files with high-cutting efficiency to work in the straight part of the canal (0.7 taper #20 and 0.10 taper #35). The apical files have triple helix and good flexibility (0.3 taper #20, 0.5 taper #15, 0.4 taper #22, 0.4 taper #25 and 0.6 taper #20).

ProTaper instruments (Dentsply Maillefer®, Ballaigues, Switzerland) present innovative files concerning taper variation (multitaper) of 3.5% to 19%. The technique which is used for the system is the crow-down technique, and the system has three root canal shaping files (shaping SX, S1 and S2), of greater taper, and three apical preparation files (finishing files) with different diameters: #20 (F1), #25 (F2) and #30 (F3).[3] Recently, Dentsply Maillefer® (Ballaigues, Switzerland) made modifications to the system and named it ProTaper Universal.[4] Therefore, it was the goal of this study to assess the shape modifications of the simulated curved canals after using ProTaper Universal, ProDesign and a hybrid technique combining both rotary systems, as well as the final mean shape for each case was also assessed.

Materials and Methods

A total of 75 Endo-training resin blocks (Dentsply Maillefer®, Ballaigues, Switzerland) with gradual curvatures of about 40 degrees, according to the Schneider[5] method were used in this study.

Working length

In order to establish the working length (WL), a K-File #10 (Dentsply Maillefer®, Ballaigues, Switzerland) was placed up to the apical end of each simulated root canal to determine patency (P). This was established by using the transparency of the resin blocks. For instrumentation sequence, 1 mm of this measure was reduced to determine the WL.

Photographic Procedures

A total of two references were determined in the resin blocks for image superimposed before and after the preparation of the simulated root canals. India ink (Acrilex®) was inserted in the artificial root canals in order to photograph them before and after preparation. The blocks were placed always in the same position, and photographed using a Nikon D7OS camera with 60 mm macro lenses, 0,23 focal length, under fluorescent lighting attached to an LPL light stand, following the same subject-to-camera distance. In order to quantify the transportations produced by the instruments, a measured section was placed along with the resin blocks. After preparation, the blocks were photographed one more time, using the initial position direction and the previously established subject-to-camera distances. The photos were digitalized and edited using (Photoshop 6.0; Adobe, San, Jose) and superimposed in order to analyze possible modifications.

Preparation of simulated root canals

The 75 blocks were randomly divided into five groups with 15 samples each and handled by a single operator, who had previous experience performing both systems. Gates-Glidden drills (Dentsply Maillefer®, Ballaigues, Switzerland) #5, #4, #3, #2, and #1 were used for all groups in the straight segment of the root canal. Endo Easy SI (Easy®, Belo Horizonte, Brazil) electric engine, started the files of both systems. For Protaper Universal Sx, S1, S2 and F3 instruments a speed of 300 rpm and a 3 N.cm torque were applied. Protaper Universal instruments F1 and F2 required 300 rpm speed and 2 N.cm torque. For ProDesign files a chip inside the device was responsible for programming files sequence, speed and torque. At each instrument change canals were abundantly irrigated with 2 ml of distilled water (Pharmakon® Uberaba, Brazil), along with 0.25 ml of bi-distilled glycerin (Farmax®, Brazil), in order to lubricate the canal and make the instrumentation easier in each block. A #10 instrument was taken up to the patency to prevent resin residues from accumulating. The blocks with artificial root canals were fixed into a mini vice (Western®) for easier handling. A dark-colored adhesive tape was placed to cover the preparation, simulating the clinical condition.

Group 1 (n=15) — preparation with (NiTi) ProTaper Universal:
- » File SX, working before curvature.
- » Gates-Glidden: 5, 4, 3, 2 and 1.
- » Files S1, S2, F1, F2 and F3 up to WL.

Group 2 (n=15) — preparation with NiTi ProDesign:
- » Black (20/07) and green (35/10) files before curvature.
- » Gates-Glidden: 5, 4, 3, 2 and 1.
- » Files #1 20/0.3 (white), #2 15/0.5 (yellow), #3 22/04 (red), #4 25/0.4 (blue), #5 20/0.6 (green) and #6 20/0.7 (black) in the WL.
- » Apical preparation #30/02 (blue).

Group 3 (n=15) — preparation with NiTi ProTaper Universal:
- » File SX, working before curvature.
- » Gates-Glidden: 5, 4, 3, 2 and 1.
- » Files S1, S2, F1 and F2 up to WL.

Group 4 (n=15) — preparation with NiTi ProTaper Universal and ProDesign hybrid technique 1:
- » ProDesign Black (20/07) and Green (35/10) files before curvature.
- » Gates-Glidden: 5, 4, 3, 2 and 1.
- » ProDesign files #1 20/0.3 (white), #2 15/0.5 (yellow), #3 22/04 (red), #4 25/0.4 (blue), #5 20/0.6 (green) in the WL.
- » F2 ProTaper Universal in the WL.

Group 5 (n=15) — preparation with (NiTi) ProTaper Universal and ProDesign hybrid technique 2:
- » ProDesign (Easy®) black (20/07) and green (35/10) files before curvature.
- » Gates-Glidden: 5, 4, 3, 2 and 1.
- » ProDesign (Easy®) files #1 20/0.3 (white), #2 15/0.5 (yellow), #3 22/04 (red), #4 25/0.4 (blue), #5 20/0.6 (green) in the WL.
- » F1 and F2 ProTaper Universal (Dentsply-Maillefer®) in the WL.

Evaluation methods

The superimposed images were increased and evaluated with Image Tool 3.0, which measures distances, angles and areas of the images. It was initially calibrated in milimeters, as a measure unit, with the measured sections placed in the blocks as reference point. In the distance icon, each millimeter was marked until it reached a total of 6 milimeters before the apical end of the simulated root canal, coinciding with the end of the curvature (Fig 1). The amount of material removed was measured in each milimeter of the curved segment (6 mm) both inside and outside, according to Uzun et al[6] (Fig 2). To calculate the difference, the following was defined:

D (difference) = Do (outer resin removed) – Di (inner resin removed)

The positive result meant the prevalence of outer and the negative result meant prevalence of inner resin removed. The closer has come to zero, the more balanced

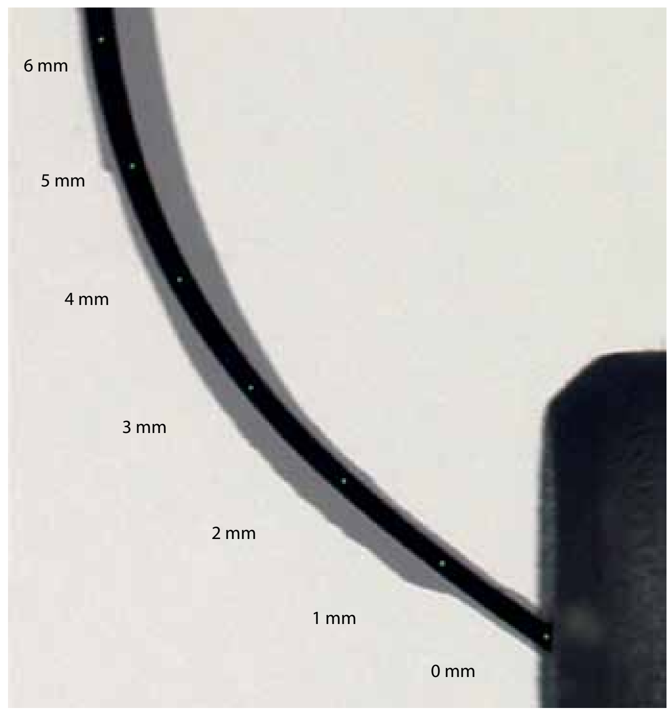

Figure 1. Values evaluated in this study.

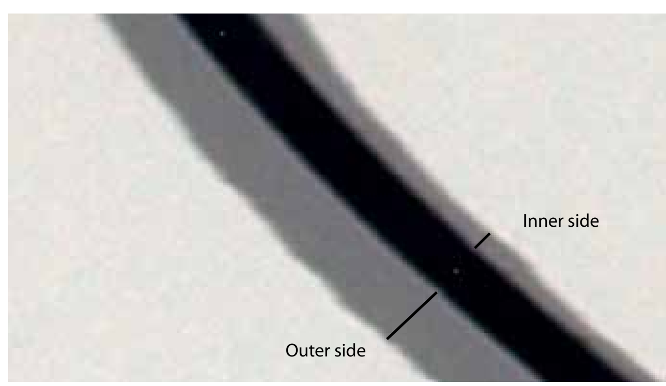

Figure 2. Measuring of removed material, inner and outer sides, at each level.

the preparation was, the further, positive or negative, the greater transportation, according to Hata et al.[7]

The quotient between inner and outer resin removed was calculated. The highest value was placed in the numerator and the lowest in the denominator. The most balanced preparation was that which was closer to 1. Aydin et al[8] indicate this systematic evaluation, however they place the lowest number in the numerator and the highest in the denominator. The superimposed were analyzed by two experienced raters, Endodontics Masters, who did not know to which group the preparation belonged. A "masking" technique was used to verify the occurrence of zip and elbow apical formation. The reference figures were revealed by Thompson and Dummer.[9] The removed resin means were used to generate a final mean of preparation for each group.

Results

Normality tests were carried to determine differences. The adoption of non-parametric Kruskal-Wallis test was applied for levels 1, 2, 4, 5 and 6 mm, whereas the ANOVA parametric test with Tukey's test (Table 1) was applied for level 3 mm.

We can observe through the mean values that outer removed resin prevailed for all groups up to the third millimeter. The remaining millimeters had greater inner curvature.

Significant differences were observed for group 1 in levels 3, 4 and 5. At levels 5 and 6, a significant inner material removed was observed for group 1 and 5.

Normality tests were carried for quotients. Kruskal-Wallis non-parametric test was adopted for levels 1, 2, 4, 5 and 6 mm and ANOVA parametric test with Tukey's test was applied for level 3 mm (Table 2). Comparisons were made at each level.

We can observe values which are far from 1 for group 1 in the three apical millimeters, except for the third millimeters. In the three remaining millimeters, we can observe more discrepant values for groups 1, 4 and 5.

Based on inner and outer material removal at all levels, a final mean shape outline was made along with an example of the transference of means to Image Tool (Fig 3).

Table 1. Statistical Inference, for compared differences at each level.

Levels/ Groups	1 mm	2 mm	3 mm	4 mm	5 mm	6 mm
Group 1	0.0880^{A}	0.1887^{A}	$0.1053^{A,b}$	-0.1213^{A}	-0.2813^{A}	-0.2540^{Ab}
Group 2	0.0853^{A}	0.0840^{B}	0.0020^{C}	-0.1393^{A}	-0.1913^{B}	-0.1347^{Cb}
Group 3	0.0120^{B}	0.0713^{Cb}	0.0687^{B}	-0.0387^{B}	-0.1887^{B}	-0.1933^{B}
Group 4	0.0007^{B}	0.1313^{Ab}	0.1220^{A}	-0.0200^{B}	-0.2080^{B}	-0.2640^{A}
Group 5	0.0767^{A}	0.1253^{Ab}	0.0760^{B}	-0.0760^{A}	-0.2433^{A}	-0.3127^{A}

Capital letters different in columns indicate significant differences.

Table 2. Statistical Inference, for quotients, compared at each level.

Levels/Groups	1 mm	2 mm	3 mm	4 mm	5 mm	6 mm
Group 1	5.5647^{A}	4.1117^{A}	2.1205^{Ab}	5.3540^{A}	5.3539^{A}	4.0819^{Bc}
Group 2	2.5853^{B}	2.4453^{B}	1.3703^{C}	2.7449^{Ab}	3.7659^{B}	2.4744^{C}
Group 3	1.9021^{B}	2.4570^{B}	1.9229^{Ac}	1.5195^{Cd}	3.8589^{B}	3.3282^{C}
Group 4	1.9317^{B}	2.9786^{Ba}	2.4726^{A}	1.2185^{Dc}	4.7617^{Ab}	7.2291^{A}
Group 5	2.0114^{B}	2.3212^{B}	1.6606^{Bc}	1.6145^{Bd}	4.8421^{Ab}	8.5153^{A}

Capital letters different in columns indicate significant differences.

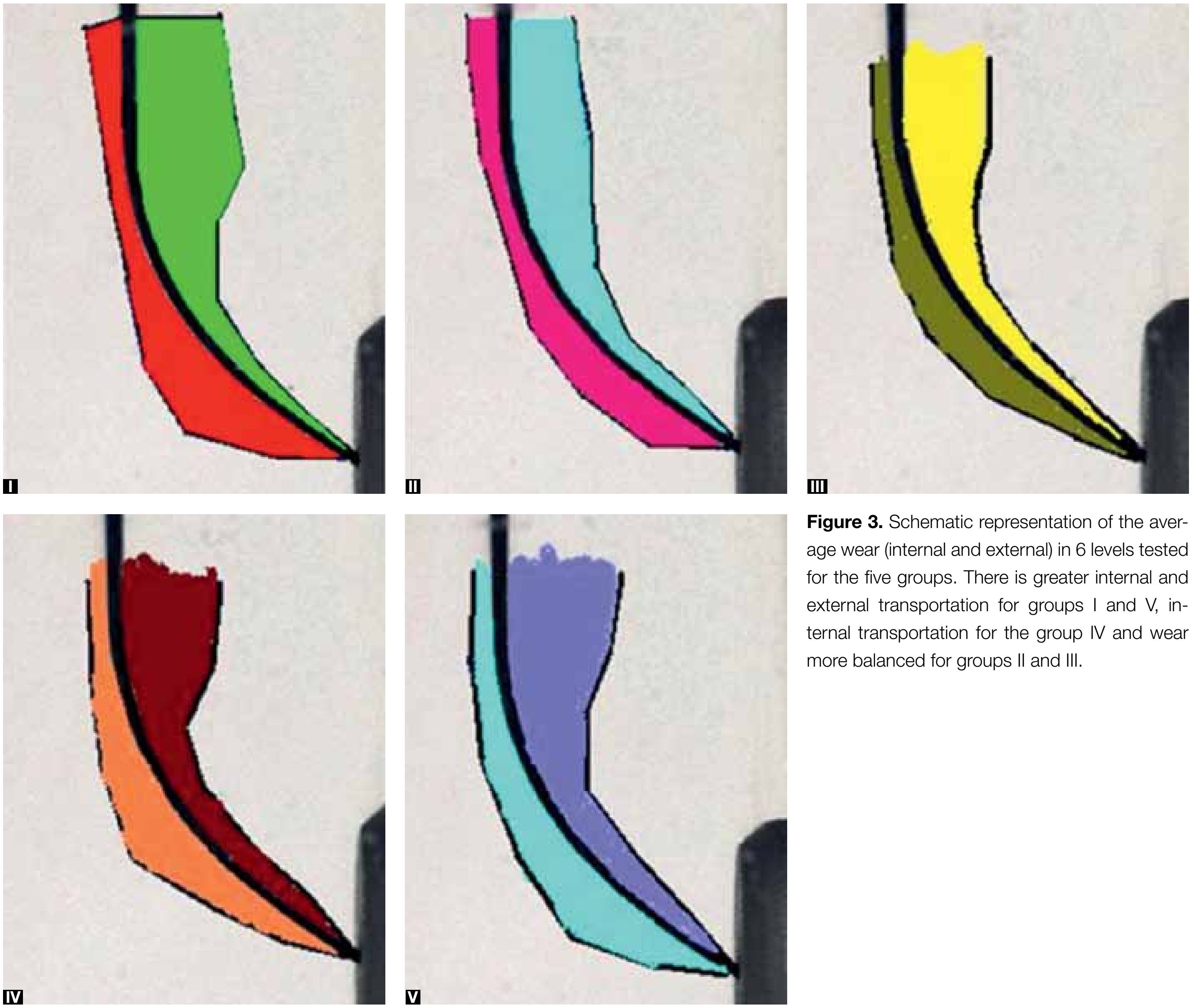

Figure 3. Schematic representation of the average wear (internal and external) in 6 levels tested for the five groups. There is greater internal and external transportation for groups I and V, internal transportation for the group IV and wear more balanced for groups II and III.

In order to obtain inter-rater agreement, Kappa test was applied results value = 1 with very good inter-rated agreement.

The occurrence of zip and elbow apical formation was also observed according to Table 3. Chi-square test was applied in order to verify the significance between comparisons. No significant differences were observed, but the amount of deformations in the ProTaper Universal, group 1, was much higher than those of the other analyzed groups.

It was observed that, when there was zip formation, mostly for group 1, the values for the difference between inner and outer resin removal, at 2 mm, were 0.25 and the quotient was 4. For group 2, the difference was 0.15 and the quotient was 4. For group 3, the difference was 0.21 and the quotient was 6. For group 4, the difference was 0.23 and the quotients were 5 and 6. For group 5, the difference was 0.16 and the quotient was 3.

Discussion

Just as observed in previous studies, the artificial root canal methodology was introduced by Weine et al.[2] in order to analyze the preparation procedures of root canals. The use of simulated curved root canals offers a standardized condition of curvature angle and length, as well as the analysis of the previous and final shapes of preparation.[7-10]

Table3. Formation of zip and elbow.

Formation	ProTaper F3	ProDesign	ProTaper F 2	ProDesign+F2	ProDesign F1+F2
Zip	5	1	1	1	1
Elbow	5	1	1	1	1

Observed more aberration for group 1.

We can observe in this *in vitro* study that, through the values of the material removal means and of the difference between inner and outer, there was greater outer removal in the three apical millimeters of the curvature and, there was greater inner material removal to all groups in the three cervical millimeters of the curvature (Table 1). These results were supported by other studies.[11,12,13] For the 2 mm level, greater transportations are observed for groups 1, 4 and 5, significant in comparison with the other tested groups. At this level, we observed that greater outer material removal and values distant from zero induced the occurrence of zip formation. For the 5 mm level, the greatest material removals were for groups 1 and 5, which were significant in comparison with the other tested groups, showing a strong tendency for perforation in inner curvature. For the 6 mm level, groups 1, 4 and 5 presented significant material removals, in comparison with the other groups, confirming the tendency of perforation. Better preparations are observed for groups 2 and 3 in the prevention of zip and perforation in inner curvature. Preparations with greater potential for aberrations formation are found in groups 1, 4 and 5 (Fig 3).

Centering ability was quantified by obtaining the quotient between the highest and the lowest value. Results closer to 1 mean that the system is better at balancing inner and outer material removal. Except for the 6 mm level, we observed a longer distance from 1 for the ProTaper Universal system up to F3 apical file (group 1). At this level, there was a greater distance for groups 4 and 5. For the 1 mm level, there was a significant difference for groups 1 and 2. It is possible to observe that the value for group 1 is twice the value of group 2, showing reduced balance. For the 2 mm level, we can see the significance of group 1 in comparison with the other groups. At 3 mm level, the significant preparations with longer distance from 1 were for groups 1 and 4. For 4 mm level, the least centered group was group 1. For 5 mm level, there was also a significant unbalanced material removal for group 1. At 6 mm level, there was greater significant level for groups 4 and 5. Therefore, ProTaper Universal system up to F3 instrument was that which provided more irregular and less centered preparations. We can observe values closer to 1 for the other groups, except for 5 mm and 6 mm levels for groups 4 and 5, which were maintaining preparation regularity (Table 2). ProTaper systems up to F2 instrument and ProDesign showed more centered preparations at all levels.

Peters et al[14] (through the use of human teeth and CT scan), Iqbal et al[15] and Veltri et al[16] (through radiographic method), and Guelsow et al[17] (through Bramante et al[18] methodology) showed preparations with low incidence of apical transportation for ProTaper system up to F3 file. A similar result was obtained by Yun and Kim[19] in simulated root canals and by Ankrum et al[20] in extracted molars, showing inner removed resin for the furcation area whereas. Schäfer and Vlassis;[11] Yoshimine et al;[12] Uzun et al[21] conducted research using simulated root canals showing that ProTaper system provides a high occurrence of zips when taken up to F3 file. Schäfer and Vlassis[22] in a similar study, but using human teeth and radiographic method before and after preparations, verified similar results for ProTaper system.

Loizides et al;[23] Zhang et al;[10] recommend a hybrid technique using ProTaper and Hero (Micro-Mega®) and show better results in "S"-shaped simulated root canals. They also observed better taper of preparations, due to the taper of ProTaper files F1 (#20 diameter tip and 0.07 taper initially) and F2 (#25 diameter tip and 0.08 taper initially). Setzer et al.[24] observed no differences in the combination of different systems in increasing the level of apical transport. It was proved that group 4, with hybrid technique, presented regular shapes in the apical region and greater taper than group 2 ProDesign using the apical preparation #30/0.2. These conditions favor cleanliness and filling quality. Special attention must be paid to displacement, at levels 5 and 6 mm, inner wall, to groups 1, 4 and 5, with tendency to form perforation in inner curved.

Visual analysis showed high incidence of zip and elbow formation for ProTaper Universal when using F3 file (group 1). This result is similar to those observed in other studies[8,11,12,17,22,25,26] Contrarily, Guelsow et al[17] showed a low incidence of irregularities for ProTaper.

It is important to be careful when transferring these results to patient preparation. Despite the countless advantages of artificial root canals, they do not simulate their complicated internal anatomy, mainly the flattening of roots in curved root canals. Cleanness is one of the factors which should be considered, since it cannot be observed in artificial root canals because they are made of resin, whereas human teeth root canals present such a complex anatomy.

Conclusion

In conclusion, based on the adopted methodology used and on the obtained results, we can conclude that: through the results of difference and quotient, a greater distance of reference values (0 to 1) was observed for groups 1, 4 and 5. A larger number of zip and elbow formation was present in group 1. The removal resin means showed more regular mean configurations for groups 2 and 3.

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The persistence of different calcium hydroxide paste medications in root canals: an SEM study

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ABSTRACT

Introduction: There is a possibility of intracanal medication remain in the root canal even after its removal prior to obturation. The present study aims to evaluate under scanning electron microscopy the persistence of residues in the root canal from calcium hydroxide medications prepared with different vehicles. **Methods:** Thirty-six bovine incisors had their crowns removed, the root canals prepared and were assigned randomly to six different experimental groups, according to the intracanal medication used. Group I (control) received no intracanal medication, whereas root canals of Group II were filled with P.A. calcium hydroxide. Group III received a mixture of $Ca(OH)_2$ and saline solution, in Group IV glycerin was used as vehicle, and Groups V and VI received $Ca(OH)_2$ mixed with propylene glycol or polyethylene glycol 400, respectively. After one week, medication was removed, roots were split and the canals observed under the scanning electron microscope. Representative photomicrographs of the apical third of each experimental group were observed and analyzed quantitatively by means of a grid, with results expressed in percentage of canal walls covered by debris. **Results:** Statistical analysis (one-way ANOVA and Tukey's post hoc test, α=0.05) revealed significant differences between groups, indicating higher amounts of $Ca(OH)_2$ residues in the canals where propylene glycol or polyethylene glycol were used as vehicles. The dentinal walls of the canals that received pure P.A. calcium hydroxide or its association to glycerin presented amounts of debris similar to the control group. **Conclusions:** $Ca(OH)_2$ P.A. based medications or its association to glycerin allows an easier removal from the root canal.

Keywords: Calcium hydroxide. Intracanal medication. Vehicles.

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Introduction

The elimination of microorganisms in the root canal environment prior to obturation is of paramount importance for predictable treatment of apical periodontitis, and literature demonstrates the necessity of using an intracanal dressing to achieve such goal[1-5].

Calcium hydroxide ($Ca[OH]_2$) has been used successfully in endodontics as a microbicide agent, due to its ionic effect observed by chemical dissociation into calcium and hydroxyl ions. The last inhibits bacterial enzymes by acting on the cytoplasmic membrane of the bacteria, generating irreversible effects, while calcium activates tissue enzymes such as alkaline phosphatase, leading to a mineralizing effect.[2,4]

The use of $Ca(OH)_2$, however, is not limited to its microbicide action. Other uses of this substance include inhibition of tooth resorption[4,6,7] and induction of repair by hard tissue formation,[4,8,9] which makes its use recommended in many clinical situations.[4] Currently, this chemical is considered the best medicament to induce hard tissue deposition and promote healing of vital pulpal and periapical tissues.[5]

The vehicle used with $Ca(OH)_2$ to create a paste grant chemical characteristics that will influence its clinical handling during application and rate of ionic dissociation and diffusion. Some authors believe that hydrosoluble vehicles have better biological behavior (antimicrobial qualities and induction of tissue repair), due to a higher ionic dissociation, whereas others advocate the use of viscous or oily vehicles, since the alkaline properties of such pastes will only be exhausted after a longer period.[5,10,11,12]

Prior to obturation of the root canal system, though, calcium hydroxide must be completely removed in order to avoid failure of the treatment.[13] Literature shows that this is a difficult, if not impossible, task. Margelos et al[14] have shown that it is necessary to combine sodium hypochlorite (NaOCl) and ethilenediamine tetracetic acid (EDTA) as irrigants with hand instrumentation to improve the removal efficiency of $Ca(OH)_2$ from root canal, but its complete elimination may not be achieved. Lambrianidis et al[15] found that even after irrigation with NaOCl and EDTA, as well as reinstrumentation with a #25 file, a considerable amount of calcium hydroxide (25 to 45%) from intracanal dressings remained attached to the canal walls. Attempts of removing such medication with nickel-titanium rotary instruments, sonic or ultrasonic irrigation or citric acid instead of EDTA also proved unsuccessful, given that $Ca(OH)_2$ still remained in the root canal.[16-19]

The persistence of $Ca(OH)_2$ in the root canal prior to obturation may lead to failure of the endodontic treatment by creation of voids in the root canal that will not be properly filled, thus affecting apical seal.[13,15,20,21] Even small amounts of $Ca(OH)_2$ remaining in the root canal may obliterate dentinal tubules affecting sealer adhesion[20,22,23] or cause adverse chemical reactions with the sealer, which may lead to an unpredictable prognosis.[14,20]

Since the vehicle used during the preparation of the calcium hydroxide-based intracanal dressing may interfere with its removal capacity, the purpose of this study is to evaluate under the scanning electron microscope the persistence of residues in the root canal from calcium hydroxide medications prepared with saline solution, glycerin, propylene glycol 400 or polyethileneglycol 400.

Material and Methods

Thirty-six bovine incisors with closed root apexes had their crowns removed and root canals instrumented up to a #50 master apical file according to the step-back technique. Irrigation was performed using 1 ml of 2.5% sodium hypochlorite between files, with a final flush of 1 ml 15% EDTA for 1 minute followed by 10 ml of distilled water. Specimens were randomly assigned to six experimental groups, according to intracanal medication to be used, as follows: GI= no medication (control); GII= $Ca(OH)_2$ P.A. powder (Synth, Diadema, SP, Brazil); GIII= $Ca(OH)_2$ mixed with saline solution; GIV= $Ca(OH)_2$ mixed with glycerin (Synth, Diadema, SP, Brazil); GV= $Ca(OH)_2$ mixed with propylene glycol 400 (Synth, Diadema, SP, Brazil); GVI= $Ca(OH)_2$ mixed with polyethylene glycol 400 (Synth, Diadema, SP, Brazil). A pediatric amalgam carrier was used in GII to place the $Ca(OH)_2$ powder inside the root canal, following compaction using a #2 Paiva endodontic condenser. The other groups had the root canals filled with the aid of a Lentulo spiral bur (Maillefer, Ballaigues, Switzerland). Radiographs of the roots were obtained both in buccal-lingual and proximal views to assure that the medication was homogeneous and no voids were produced during its introduction. The canal openings were then sealed with Coltosol® (Coltène, Whaledent, Switzerland).

After storage for seven days at 37°C, 100% humidity, samples were irrigated 1 mm short of working length with 5 ml of 2.5% sodium hypochlorite alternated with 5 ml of 15% EDTA, using the master apical file to reach the working length. A final irrigation with saline solution was performed and samples were processed for observation under the scanning electron microscope, at 500x magnification. Three representative photomicrographs of the apical third of each sample were obtained and analyzed quantitatively for debris, with the aid of a 10x10 grid. Results were recorded as percentage of debris covering the root canal walls, and statistical analysis was performed (one-way ANOVA and Tukey's post hoc test, α=0.05).

Results

Table 1 presents the average of debris found in all experimental groups, according to SEM observations.

Statistical analysis (one-way ANOVA, α=0.05) revealed statistical significant differences between groups ($p<0.001$). Tukey's post-test indicated lower amounts of debris found for groups I, II and IV, while higher amounts were found for groups V and VI. Group III (association with saline solution) presented intermediary amounts of debris.

Table 1. Mean amount of debris found in the apical third of the experimental groups, in percentage.

Group	amount of debris
I) no medication (control group)	1.6% (±0.55%)[a]
II) $Ca(OH)_2$ powder	9.6% (±4.72%)[a]
III) $Ca(OH)_2$ + saline solution	16.0% (±10.5%)[b]
IV) $Ca(OH)_2$ + glycerin	10.8% (±2.86%)[a]
V) $Ca(OH)_2$ + propylene glycol 400	28.0% (±11.8%)[c]
VI) $Ca(OH)_2$ + polyethylene glycol 400	19.4% (±15.3%)[c]

Same letters indicate statistical similarity ($p>0.05$).

Discussion

Calcium hydroxide-based medications are routinely used in endodontics to eradicate microorganisms from the root canal system, which due to its complex anatomy may lodge such pathogens even after careful instrumentation and irrigation, leading to failure.[1-4] Various vehicles associated to $Ca(OH)_2$ have been proposed, and a consensus seems still far from being reached.[5]

Most authors agree that such medication must be removed from the root canal prior to filling since it may interfere with the quality of the obturation, especially the apical seal,[13-23] while other studies indicate that the persistence of $Ca(OH)_2$ does not promote a higher apical leakage.[24,25] However, Kim and Kim[20] point out that these studies also noted that when calcium hydroxide dressing was retained in the canal, apical leakage increased with time. The fact that methylene blue dye may suffer discoloration when in contact with $Ca(OH)_2$ may also lead to false positive results, which might invalidate some of the previous findings.[26,27]

The complete removal of $Ca(OH)_2$ prior to obturation by the clinician is impossible to verify, since this material has the same radiographic aspect as that of dentin.[23] According to previous studies, even minute concentrations of $Ca(OH)_2$ covering the root canal walls may interfere with the setting of zinc oxide-eugenol based sealers.[13,20] Resin-based sealers also may suffer adverse effects from such intracanal medication.[21]

The present study evaluated the persistence of $Ca(OH)_2$ medication in the root canal walls at a microscopic level. The choice of using bovine incisors was due to their wide root canal, which would provide a standardized, generous space for irrigation, thus creating the most favorable conditions possible for the medication removal. Anatomical complexities would retain mechanically more intracanal medication,[19] which might lead to biased results. The choice of using NaOCl and EDTA as irrigants and the master apical file at the working length also constitutes an attempt to remove as much medication as possible from the root canal walls.

Results indicate that, despite such favorable conditions, $Ca(OH)_2$ still persists inside the canal after its removal attempts. This is in agreement with previous studies that found the removal of calcium hydroxide-based dressings extremely difficult or even impossible.[13-21,23]

The use of pure $Ca(OH)_2$ as intracanal dressing, although reported in some studies,[18,28] seem to be both impractical clinically in narrow canals and not desirable, since ionic diffusion would be minimal. In the present study, it was used merely as a control, to allow comparison with other formulations. Although the lower persistence in the root canal system reported in the results, the use of such medication without a vehicle does not seem to be suitable or desirable clinically.

Propylene glycol or polyethylene glycol used as vehicles provide a viscous consistency to the paste, which facilitates the insertion in the root canal, leading some authors to prefer this formulation. The slow release of ions and resorption by the surrounding tissues are also among the qualities advocated.[5,11,12] However, results suggest that removal of viscous pastes may be more difficult than other formulations, causing an excess of medication remaining at the apical level of the root canal. Similar findings were found by Lambrianidis et al[15] and Nandini et al,[18] but using commercially available pastes based on methylcellulose or silicone oil, respectively. Other authors found no differences regarding $Ca(OH)_2$ medication persistence associated to different vehicles.[23,28]

Association of $Ca(OH)_2$ with saline solution showed to be easier to remove from the root canals than propylene or polyethylene glycol, but still persisted in greater amounts when compared to glycerin used as vehicle. Other studies may be necessary to understand the reasons of the lower amounts of $Ca(OH)_2$ found on the $Ca(OH)_2$ + glycerin group (GIV).

Conclusions

1. Pure calcium hydroxide based medications or its association to glycerin allows an easier removal from the root canal.
2. The association of $Ca(OH)_2$ with polyethylene glycol or propylene glycol 400 determines a higher persistence of the medication inside the canal prior to obturation.
3. None of the intracanal medications could be totally removed from the root canals.

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SEM and microbiological analysis of dirt of endodontic files after clinical use and your influence on sterilization process

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ABSTRACT

Objective: The aim of this study was to assess the level of cleaning of endodontic files after its use in root canals preparation and their influence on the sterilization process. **Methods:** Fifty files were divided into two groups: one group of 25 files for analysis in scanning electron microscopy (SEM) for verification of cleaning and another group of 25 files for microbiological analysis in thioglycolate and BHI after sterilization. **Results:** The results showed that endodontic files had different degrees of dirt on his active part through evaluation by scanning electron microscopy. The bacterial growth wasn't detected through microbiological test after sterilization. **Conclusion:** It was concluded that despite the significant presence of dirt on endodontic files in their active part, this dirt don't interfere in the sterilization process.

Keywords: Dirt. Endodontic files. Microbiological test. Scanning electron microscopy.

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Introduction

The success of endodontic therapy is grounded not only in the correct diagnosis, but also the proper planning and technical implementation, and especially in caring for the maintenance of the aseptic chain during the patients care.

The endodontic instruments are used to remove the remnants of pulp tissue during the procedures of cleaning and shaping of the root canal system. These instruments can be recycled for reuse after its first use. In a large study was reported that 88% of general practitioners dentists in the UK re-process the endodontic files after use[1].

The mandatory conduct of biosecurity recognizes that the endodontic instruments, to be reused, they must go through a cleaning process before sterilization[2], since the presence of organic matter and/or debris on the instruments may interfere with the sterilization process. These organic compounds creates barriers to protect the microorganisms, which may prevent the penetration of the sterilizing agent[3].

The procedures of pre-cleaning and autoclave can be used to sterilize endodontic instruments[4,5]. However, the complex architecture of endodontic files could difficult these procedures[5]. Dental structures and organic debris have been observed on the surface of rotary instruments, especially in the cracks[6]. According to a previous study, 66% of endodontic files retrieved dental general practitioners remained visibly contaminated[7]. Thus, there is the possibility of cross contamination associated with the inability to properly clean and sterilize them, and suggested that these instruments should be single-use devices.

Therefore, the aim of this study was to determine the presence of debris left on the surface of endodontic files after performing a cleaning process and analyze its influence on the sterilization process.

Materials and methods

Fifty endodontic files K #25 were selected for this study, regardless of their trademark. The samples were divided into two groups (n = 25) according to the method of analysis, prior use and performance of disinfection protocol, according to Table 1.

Table 1. Distribution of samples in groups.

Group	Method	N	Prior use	Clean	Sterilization
G1	SEM	25	Yes	Yes	Yes
G2	Culture	25	Yes	Yes	Yes

The endodontic instruments were obtained directly from students of the School of Dentistry of Pontifical Catholic University of Rio Grande do Sul (PUC-RS, Porto Alegre, Brazil). The cleaning protocol used consisted of brushing with chlorhexidine gluconate 2% (Globomedia, Sacomã, SP, Brazil), washing in water and drying. Prior to analysis, samples were placed in plastic Eppendorf tubes (Eppendorf AG, São Paulo, Brazil) and sterilized by autoclaving (Dabi Atlante, Ribeirão Preto, Brazil), for 30 minutes at a temperature of 120°C.

Analysis in Scanning Electron Microscopy

The first group of endodontic files was removed from the Eppendorf plastic tubes with clinical tweezers and manipulated only by the cable, avoiding any contact of the active part of the instrument. The cables of instruments were removed through a wire cutter and its metal rods, made by the blade and the intermediate portion, were fixed in stubs for further observation.

After this process, samples were taken to a scanning electron microscope. The initial portion of the active blade of each instrument was evaluated under magnification of 150 X and 15 kV, recording the images for each instrument.

The images were evaluated by four examiners previous calibrated by Kappa test for inter-examiner agreement. A numeric score was assigned for each image, representing its degree of dirt for each instrument: 1 = no residues in the file, 2 = file almost clean surface, i.e., with low residue, 3 = surface file with an average amount of waste, and 4 = the surface of the file with a large amount of waste.

The data were submitted to Kruskal-Wallis test, using the mode to qualitative assessment on a significance level of 1%.

Microbiological Analysis of Contamination of Files

All procedures were performed under strict aseptic conditions inside a laminar flow camera. Each endodontic file was removed from the Eppendorf plastic tube with a sterile tweezers and then introduced into a glass tube containing BHI (Brain Heart Infusion, Himedia, Curitiba, PR, Brazil). Then it was removed and placed in a test tube containing thioglycolate broth (Himedia, Curitiba, PR, Brazil). As a negative control, two tubes of BHI liquid and thioglycolate were used. These tubes didn't receive samples. The positive control was performed by inoculating strains of periodontal pathogens from clinical specimens and isolates of Enterococcus spp. The tubes were incubated in a microbiological stove, in the presence of oxygen at 37°C for 72 hours. The presence of microorganisms was confirmed by observing turbidity of the liquid culture medium after 24, 48 and 72 hours. The negative samples were those which do not lead to change in the culture medium, whereas the positive samples were those that caused the turbidity of it.

To prove the sterility of files, after observing the presence or absence of turbidity in liquid media, was made the inoculation in solid medium. A 10μl aliquot of BHI was inoculated on the surface of the culture medium (agar plain), allowed to dry and incubated aerobically at 37°C. The same procedure was performed with sodium thioglycolate, but the plates were incubated in microaerophilic by the method of the candle flame.

Results

The results showed that in group 1 the endodontic files showed different degrees of dirt after performing the same cleaning protocol (Fig 1) providing, in most cases, a surface with large quantities of waste, represented by score 4 (Graph 1).

Moreover, the results didn't show presence of bacterial growth on the surface of endodontic files for 24, 48 and 72 hours after incubation, both in BHI and in thioglycolate medium, except the positive control where there was the presence of growth bacteria in all periods of compliance and in both culture media (Graphs 2 and 3).

Discussion

The endodontic instruments are used to remove the remnants of pulp tissue during the procedures of cleaning and shaping of the root canal system. These instruments are submitted by a cleaning process before sterilization to be reused, with the aim of removal of organic matter and waste tissue in the instruments.

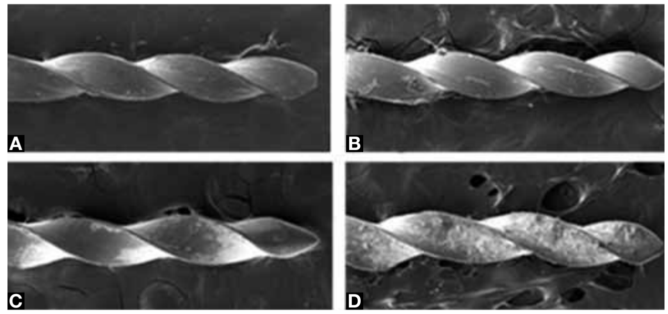
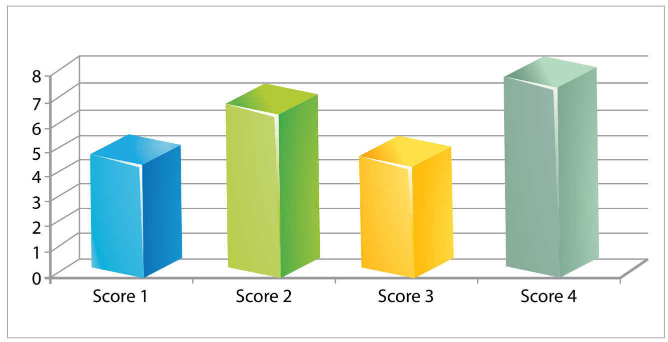
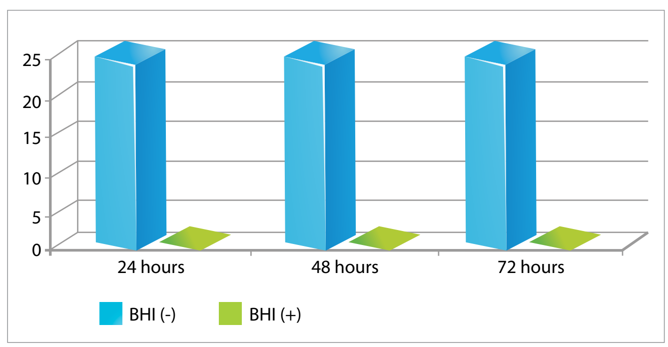


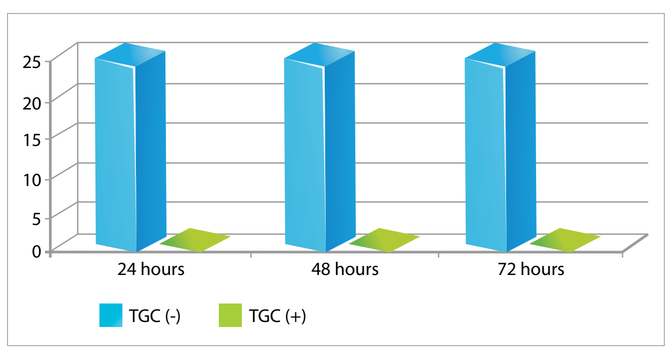
Figure 1. Scores for determining the amount of dirt on the surface of endodontic files: **A**) Score 1 - no residues in the file; **B**) Score 2 - file almost clean surface, i.e., presenting a small quantity of waste; **C**) Score 3 - surface of the file with an average amount of waste and, **D**) Score 4 - surface of the file with a large amount of waste.



Graph 1. Assessment of the degree of contamination of endodontic files.



Graph 2. Presence / absence of bacterial growth on the surface of endodontic files in culture media BHI/Time.



Graph 3. Presence / absence of bacterial growth on the surface of endodontic files in culture media Thioglycolate TGC/Time.

Several studies approach the cleaning techniques of endodontic files, including brushing, enzymatic cleaners and ultrasonic aid. However, these methods aren't able to clean completely the instrument, leaving it free of any residue, although the best results have been obtained by combining the resources of brushing and ultrasonic.[2,8,9,10]

The ultrasonic cleaning has some advantages over the manual, such as higher cleaning efficiency; reduces the aerosolization of infectious particles released during the brushing; instruments with reduced incidence, increased cleaning, including removal of oxidation, better use of time and reduction of manual work.[3,11,12,13]

The files collected for this study were subjected to cleaning by brushing performed by students of the School of Dentistry of Pontifical Catholic University of Rio Grande do Sul. SEM analysis demonstrated that 20% of files were included on the score 1, 28% in score 2, 20% in score 3 and 32% in score 4. This may be related to the fact that the feature was not used to perform ultrasonic cleaning of endodontic files, showing them a significant degree of dirt on their surfaces.

Previous study states that the presence of organic matter and/or debris on the instruments may interfere with the sterilization process, because it creates barriers to protect the microorganisms, which may prevent the penetration of the sterilizing agent.[3] However, these findings aren't in agreement with the findings in our study, where was shown that, despite the presence of dirt and organic matter on the surface of endodontic files, no bacterial growth was detected after the sterilization process of them. This can be explained by the efficient sterilization process that is able to reduce and eliminate all forms of microbial content present on the surfaces of endodontic instruments.

Results similar to our study were found by previous study which compared the microbiological conditions of files used by undergraduate students in six Schools of Dentistry of Rio Grande do Sul.[14] The results showed that 53 samples were sterile of a total of 60 samples examined, whereas 7 were contaminated. The collected endodontic files obtained 100% of negative cultures only in two schools.

According to the limitations of this study, was concluded that despite a significant presence of dirt on the surface of endodontic files after cleaning, this factor doesn't influence the process of sterilizing them.

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Influence of cone beam computed tomography on dens invaginatus treatment planning

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ABSTRACT

The achievement of endodontic success is associated with the accurate diagnosis. To establish the diagnostic hypothesis based on periapical radiograph is a challenge for all different dentistry specialties. The visualization of three dimensional structures, available with cone beam computed tomography (CBCT), favors precise definition of the problem and treatment planning. The aim of this manuscript is to present a case report of dens invaginatus treatment planning changed by 3-D CBCT images. The complete and dynamic visualization regarded the correct endodontic-periodontal structures, suggesting type 2 dens invaginatus associated with radiolucent areas, and periodontal compromising. The adequate examination using imaging exams should be always made in conjunction with the clinical findings. The accurate management of CBCT images may reveal abnormality which is unable to be detected in periapical radiographs. The choice of clinical therapeutics for these dental anomalies was influenced by CBCT views which showed bone destruction not previously visible in initial periapical radiograph. Based on the necessity of extensive restorative treatment, the option of treatment was the extraction of this tooth and oral rehabilitation.

Keywords: Dens invaginatus. Dental anomaly. Cone beam computed tomography. Endodontic diagnosis.

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Introduction

Dens invaginatus is an anomaly of development (malformation) of teeth resulting from a infolding of dental papilla during tooth development. The affected tooth shows a deep infolding of enamel and dentine starting from the foramen coecum or even the tip of cusps and which may extend deep into the root.[1]

Every tooth may be affected, but the maxillary lateral incisor is the most affected and the bilateral occurrence is common. The root canal therapy may present severe difficulties and problems because of the complex anatomy of these teeth.[2-6] Since the second period of 19th century the dental literature has published about this malformation with the following synonyms: dens in dens, invaginated odontome, dilated gestant odontome, dilated composite odontome, tooth inclusion, dentoid in dens.[1-7]

Hülsmann,[1] based on several studies, presents seven possibilities about aetiology of dens invaginatus malformation and these etiologies are controversial and still today remains unclear. These theories about etiology of dens invaginatus have been proposed to explain these dental malformation: growth pressure of dental arch results in buckling of the enamel organ; the invagination results from a focal failure of growth of the internal enamel epithelium while the surrounding normal epithelium continues to proliferate and engulfs the static area; the invagination is a result of a rapid and aggressive proliferation of a part of the internal enamel epithelium invading the dental papilla; Oehlers[8] considered that distortion of the enamel organ during tooth development and subsequent protrusion of a part of the enamel organ will lead to the formation of an enamel-lined channel ending at the cingulum or occasionally at the incisal tip; the latest might be associated with irregular crown form; a fusion of two tooth-germs; infection; traumatic dental injury; genetic factors. Alani and Bishop[7] reported recently that the exact aetiology of dens invaginatus is unknown although a genetic cause is probably the most likely factor.

In many cases a dens invaginatus is detected by routine radiograph examination, and it may be easily overlooked because of the absence of any significant clinical signal of the anomaly. This is unfortunate as the presence of an invagination is considered to increase the risk of caries, pulpal pathosis and periodontal inflammation.[2-6]

Cone beam computed tomography (CBCT) has permitted lately the third dimension into dentistry, being a benefit to all the areas of dentistry, up to this time had not used the advantages of medical CT, due to lack of specificity. CBCT is an important tool in diagnostic, with non-destructive and non-invasive characteristics,[9,10] and this diagnosis tool allows visualization of a three-dimensional image, in which a new plane has been added: depth. Its clinical application allows high accuracy and is directed towards nearly.[11-15]

This article discusses a case report in which the 2-D radiography shows a standard aspect of type 2 dens invaginatus in peg shaped lateral incisor that in an initial moment seems to be possible an endodontic treatment. The 3-D images had resulted in additional information which had not been previously seen with the commonly used 2-D radiography.

Case Report

A 20-year-old man was referred to the clinical service of Faculty of Dentistry of Federal University of Goiás, in order to assess and clarify an oral health problem, and sporadic discomfort during mastication. The medical history was negative for concomitant disease, and it was not contributory. Clinical examination revealed presence of periodontal inflammation. There was no spontaneous symptom or edema in the teeth, but it was detected a large mobility in maxillary left lateral incisor. Vitality pulp test showed the dental pulp to be nonvital. Periapical radiographic revealed a type 2 dens invaginatus, associated with periapical radiolucency. Considering the discomfort of patient, periodontal, orthodontics and endodontics problems, it was suggested to perform CBCT imaging with i-CAT Cone Beam 3D imaging system (Imaging Sciences International, Hatfield, PA, USA). The volumes were reconstructed with isotropic-isometric voxels measuring 0.20 mm - 0.20 mm - 0.20 mm. The tube voltage was 120 kVp and the tube current 3.8 mA. Exposure time was 40 seconds. Images were examined with the scanner's proprietary software (Xoran version 3.1.62; Xoran Technologies, Ann Arbor, MI, USA) in a PC workstation running Microsoft Windows XP professional SP-2 (Microsoft Corp, Redmond, WA, USA), with processor Intel® CoreTM 2 Duo-6300 1.86 Ghz (Intel Corporation, USA), NVIDIA GeForce 6200 turbo cache videocard (NVIDIA Corporation, USA) and Monitor EIZO - Flexscan S2000, resolution 1600x1200 pixels (EIZO NANAO Corporation Hakusan, Japan). The maxillary left lateral incisor was focused and scans were obtained in different planes (sagittal, coronal and axial) of 0.2 mm thickness.

In sagittal and axial CBCT images it may be observed the presence of dens invaginatus type 2 of Oehlers, suggesting clearly infolding of the enamel and dentine. Periapical radiolucency with presence of the periapical bone cortical destruction was detected in palatal surface, and loss of buccal bone cortical until apical third could be also visible (Fig 1). Axial CBCT images in apical, middle and coronal thirds from dens invaginatus showed a central position into the tooth (Fig 1). Note apical, palatal and buccal bone cortical destruction in CBCT images reconstructions (Fig 2).

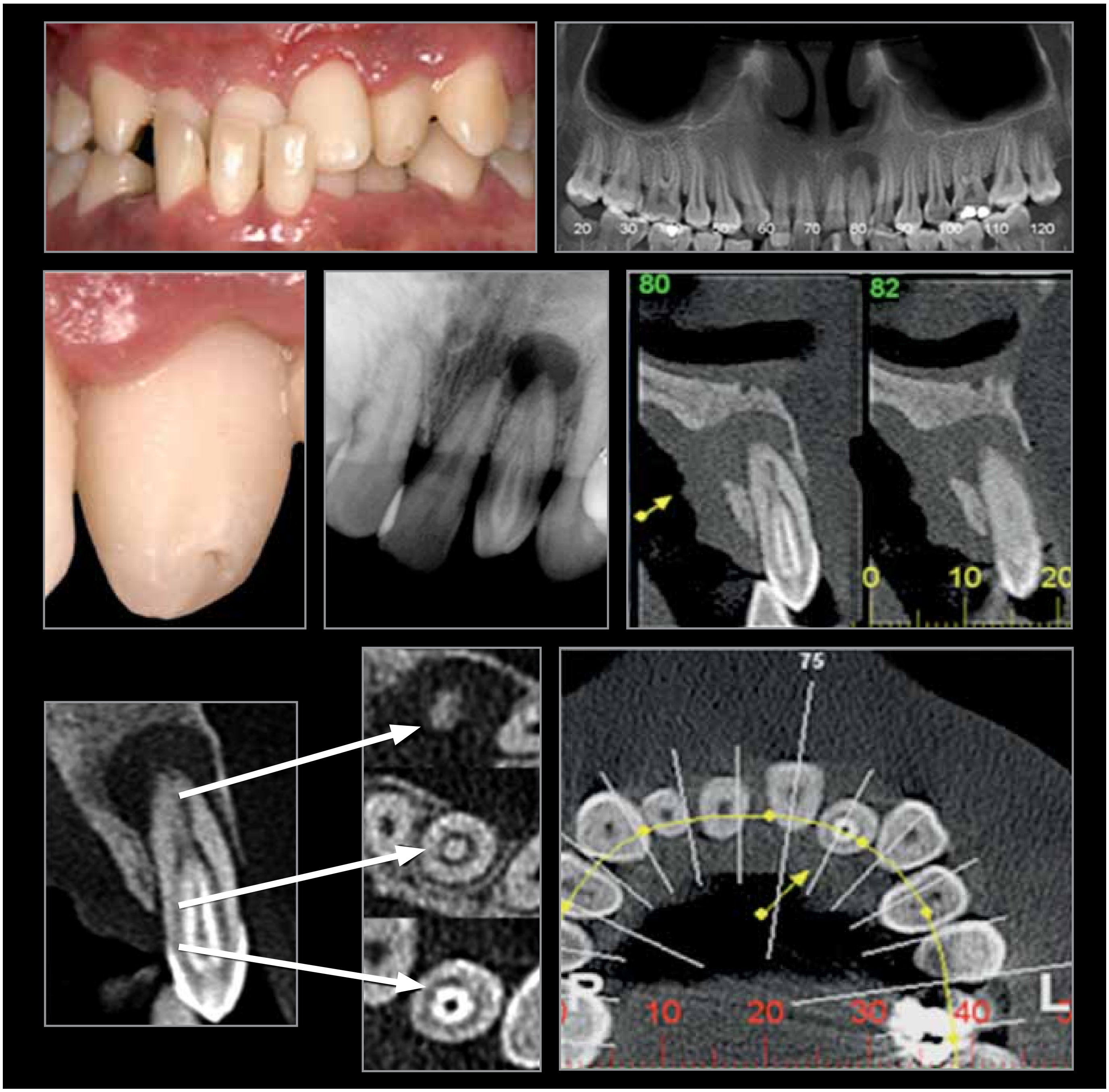

Figure 1. In sagittal and axial CBCT views it may be observed the presence of dens invaginatus type 2 of Oehlers. Periapical radiolucency with presence of the periapical bone cortical destruction was detected in palatal surface, and loss of buccal bone cortical until apical third can be also visible.

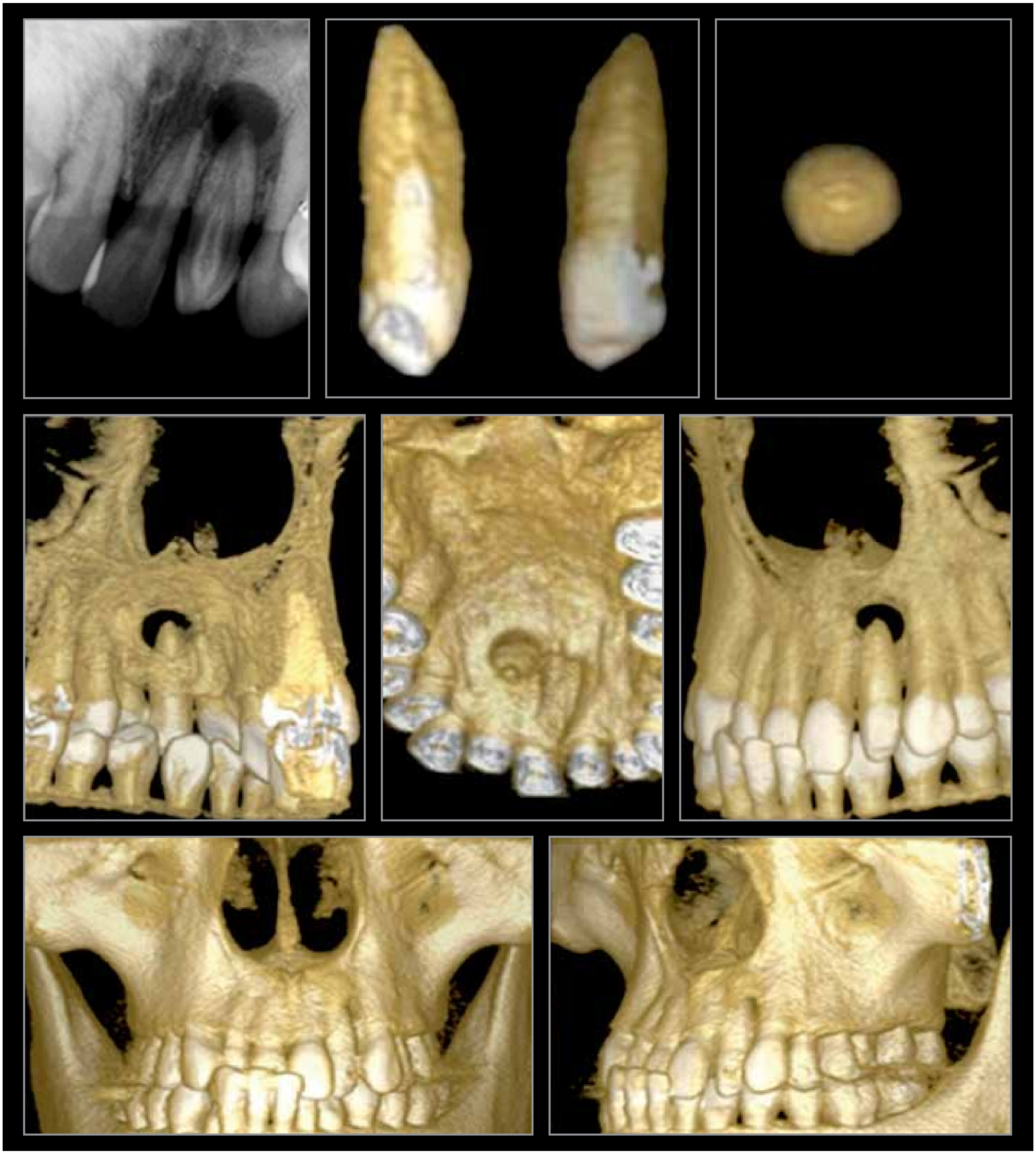

Figure 2. CBCT images reconstructions. Note apical, palatal and buccal bone cortical destruction.

Discussion

In many cases a dens invaginatus is detected by radiograph examination. Clinically, unusual crown morphology (peg shaped) or a deep foramen coecun may be important to indicate the probability of a dens invaginatus.[2] As the maxillary incisors are the most susceptible teeth to present dens invaginatus and these teeth should be investigated thorough clinical and radiographic exams. If one tooth is affected in a patient, the contra lateral tooth should be investigated. It is necessary to acquire radiographs for the maxillary lateral incisors with peg shaped, because Siqueira et al[5] found that 10 per cent of these teeth may be associated with dens invaginatus.

The most commonly used classification was proposed by Oehlers:[8] dens invaginatus Type I - an enamel-lined minor form occurring within the confines of the crown not extending beyond the amelocemental junction; dens invaginatus Type II - an enamel-lined form which invades the root but remains confined as a blind sac (it may or may not communicate with dental pulp); dens invaginatus Type III – a form which penetrates through the root perforating the apical area showing a "second foramen" in the apical or periodontal area. There is no immediate communication with the pulp. The invagination may be completely lined by enamel and frequently cementum will be found lining the invagination.

As pulpal involvement of teeth with coronal invaginations may occur a short time after tooth eruption, the early diagnosis is very important to instigated preventive treatment. The Table 1 describes the summary of treatment of different type of dens invaginatus observed in dental literature.[4,6] Ridell et al[16] evaluated the prognosis for pulp survival in the teeth with dens invaginatus subjected to prophylactic invagination treatment. The dental records of all patients referred to the Eastman Dental Institute, Stockolm, Sweed, with diagnosis of dens invaginatus between the years 1969-1997 were reviewed. Five teeth in 66 patients had been subjected to prophylactic invagination treatment. The retrospective evaluation was based on an examination of the radiographs available from the follow-up. They founded: (a) patients with 1 tooth affected (64.8%); with 2 teeth (29.7%), with 3 teeth (2.2%) and with 4 teeth (3.3%); (b) teeth with dens invaginatus - maxillary central incisors 13%, maxillary lateral incisors 85.5%, maxillary pre-molar 0.8% and maxillary molars 0.8%; (c) dens invaginatus founded according to the Oehlers' classification:[8] Type I (15.35%); Type II (79.4%) and Type III (5.3%). After prophylactic invagination treatment they observed 71% of success and a 9% of failure in an observation period of 6-128 months.

Table 1. Summary of treatment of different type of dens invaginatus.

Dens Invaginatus	Characteristics	Treatment observed in dental literature
Type I (Oehler, 1957)	An enamel invagination is confined within the crown before enamelcemental junct	Prevention and clinical and radiograph control; Application of sealant in invagination; Restoration of teeth
Type II (Oehler, 1957)	The invagination extends to the amelocemental junction and may or may not present a communication with dental pulp	Restoration of invagination if dental pulp is normal; Endodontic therapy; Combined endodontic-surgical treatment
Type III (Oehler, 1957)	The enamel-lined invagination penetrates the entire root usually without a communication with dental pulp	Endodontic therapy; Surgery therapy; Combined endodontic-surgical treatment; Extraction

Hamasha and Alomari,[17] in Jordania, collected 3024 radiographs from a random sample of 1660 patients showing 9377 teeth. A tooth was considered as having dens invaginatus if an infolding of a radiopaque ribbon-like structure equal in density to enamel was seen extending from the cingulum into the root canal. The teeth with dens invaginatus were found in 49 subjects out of 1660 subjects examined. The prevalence was 2.95%. Bilateral dens invaginatus was seen in 12 patients, whereas unilateral dens invaginatus was demonstrated in 37 patients. Maxillary lateral incisor was the most common tooth affected with this condition, which represented 90% of cases.

The introduction of CBCT brings the revolution of information in health area, which have contributed in planning, diagnosis, therapeutic and prognosis of several dental alterations.[9-15] Radiographic image corresponds to a two-dimensional aspect of a three-dimensional structure, which had a potential to bring errors of interpretation.[18] The planning, diagnosis and prognosis of endodontic therapy involve the interpretation of images. New methods using CBCT scans to investigate apical periodontitis and root resorption and a new tool to use in several research areas are suggested.[12-16] In two articles recently published, the authors describe the use of CT[7] and CBCT[17] in the management of the dens invaginatus. Patel[19] reported an interesting case with chronic periradicular periodontitis associated with an infected invagination in an immature mandibular lateral incisor tooth. CBCT images showed absence of communication between the invagination and the main root canal. The endodontic treatment was carried out on the invagination and the root canal with a vital pulp was left untreated, thus allowing the tooth to mature and to continue its development.

CBCT allows visualization of a three dimensional image, in which a new plane has been added: depth. Its clinical application allows high accuracy and is directed towards nearly all the areas of dentistry — surgery, implant, dentistry, orthodontics, endodontics, periodontics, temporomandibular dysfunction, image diagnosis, etc. The real view of the association of these indicators with the clinical aspects projects a fourth dimension, marked by the requirement of time and space.[20]

In the present case report, the real periapical bone cortical destruction was detected in palatal surface, and the loss of buccal bone cortical until apical third can also be visible (Figs 1, 2 and 3). These aspects were not visualized on 2-D initial periapical radiography. In function of periodontal conditions presented (high mobility, big bone loss in buccal, distal and palatal sides), and the necessity of extensive restorative treatment, the option of treatment was the extraction of this tooth and oral rehabilitation.

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— To be submitted, all manuscripts must be original and not published or submitted for publication elsewhere. Manuscripts are assessed by the editor and consultants and are subject to editorial review. Authors must follow the guidelines below.

— All articles must be written in English.

GUIDELINES FOR SUBMISSION OF MANUSCRIPTS

— Manuscritps must be submitted via www.dentalpressjournals.com.br/rdpendo. Articles must be organized as described below.

1. Title Page
— Must comprise the title in English, an abstract and keywords.
— Information about the authors must be provided on a separate page, including authors' full names, academic degrees, institutional affiliations and administrative positions. Furthermore, the corresponding author's name, address, phone numbers and e-mail must be provided. This information is not made available to the reviewers.

2. Abstract
— Preference is given to structured abstracts in English with 250 words or less.
— The structured abstracts must contain the following sections: INTRODUCTION: outlining the objectives of the study; METHODS, describing how the study was conducted; RESULTS, describing the primary results, and CONCLUSIONS, reporting the authors' conclusions based on the results, as well as the clinical implications.
— Abstracts in English must be accompanied by 3 to 5 keywords, or descriptors, which must comply with MeSH.

3. Text
— The text must be organized in the following sections: Introduction, Materials and Methods, Results, Discussion, Conclusions, References and Figure legends.
— Texts must contain no more than 4,000 words, including captions, abstract.
— Figures and tables must be submitted in separate files (see below).
— Insert the Figure legends also in the text document to help with the article layout.

4. Figures
— Digital images must be in JPG or TIF, CMYK or grayscale, at least 7 cm wide and 300 dpi resolution.
— Images must be submitted in separate files.
— In the event that a given illustration has been published previously, the legend must give full credit to the original source.
— The author(s) must ascertain that all figures are cited in the text.

5. Graphs
— Files containing the original versions of graphs must be submitted.
— It is not recommended that such graphs be submitted only in bitmap image format (not editable).
— Drawings may be improved or redesigned by the journal's production department at the discretion of the Editorial Board.

6. Tables
— Tables must be self-explanatory and should supplement, not duplicate the text.
— Must be numbered with Arabic numerals in the order they are mentioned in the text.
— A brief title must be provided for each table.
— In the event that a table has been published previously, a footnote must be included giving credit to the original source.

— Tables must be submitted as text files (Word or Excel, for example) and not in graphic format (non-editable image).

7. Copyright Assignment

— All manuscripts must be accompanied by the following written statement signed by the main author: "Once the article is published, the undersigned author hereby assign all copyright of the manuscript [insert article title here] to Dental Press International. The undersigned author warrant that this is an original article and that it does not infringe any copyright or other third-party proprietary rights, it is not under consideration for publication by another journal and has not been published previously, be it in print or electronically. I hereby sign this statement and accept full responsibility for the publication of the aforesaid article."

— This copyright assignment document must be scanned or otherwise digitized and submitted through the website, along with the article.

8. Ethics Committees

— Articles must, where appropriate, refer to opinions of the Ethics Committees.

9. References

— All articles cited in the text must appear in the reference list.

— All listed references must be cited in the text.

— For the convenience of readers, references must be cited in the text by their numbers only.

— References must be identified in the text by superscript Arabic numerals and numbered in the order they are mentioned in the text.

— Journal title abbreviations must comply with the standards of the "Index Medicus" and "Index to Dental Literature" publications.

— Authors are responsible for reference accuracy, which must include all information necessary for their identification.

— References must be listed at the end of the text and conform to the Vancouver Standards (http://www.nlm.nih.gov/bsd/uniform_requirements.html).

— The limit of 30 references must not be exceeded.

— The following examples should be used:

Articles with one to six authors

Vier FV, Figueiredo JAP. Prevalence of different periapical lesions associated with human teeth and their correlation with the presence and extension of apical external root resorption. Int Endod J 2002;35:710-9.

Articles with more than six authors

De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, et al. A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res. 2005 Feb;84(2):118-32.

Book chapter

Nair PNR. Biology and pathology of apical periodontitis. In: Estrela C. Endodontic science. São Paulo: Artes Médicas; 2009. v.1. p.285-348.

Book chapter with editor

Breedlove GK, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services; 2001.

Dissertation, thesis and final term paper

Debelian GJ. Bacteremia and fungemia in patients undergoing endodontic therapy. [Thesis]. Oslo - Norway: University of Oslo, 1997.

Digital format

Oliveira DD, Oliveira BF, Soares RV. Alveolar corticotomies in orthodontics: Indications and effects on tooth movement. Dental Press J Orthod. 2010 Jul-Aug;15(4):144-57. [Access 2008 Jun 12]. Available from: www.scielo.br/pdf/dpjo/v15n4/en_19.pdf

1. Registration of clinical trials

Clinical trials are among the best evidence for clinical decision making. To be considered a clinical trial a research project must involve patients and be prospective. Such patients must be subjected to clinical or drug intervention with the purpose of comparing cause and effect between the groups under study and, potentially, the intervention should somehow exert an impact on the health of those involved.

According to the World Health Organization (WHO), clinical trials and randomized controlled clinical trials should be reported and registered in advance.

Registration of these trials has been proposed in order to (a) identify all clinical trials underway and their results since not all are published in scientific journals; (b) preserve the health of individuals who join the study as patients and (c) boost communication and cooperation between research institutions and with other stakeholders from society at large interested in a particular subject. Additionally, registration helps to expose the gaps in existing knowledge in different areas as well as disclose the trends and experts in a given field of study.

In acknowledging the importance of these initiatives and so that Latin American and Caribbean journals may comply with international recommendations and standards, BIREME recommends that the editors of scientific health journals indexed in the Scientific Electronic Library Online (SciELO) and LILACS (Latin American and Caribbean Center on Health Sciences) make public these requirements and their context. Similarly to MEDLINE, specific fields have been included in LILACS and SciELO for clinical trial registration numbers of articles published in health journals.

At the same time, the International Committee of Medical Journal Editors (ICMJE) has suggested that editors of scientific journals require authors to produce a registration number at the time of paper submission. Registration of clinical trials can be performed in one of the Clinical Trial Registers validated by WHO and ICMJE, whose addresses are available at the ICMJE website. To be validated, the Clinical Trial Registers must follow a set of criteria established by WHO.

2. Portal for promoting and registering clinical trials

With the purpose of providing greater visibility to validated Clinical Trial Registers, WHO launched its Clinical Trial Search Portal (http://www.who.int/ictrp/network/en/index.html), an interface that allows simultaneous searches in a number of databases. Searches on this portal can be carried out by entering words, clinical trial titles or identification number. The results show all the existing clinical trials at different stages of implementation with links to their full description in the respective Primary Clinical Trials Register.

The quality of the information available on this portal is guaranteed by the producers of the Clinical Trial Registers that form part of the network recently established by WHO, i.e., WHO Network of Collaborating Clinical Trial Registers. This network will enable interaction between the producers of the Clinical Trial Registers to define best practices and quality control. Primary registration of clinical trials can be performed at the following websites: www.actr.org.au (Australian Clinical Trials Registry), www.clinicaltrials.gov and http://isrctn.org (International Standard Randomized Controlled Trial Number Register (ISRCTN). The creation of national registers is underway and, as far as possible, the registered clinical trials will be forwarded to those recommended by WHO.

WHO proposes that as a minimum requirement the following information be registered for each trial. A unique identification number, date of trial registration, secondary identities, sources of funding and material support, the main sponsor, other sponsors, contact for public queries, contact for scientific queries, public title of the study, scientific title, countries of recruitment, health problems studied, interventions, inclusion and exclusion criteria, study type, date of the first volunteer recruitment, sample size goal, recruitment status and primary and secondary result measurements.

Currently, the Network of Collaborating Registers is organized in three categories:

- Primary Registers: Comply with the minimum requirements and contribute to the portal;
- Partner Registers: Comply with the minimum requirements but forward their data to the Portal only through a partnership with one of the Primary Registers;
- Potential Registers: Currently under validation by the Portal's Secretariat; do not as yet contribute to the Portal.

3. Dental Press Endodontics - Statement and Notice

DENTAL PRESS ENDODONTICS endorses the policies for clinical trial registration enforced by the World Health Organization - WHO (http://www.who.int/ictrp/en/) and the International Committee of Medical Journal Editors - ICMJE (# http://www.wame.org/wamestmt.htm#trialreg and http://www.icmje.org/clin_trialup.htm), recognizing the importance of these initiatives for the registration and international dissemination of information on international clinical trials on an open access basis. Thus, following the guidelines laid down by BIREME / PAHO / WHO for indexing journals in LILACS and SciELO, DENTAL PRESS ENDODONTICS will only accept for publication articles on clinical research that have received an identification number from one of the Clinical Trial Registers, validated according to the criteria established by WHO and ICMJE, whose addresses are available at the ICMJE website http://www.icmje.org/faq.pdf. The identification number must be informed at the end of the abstract.

Consequently, authors are hereby recommended to register their clinical trials prior to trial implementation.

Yours sincerely,

Carlos Estrela
Editor-in-Chief of Dental Press Endodontics
ISSN 2178-3713

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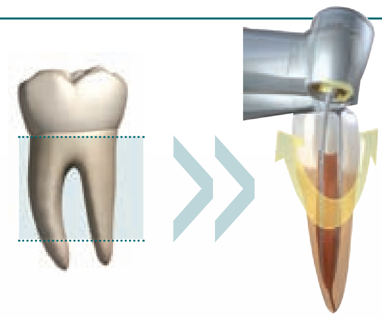

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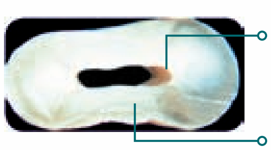

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