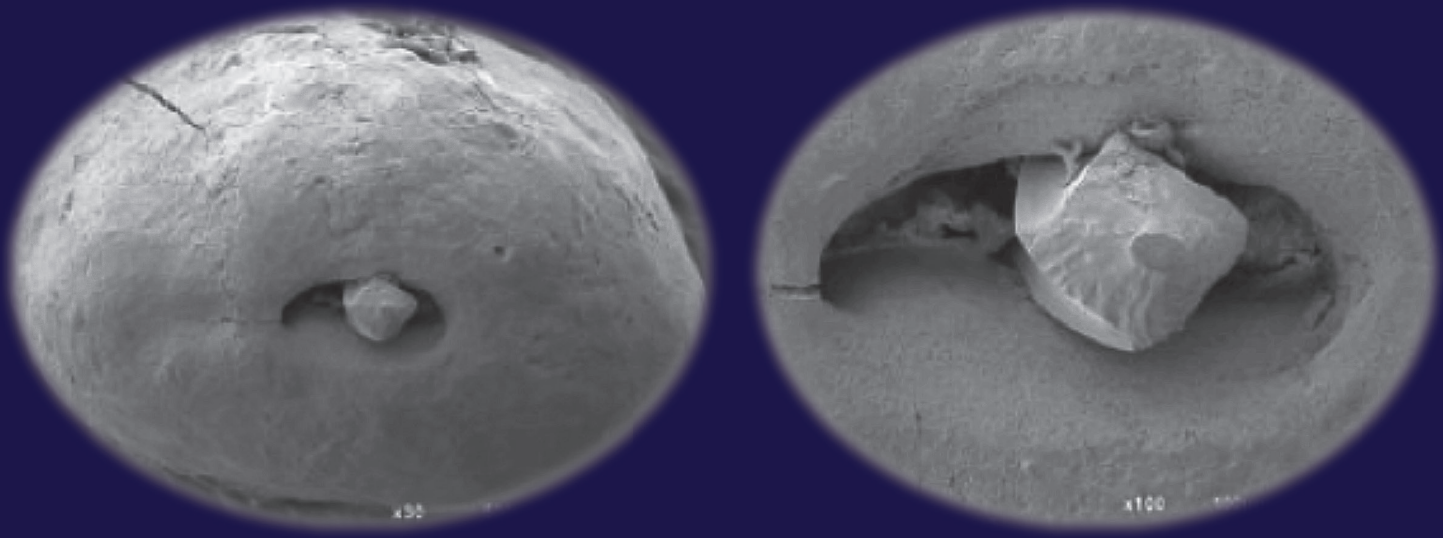
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Care in the development of a scientific text

Endodontics journals, in every continent, have received an extraordinary amount of research developed by Brazilians. This international visibility and credibility have often been highlighted, as in a recent editorial published in Dental Traumatology (*Impressive research development in dental traumatology from Brazil*. Dent Traumatol. Aug 2012, 28[4]:255), by its editor-in-chief, Prof. Dr. Lars Andersson. Such an important comment like this reinforces the need for, each time more, enhancing and improving studies developed here.

The search for new therapeutic strategies able to promote diagnosis and treatment alternatives justifies this researches. Starting from a well elaborated project, a clear and relevant hypothesis, a pilot study able to minimize future problems and that indicates the validity and vulnerabilities of the study, the experiment is developed. After obtaining the results, the transcription phase is set, in a way it is well understood, with adequate and legible scientific and literary structure.

Among cares deserving to be remembered, there are: A title describing directly the study content; an introduction covering important information about the topic and possible innovations; a clear hypothesis that evidences a good problem to be solved; a methodology described in details, so that it can be reproduced; a data analysis with appropriate statistical treatment; a logical, organized and informative presentation of the results, avoiding duplication of pictures, graphs and tables; a discussion of methods and results with rich and precise correlations; a clear conclusion seeking to answer the problem formulated. References and scientific text should be strictly in accordance with the standards of the journal. Once the text is ready, it must be read, reread and corrected, as for the scientific aspects and grammar, prior to submission.

An article with good readership scores, besides being methodologically well-structured should bring contributions to the health process.

Carlos Estrela
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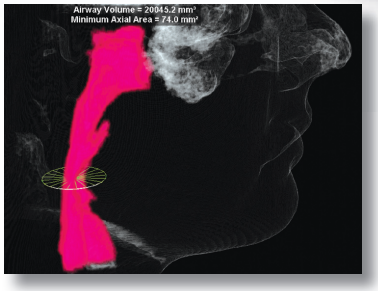
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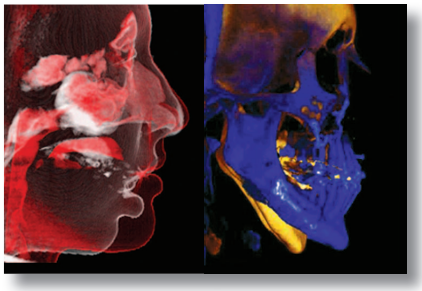
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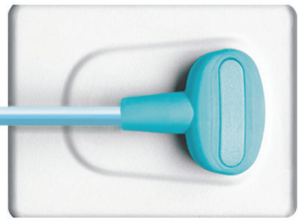
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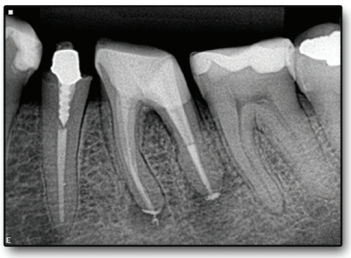


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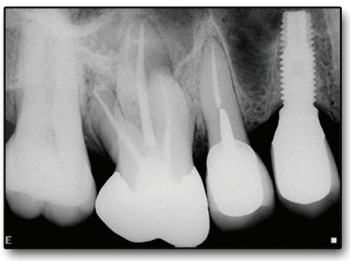
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Bone reaction capability and the names of inflammatory bone disorders in endodontic clinical practice

Alberto **CONSOLARO**[1]
Renata Bianco **CONSOLARO**[2]

ABSTRACT

Reactional inflammatory bone diseases are common in the jaws and are associated with periapical lesions. A chronic dentoalveolar abscess represents a chronic purulent osteitis, just like periapical granuloma is a chronic granulomatous osteitis. Imagiologically, chronic inflammatory periapical injuries are osteitis which manifest themselves either as bone rarefactions, either as sclerotic areas. The terms "rarefying diffuse lesion" or "sclerosing at the periapex" are used in reports to identify chronic inflammatory periapical lesions that represent true reactive inflammatory bone diseases with specific names by the direct relationship with the teeth as dentoalveolar abscess and periapical granulomas. When teeth are extracted they can leave imagiologically detected structural changes, such as bone sclerosis and rarefactions, without the possibility of establishing a cause and effect relationship, making it hard to provide a secure diagnosis. In planning, a previous diagnosis of bone status implies recognizing injuries and pathological situations. The standardization of nomenclature and concepts can facilitate communication and the establishment of uniform protocols and behaviors.

Keywords: Osteitis. Osteomyelitis. Periostitis. Osteonecrosis. Periapical diseases.

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A normal bone: understanding the structure and functions

The bone may be analyzed as an anatomical piece or as an organ that forms blood cells, due to the bone marrow, but it can be studied as a tissue with specific functions and structures, including cells.

To meet the functional requirements related to movement and protection, as well as the mechanisms of ionic balance of the organism, at every moment storing and repositioning many mineral components in blood plasma, human skeleton completely renews itself every period of 2 to 4 years in children, and from 4 to 10 years in adults.

Bones present soft and hard tissues. At the mineralized part of the bone, such as corticals and trabeculae, are included osteoblasts, osteocytes and osteoclasts. The periosteum, endosteum and bone marrow are not mineralized and they fill the spaces delimited by the mineralized parts.

The periosteum, a real natural membrane, is composed of a fibrous connective tissue densely collagenized in its external part, while its inner part is intersected by collagen fibers that penetrate and merge with the cortical mineralized bone matrix, fixing it firmly to the external bone surfaces. Inside it, at the interface with the cortical bone, the periosteum presents itself richly cellularized with abundant osteoblasts and clasts as well as by young cells, undifferentiated and even bone tissue stem cells or reserve cells. Every bone blood supply passes through, necessarily, the periosteum.

In turn, the endosteum, a very fragile membrane tissue, covers the surface of the trabeculae and inner parts of corticals. Also consists of tissue with few collagen fibers and rich in osteoblastic reserve cells, covering cells and/or osteoprogenitor cells, also bone tissue stem cells. The endosteum, while covering the bone trabeculae, continues naturally with the fibrous or adipose hematopoietic tissue, filling the marrow spaces.

The bone lodges in its structure the hematopoietically active bone marrow when it is red; or inactive, when replaced by fibrous or adipose tissue and then presents itself yellow or grayish white.

Osteoblasts, osteocytes and osteoclasts are mature bone cells which, in line with other components, such as macrophages, promote bone remodeling and at the same time contribute to fulfill its functions in this tissue. In the process of bone remodeling, besides the cells there are three key enzymes which serve as parameter to measure metabolic activity in the human skeleton: acid phosphatase, located on osteoclasts, which also release collagenase, both involved in bone resorption; on the other hand, there is the alkaline phosphatase localized in osteoblasts, related to bone formation.

Adaptive and reactive capacity of bone tissue: understanding sclerosis and rarefactions in periapical trabeculate!

Bone adaptations to situations and functions represent its reactive capability and, when extrapolated, it originates a group of bone diseases named reactional or inflammatory, which are clinically very important in clinical practice, due to:

1. Their frequency.
2. The sequelae resulting from their occurrence.
3. The possibility of resulting from unnoticed important dental changes.
4. The possibility of deriving from professional interventions such as bone surgeries, inappropriate treatments and lack of precise identification of patient's organic deficiency in the medical history and systemic review.

Bone reactive capability, resistance to stimuli or aggressions depends on the following three key factors:

1) Local bone morphology

A more compact or dense cancellous bone presents small marrow spaces that provide little room for the more abundant inflammatory exudates. In a very short time, inflammation may increase the pressure within the small medullary spaces, compressing vessels, complicating venous return and leading more rapidly the medullary tissue to necrosis. A bone marrow tissue area with necrosis can be the ideal place for bacteria to lodge and build microbial biofilms.

Bone tissue with more sparse or loosely distributed trabeculae, facing aggression, provide more spaces for infiltration and inflammatory exudate, allowing a longer time, which increases its defensive ability to eliminate the aggressors from the site.

It can be said that the more compact bone tissue is much stronger physically, but biologically fragile, for it requires a very fast and extremely efficient functioning of the inflammatory process. The contrary also occurs with less compact and more cancellous bone: it gives longer time and space for the inflammation tools — the exudate and the infiltrate — to act against the aggressors.

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2) Aggression intensity and duration

A mild and constant irritation, sometimes referred to as chronic, like all aggressors, promotes an initial acute inflammation, but soon it evolves into a mild or moderate chronic phase, with a restricted accumulation of mediators at the site.

Many mediators of the inflammatory exudate are inducers of bone resorption, but present bipolar effects: when in high concentration, induce predominantly clastic activity, but when at low levels in the same environment bone, induce synthesis of osteoblastic activity with new bone formation, predominating in trabecular and cortical surfaces.

The mediators that induce new bone formation on trabecular and subperiosteal surfaces gradually change the local bone morphology, that remains organized. As the irritation increases its harmful power, the neoformation reaction may also occur, but in a not so organized way.

The rapid and intense irritation, also referred to as acute, promotes an initial acute inflammation, but much more exudative and rich in mediators to induce bone resorption and it may induce necrotic areas on bone marrow and endosteal tissue, and osteocytes.

It can be said that chronic or mild irritation induces bone formation or productive reactions predominant of synthesis phenomena, whereas acute or severe aggressions cause bone resorptive, osteolytic or destructive reactions.

3) Host systemic state

The host systemic state may be determinative in bone reactions against aggressions. Overall, osteomyelitis occurs only in systemically compromised patients or with extensive sclerotic local bone diseases.

The three most common causes of osteomyelitis are:

a) Trauma with open fractures.
b) Bone surgery in contaminated environments.
c) Bone surroundings with infectious foci in other tissues, such as in infected channels.

In the jaws, these three situations occur in thousands of people in daily dental practice and the number of osteomyelitis does not stand out compared to other skeletal structures.

When the patient is systemically normal, the same causes that would induce osteomyelitis will promote osteitis, also an inflammatory process but localized and focal, with less consequences, because the osteolytic areas are restricted and small, predominating areas with bone sclerosis, and the symptomatology is very low. The prognosis of osteitis is very good.

Reactional or inflammatory bone diseases: name and concepts applied to periapical and periodontal areas

The reactional inflammatory bone diseases may represent the exhaustion of the adaptive capability of the bone against external or internal aggressors. The terminology / nomenclature used to identify reactional and inflammatory bone diseases is very important to standardize diagnostic procedures, treatment protocols and monitoring, as well as to understand imagiologic reports and studies on the subject.

Before conceptualizing each of the names applied to identify them, it is very important to distinguish the terms “disease” and “lesion,” since they have a lot in common, but different meanings.

The diseases, or clinical entities, are changes and biological processes outside the structural and functional normality of tissue and organs. Diseases, or clinical entities, represent specific and repetitive signs which allow identification or diagnosis by any qualified professional. Diseases induce transient or permanent structural changes in anatomical tissues and organs, changes called lesions. Lesion means any anatomical alteration of any kind, in tissues or organs, i.e., it represents a term with broad and daily use.

The reactional and inflammatory bone diseases may be denominated according to their diagnosis:

» Osteomyelitis: Characterized by bone inflammation with sudden and symptomatic onset, that may involve the three structural components — the mineralized part, tissue components of the medullary spaces and periosteum.[2,7,8,10,15] Its origin is predominantly microbial, but may be physical and chemical, such as substances leaked to the periapex via channel during irrigation procedures. Generally, the bone area affected is broad and diffuse, with predominantly osteodestructive phenomena.

Osteomyelitis rarely occurs in systemically healthy patients. Almost all cases have an underlying disease such as diabetes mellitus, immunosuppression, anemia, among others; or even the patient presents at the site

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of osteomyelitis a sclerosing advanced disease such as florid cemento-osseous dysplasia, for example. In endodontics, periapical inflammatory processes rarely evolve into osteomyelitis, for systemic conditions of patients generally are not weakened or so compromised to significantly reduce its defensive capabilities.

» Osteitis: Characterized by an usually asymptomatic inflammatory process that slowly may also involve up to the three structural components of bone, but, in general, is a localized and predominantly osteoproductive phenomena.[1,5,6,9,11,12,14,17] In general, the causes of osteitis have low intensity and long duration.

These diseases are common in periapex, especially when the root canal is infected. Acute or chronic periapical lesions, when considered only bone structures, are perfect examples of osteitis, but when involving tooth roots they receive specific names, such as dentoalveolar abscesses and periapical granulomas.

In long term occlusal trauma, it is not difficult to observe in periapex and/or in lateral alveolar bone and in the bone crest itself, imagiological findings indicating chronic and sclerosing focal osteitis. Not only microbial causes can induce osteitis, occlusal trauma represents an etiology of physical nature.

» Periostitis: Characterized by the inflammatory response of periosteum against aggressive agents that act directly or indirectly on its structures. When the cause is acute or intense and acts directly, it can be destructive and part of other broader processes such as abscess and dentoalveolar osteomyelitis.[4,13,16,18,19,20] However, when the cause is chronic, with low-intensity and long duration on its structures, the periosteum involved reacts producing new bone layers on cortical surface, and it is known as periostitis ossificans or productive, once mistakenly named as Garrè's osteomyelitis.

In children, adolescents and young adults with long-term chronic periapical lesions, or chronic pericoronitis, the periostitis ossificans explains the hardened swellings on the corresponding cortical surface and which promote facial asymmetries. After treatment, these "swellings" may disappear over a few weeks or months without any "drainage" or corrective surgery. Imagiologicaly, they are evidenced only with underexposure and occlusal views due to the low degree of organization and mineralization of the reactive bone deposited on cortical surface.

» Osteoradionecrosis and osteoradiomyelitis: It can be considered a specific or special variant of osteomyelitis in areas of osteonecrosis by radiation, usually for therapeutic purposes in oncology.[3,10] Based on animal studies, osteoradionecrosis can be considered an state in which the irradiated bone presents itself for some years with:

a) Chronic hypoxia, promoted by obliterative endarteritis obstructing the passage of blood to the cells, because part of the vascular lumens is occupied by the increased thickness of the inner wall of blood vessels.
b) Hypovascularization, since the entire cell population in this site is decreased, including endothelial cells, which form the internal lining of blood vessels.

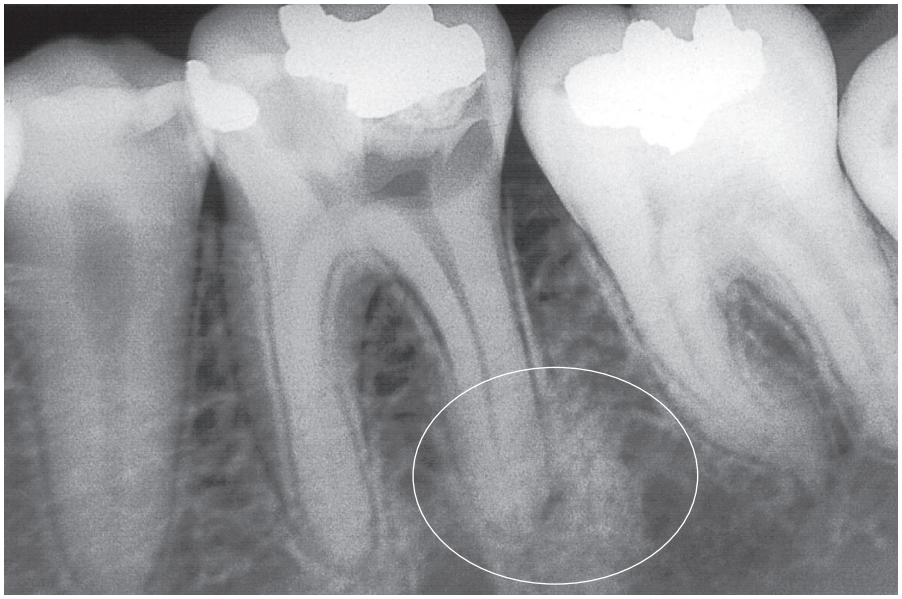


Figure 1. In periapical bone tissue, inflammation corresponds to chronic focal sclerosing osteitis related to necrotic pulp and chronic inflammatory periapical lesions. Radiopaque areas are predominant, corresponding to thickening of trabeculae and reduced marrow spaces. Smaller and irregular rarefying areas immediately around the opening of the root canal in which the low intensity and long duration aggressors come out. From a clinical and dental point of view, these signs can be diagnosed as chronic inflammatory periapical lesions compatible with periapical granuloma. There was no fistula.

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c) Local hypocellularity in the irradiated area, due to small mitotic index of the region, reducing its ability to be reactive and reparatory.
d) Death of osteocytes, a very important cell in bone histophysiology. Each osteocyte connects with other 40-50 cells, forming an intercommunicating network with trabecular and cortical surfaces, thus controlling the bone shape, its subperiosteal responses and also strongly influencing the local and systemic ionic balance.

For a period of 5 to 10 years, the irradiated bone tissue presents a low defensive and reactional capability, and at the same time its reparatory potential decreases. After this period, the irradiated region tends to return to its previous reparatory potential. In these bone conditions, bacteria and other not as aggressive aggressors find local and conditions to proliferate in this weakened bone structure and the inflammatory process becomes insufficient to contain them, thus establishing a secondary acute suppurative osteomyelitis and then chronic, also known as osteoradiomyelitis, although it is a term rarely used in the literature. It seems logical to distinguish that osteoradionecrosis is the state of the irradiated bone and osteoradiomyelitis corresponds to the osteomyelitis or inflammation in that modified bone.

» Osteonecrosis: Increased bone surgery and placement of osseointegrable dental implants widely promoted the use of some terms hitherto little used by clinicians, such as osteonecrosis.

Osteonecrosis can be conceptualized as bone death without infection and induced by many factors such as trauma, excessive heat, thrombi and emboli, radiation, grafts and chemical products.[3,10]

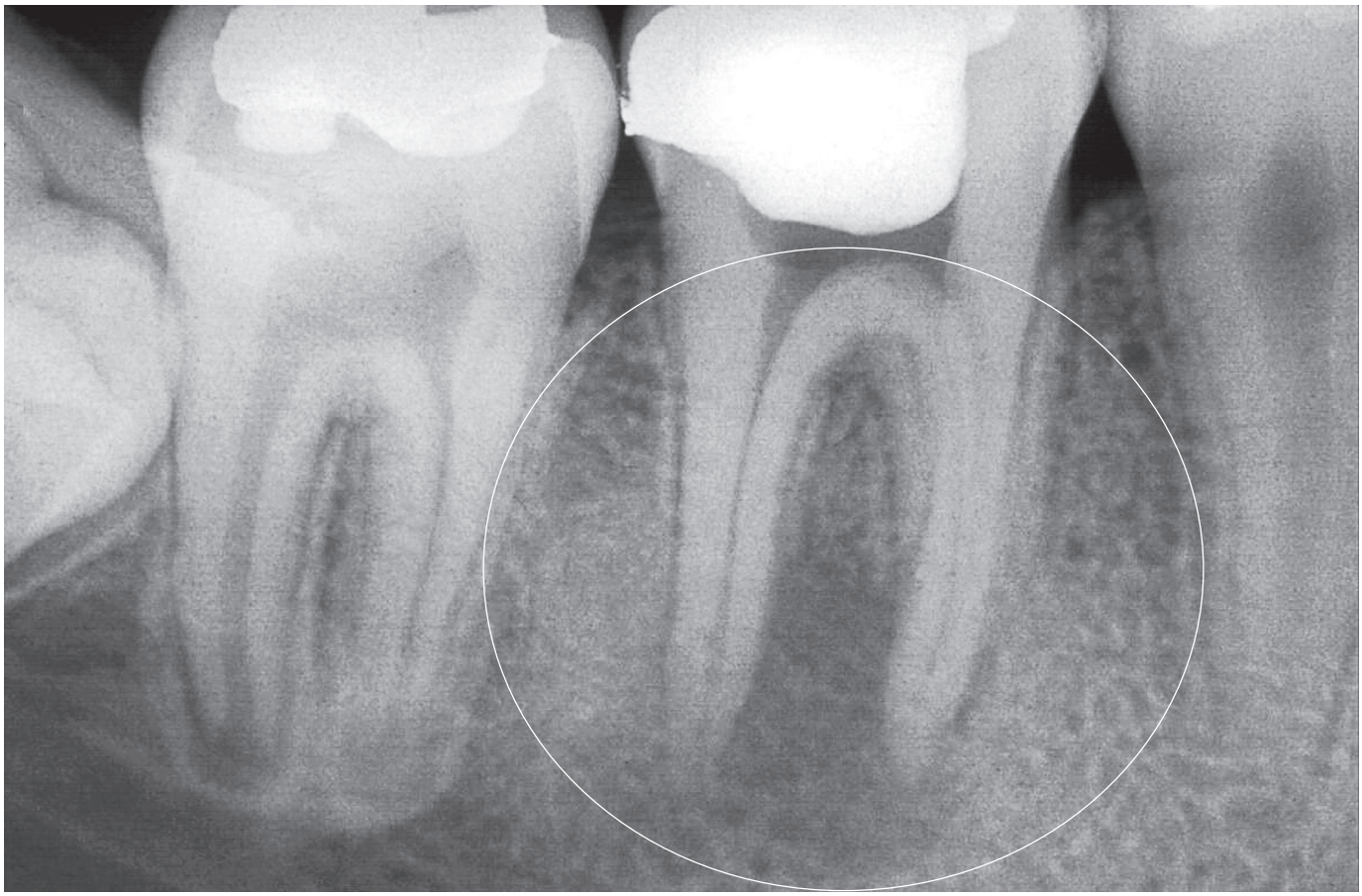


Figure 2. In bone environment, the lesion may be diagnosed as chronic focal rarefying osteitis related to necrotic pulp and chronic inflammatory periapical lesions with imagiologic bone rarefaction predominance with discrete sclerotic peripheral areas. From a clinical and dental point of view, this scenario can be diagnosed as chronic inflammatory periapical lesions compatible with chronic dentoalveolar abscess. The radiolucent areas are more extensive than in the case of Figure 1, and it indicates that the aggressor has a higher pathogenicity, comparatively. Clinically, there was intraoral fistula.

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However, the term "necrosis", conceptually, should only be applied to cells, as it represents cell death in a living organism without any genetic involvement in its occurrence. The bone, as an anatomical organ, is not in necrosis, but is biologically unfeasible as a tissue in the context of our body.

Many agents may act on bone and kill its cells, by necrotizing them, and if this occurs with osteocytes, which are its most internal cells and protected by mineralized matrix, it can be said that the bone became biologically unfeasible. It can be argued that a bone without osteocytes needs to be completely restored, it is with no biological viability, should be resorbed and replaced by new bone tissue rich in osteocytes.

Physical agents may act on bone tissue, such as radiation and excessive heat, and also chemical products. Both types of aggression can lead to osteocyte necrosis and therefore such injured areas can receive the name of osteonecrosis, but not as a clinical condition or disease.

The death of osteocytes and/or an area with osteonecrosis induces on its periphery, and then inside it, an inflammation localized and restricted to a certain area and with low symptomatology. Bone tissue made unviable by osteocytes death has a certain aggressiveness to the surrounding tissues, induces a low symptomatic and restricted inflammatory process in this bone area, i.e., induces an osteitis. In short, osteonecrosis induces and resolves itself after an osteitis.

After a few days of aseptic inflammation at the site, or osteitis in cancellous bone, it evolves to the formation of granulation tissue by migration, proliferation and invasion of endothelial and osteoblastic surrounding cells, permeating the medullary spaces of bone necrosis area. Gradually, the newly formed part replaces the old bone.

In cortical osteonecrotic areas, osteitis occurs almost in its interface, and gradually the clasts resorb and invade the small spaces, and the neighboring osteoblasts will be forming and entering in the osteonecrotic area, merging the old part with the new one, until all necrosed tissue is replaced. The formation of granulation tissue is very small and restricted to external surfaces.

Osteonecrosis, and subsequent osteitis, may delay or prevent bone healing and osseointegration; they are:

a) Osteonecrosis by local hyperthermia: situation where part of the bone in a surgical area loses its viability by dehydration or denaturation, which means loss of water with coagulation of proteins and loss of vitality of cells. The heat can be generated by electric surgical saws and worn cutters while placing implants. This tissue will be reabsorbed by clasts so it can be replaced by normal bone in a repair context. During this period while it is not fully resorbed, it will be infiltrated by poly and mononuclear leukocytes, as well as by clasts. At the local, exudate or inflammatory edema will also be present, characterizing a chronic osteitis induced by heat excess or local hyperthermia. Since the cause has been eliminated with the surgery, the only option is to wait until the body repair the area so it can be reevaluated the possible consequences, if any.
b) Chemical osteonecrosis: chemical products can be poured on bone environment, such as chlorinated soda, used in endodontic irrigation. The acute inflammatory process can dilute the chemical, which will disappear in a few days. The local chronic osteitis, its macrophages, phagocytes, along with clasts, promote cleaning of the area, which gradually repair, unless it is secondarily infected by bacteria. In many cases, this process has been named chemical osteomyelitis, but the process is restricted to the site and without systemic involvement, proving wrong the use of the term osteomyelitis.
c) Drug-induced osteonecrosis: Term used to identify areas of unviable bone believed to be associated with the use of bisphosphonates, especially in cancer patients. These drugs do not kill other osteocytes or bone cells, nor obliterate the vessels and even depress the immune system.

However, cancer patients are systemically debilitated, especially due to the numerous and powerful medicines ingested, as well as by chemotherapy and radiotherapy that they undergo. Patients get immunosuppressed, and their tissues with few vessels and cells. Any microorganism that gets to the bone of this type of patient may induce suppurative osteomyelitis clinical signs, whose evolution leads to fistula formation and bone losses. The ingestion of the bisphosphonate drug represents a superimposition in the situation and not a primary cause of this osteonecrosis, although many surgeons insist on assigning

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such problems in cancer patients to bisphosphonates, which in biological and medical fields is not considered. Patients with cancer have several local and systemic debilitating conditions that increase their susceptibility to osteomyelitis.

Other often used terms in bone biopathology

In the jaws, rarely the systemic metabolic bone diseases alter the morphology of corticals and trabeculate, for the rate or velocity of bone turnover is very low. When affecting the jaws, the disease is highly evolved, or terminal. Long before this stage, other medical problems led the patient to seek for treatment and medical advice. Still, some concepts about skeletal states are important because they are often used or mentioned when describing pathological states of varied nature in the jaws. They are:

1. Osteopenia: Bone condition characterized by thinner and shorter trabeculae and thinner cortex, increasing the susceptibility to fractures. Several systemic metabolic bone diseases may promote this bone state, which is very difficult to happen in the jaws, being more often diagnosed in long bones. It is not a disease but a condition or bone state.
2. Osteoporosis: Bone condition characterized by osteopenic bone with subclinical or clinically diagnosed fractures. It can be argued that osteopenia can progress to osteoporosis, but not necessarily. This term is necessarily related to the presence of fractures in osteopenic bone. Osteoporosis can be classified into primary, when it is post-menopausal, and secondary, when results from endocrine or kidney disorders. Other factors associated with osteoporosis are smoking habit, alcoholism, low intake of calcium and surgical or premature menopause. It is not a disease but a condition or bone state that can be induced by various diseases.
3. Osteomalacia: Bone state characteristic of adults, resulting from a disturbance with inadequate mineralization of newly formed bone matrix. The term osteomalacia means soft bones. The osteopenic osteomalacia has a pattern with fractures or pseudo-fractures. Several diseases can lead to osteomalacia, often related to impaired metabolism of vitamin D.
4. Osteopetrosis: Group of at least nine rare congenital developmental disorders, characterizing a disease induced by an autosomal dominant developmental disorder, also known as stone bone or marble bone disease, which reflects his intense radiopaque appearance. The texture is very hard, like a rock, including the cartilaginous areas. The bone is easily fractured. There is almost no marrow and it is even described the splenomegaly and hepatomegaly with enlarged lymph nodes to compensate for lack of hematopoiesis, besides severe anemia. In the end there is compression of nerves in the foramen, also promoting facial paralysis.
5. Sclerosis or bone condensation: State with increase in thickness and number of bone trabeculae, decreasing the size and appearance of medullary spaces in radiographs and CT scans.

Conclusions

The reactional and inflammatory bone diseases are very common in the jaws and most of them are related or associated directly or indirectly with endodontic and periodontal changes.

The periapical lesions can be direct causes of inflammatory reactional bone lesions or may constitute the osteitis itself. For example, a chronic dentoalveolar abscess is a purulent chronic osteitis, just like a periapical granuloma is a chronic granulomatous osteitis. Imagiologicaly, chronic inflammatory periapical lesions are osteitis manifested as bone rarefactions with random and diffuse associated sclerosing areas, or peripherally, in a more organized way.

Often it is observed, in imagiologic reports, terms as diffuse rarefying lesion in the premolar periapex or the use of names such as chronic rarefying or sclerosing osteitis in the molar periapex. These terms are generally applied in these reports to identify inflammatory chronic periapical lesions that represent true bone inflammatory reaction, with specific names by the direct relationship with the teeth. These specific names would be dentoalveolar abscess and periapical granulomas, for example.

When teeth are extracted, structural changes may be imagiologicaly detected, such as bone sclerosis and rarefactions, with no possibility of establishing a cause and effect relationship, hindering the diagnosis in many cases. Knowing bone biopathology has fundamental importance, since teeth are inserted into the

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bone, moving naturally or orthodontically through the bone, as well as jaws often receive and adapt themselves to dental implants.

In rehabilitation, orthodontic and pre-surgical planning, previous diagnosis of maxillary bone status implies recognizing injuries and pathological situations involving them in clinical practice. The standardization of nomenclature and concepts can facilitate communication and the establishment of protocols and uniform behaviors.

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Effects of modified Portland cement and MTA on fibroblast viability and cytokine production

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ABSTRACT

Objective: The aim of this study was to investigate the effects of a new Portland Cement formulation (CPM) comparing it to Angelus MTA on cell viability and IL-1β and IL-6 release by mouse fibroblasts. **Methods:** Polyethylene tubes filled with these materials were placed into 24-well cell culture plates with mouse fibroblasts. Empty tubes were used as control. After 24 hours, MTT assay was used to evaluate the cell viability. For cytokine assay, mouse fibroblasts were incubated in 24-well flat-bottom plates with set material disks at the bottom or without material, as control. After 24 hours, culture media were collected for cytokine evaluation by using ELISA. **Results:** CPM and Angelus MTA did not inhibit the cell viability. Both materials induced IL-6 and IL-1β release and the amount was statistically significant compared with the control group. **Conclusions:** Both materials were not cytotoxic in fibroblast culture and induced IL-6 and IL-1β release.

Keywords: MTA. Cytotoxicity. Dental materials.

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Introduction

Mineral trioxide aggregate (MTA) was developed by Torabinejad in the early 1990's; the first study on this material was published by Lee et al.[1] The main MTA components are tricalcium oxide, tricalcium silicate, bismuth oxide, tricalcium aluminate, tricalcium oxide, tetracalcium aluminoferrite and silicate oxide. It was introduced to be used in pathological or iatrogenic root perforations and in root-end cavities.[1-9]

Studies have shown that MTA promotes favorable tissue reactions that are characterized by absence of severe inflammatory response, presence of a fibrous capsule, and induction of mineralized tissue repair.[8-13] However, MTA has working properties that are less than ideal. The resulting cement from the mixing of powder with water is difficult to manipulate and its setting time has been reported to be almost 3 hours, whereas the working time is less than 4 minutes.[14,15] Additional moisture is also required to activate the setting of the cement.[14]

In 2004, CPM ('Cimento Portland Modificado' or Modified Portland Cement) was developed in Argentina (Egeo S.R.L., Buenos Aires, Argentina), which is stated to be similar to MTA. The powder also consists of thin hydrophilic particles that form a colloidal gel in presence of moisture, that becomes solid to form a hard cement in one hour. The main components are tricalcium silicate, tricalcium oxide, tricalcium aluminate and other oxides.[3] However, according to the manufacturer, calcium carbonate was added to reduce the pH after set from 12.5 to 10.0 aiming to limit the surface necrosis but allowing the alkaline phosphatase action.

There are some experimental models used to evaluate the biocompatibility of endodontic materials such as cell culture,[16] which has the advantage of being relatively inexpensive, rapid and reliable.[17,18] However, there have been no studies in the literature evaluating cell viability and cytokine production induced by CPM. Thus, the aim of this study was to determine the effects of the CPM and Angelus MTA on cell viability in fibroblasts and to assess the effects of these materials on IL-6 and IL-1β releasing.

Materials and Methods

Cell culture

L929 mouse fibroblasts were grown in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum (GIBCO BRL, Gaithersburg, MD) streptomycin (50 g/mL), and 1% antibiotic/antimycotic cocktail (300 U/mL, 300 mg/mL streptomycin, 5 mg/mL amphotericin 100 g/mL) (GIBCO BRL, Gaithersburg, MD) under standard cell culture conditions (37 °C, 100% humidity, 95% air and 5% CO_2).

Test material

The materials used in this study were CPM (Egeo SRL, Buenos Aires, Argentina) and Angelus MTA (Angelus, Londrina, Brazil), that were prepared according to the manufacturers' recommendations.

Cytotoxicity testing

L929 fibroblasts were seeded into the 24-well plates ($3x10^4$ cells/1 mL medium per well). Cells were incubated for 24 hours in a humidified air atmosphere of 5% CO_2 at 37 °C. The test materials were placed in polyethylene tubes (BARD, C.R.; Bard Ireland Ltda., Galway, Ireland) with a 1.1-mm inner diameter and 10-mm length, and inserted into the fibroblast culture. Six wells were used for each material, and an empty tube was used as the control. Exposure of the cell cultures was stopped by discarding the exposed media after 24 hours. Viable cells were stained with formazan dye (3-[4.5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) (MTT) (Sigma Chemical Co, St Louis, MO). MTT was dissolved in phosphate-buffered saline at 5 mg/mL and filtered in order to sterilize and remove a small amount of insoluble residue. At the times indicated later, stock MTT solution (20 mL per 180 mL medium) was added to all wells of an assay, and plates were incubated at 37°C for 4 hours. The medium was then removed by the inversion of the plate and the dumping of 200 μL of isopropyl alcohol, which was added to the wells and mixed during 30 minutes in order to dissolve dark blue crystals. The blue solution was transferred to a 96-well plate, and the absorbance was read in the microplate reader by using a test wavelength of 570 nm.[19]

Cytokine assay

For cytokine assay, the tested materials were inserted into the wells of 24-well flat bottom plates (Corning) and condensed to disks that were approximately 1-mm thick and with the same diameter of the wells. The material was allowed to set for 2 weeks in cell culture medium at 37 °C. The medium was changed every day during this time.

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L929 fibroblasts were seeded into the wells (10^6 cells/1 mL medium per well) with the material disks in the bottom. The plates were incubated for 24 hours. After incubation, the culture media were collected and analyzed for IL-1β and IL-6 content by ELISA (R&D Systems, Inc, Minneapolis, MN). Cells cultured without tested material served as negative controls.

Statistical analysis

The results were statistically analyzed by analysis of variance with Bonferroni correction ($p<0.05$).

Results

ELISA assay revealed that the average IL-6 (pg/mL) release was statistically higher when the cells were cultured in the presence of CPM and Angelus MTA than in the control for 24 hours, but they were not statistically different from each other ($p>0.05$) (Fig 1). IL-1β release for the Angelus MTA and CPM was statistically higher than for the control, but there were no statistically difference between them ($p>0.05$) (Fig 2). After 24 hours, CPM and Angelus MTA did not inhibit the cell viability, maintaining the same level as the control group ($p>0.05$) (Fig 3).

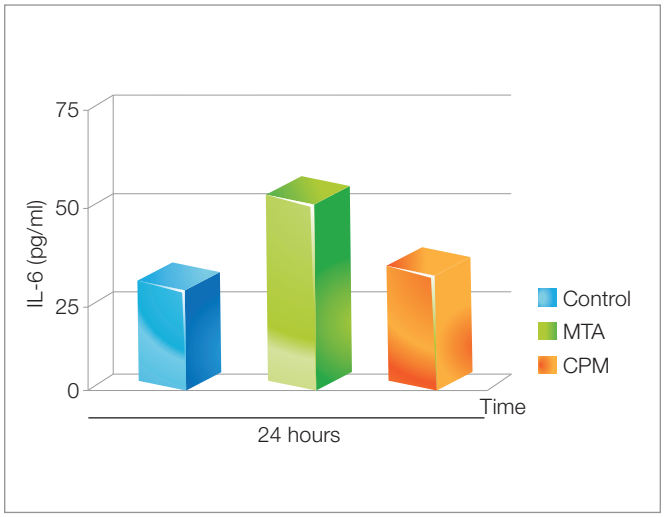


Figure 1. Mean levels of IL-6 raised when the cells were grown in the presence of the materials. There was statistically significant difference ($p<0.05$) between the experimental materials and the control group, but not between the materials.

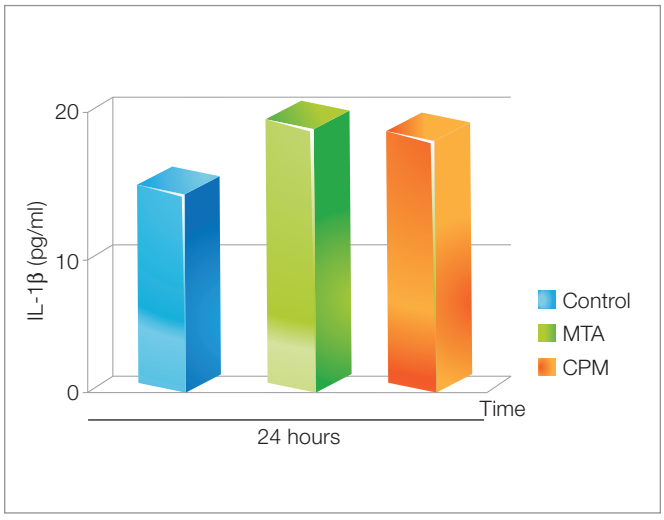


Figure 2. There was difference between the experimental materials and the control group ($p<0.05$), but not between the materials ($p>0.05$) for the levels of IL-1β.

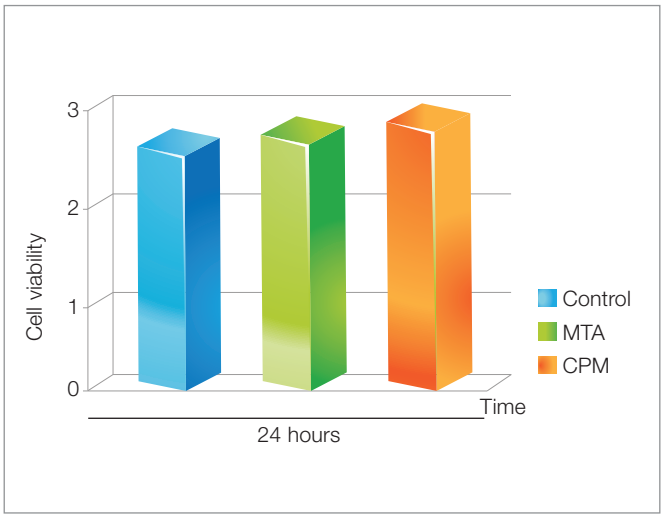


Figure 3. Viability of fibroblasts was not statistically different ($p<0.05$) between the experimental materials and the control group. These results were expressed as means of the absorbance (A_{570nm}) ± SD of each material and the control group.

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Discussion

Endodontic materials should have adequate biological and physicochemical properties. The toxic effects of materials used for endodontic therapy are of particular concern once they can cause degeneration of the periapical tissue and delay wound healing.[20]

In this study, cell viability was determined by MTT assay based on the ability of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble tetrazolium MTT salt into dark blue formazan crystals. Simplicity, rapidity, and precision are advantages of this method. In addition, it does not require radioisotopes.[19,21] Statistical analyses of the MTT assay data showed no significant difference to the CPM in 24 hours. MTA has been recommended to seal all pathways of communication between the root canal system and the external surface of the tooth. The results in the present study agree with previous work showing that MTA was not cytotoxic.[22-25]

Concerning CPM, according to the manufacturer, this material has similar or better physical, chemical and biological characteristics compared to MTA, with the same clinical indications.[26] In this study, the CPM cytotoxicity was not statistically different from the control group. The result can be explained by the similarity in the composition of CPM and MTA based on Portland cement. The pH reduction from 12.5 to 10.0 did not affect cell viability.

Synthesis of cytokines is complex, and their expression and effects are governed by many factors including other cells and chemical mediators.[27] Previous studies have shown that MTA stimulated IL-1β production by osteoblasts.[28,29,30] IL-1β is a cytokine that mediates bone resorption and it is synthesized by various cells including macrophages close to the bone resorption and osteoclasts.[31] In this study, all materials induced statistically more IL-1β release than the control group. IL-6, on the other hand, is a cytokine that mediates the host response to injury and infection, and it is secreted during the inflammatory process in order to regulate various aspects of the immune response, the acute phase of the reaction, and the control of blood infection.[32] Animals depleted of IL-6 showed larger periapical lesions than normal rats.[32] According to these results, all materials induced statistically more IL-6 release than the control group, which shows that they can play an important role on controlling the inflammation and promoting the healing process.[5]

It was possible to conclude that CPM and Angelus MTA did not inhibit L929 fibroblasts viability. Both materials induced statistically more IL-1β and IL-6 releasing than the control group.

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Assessment of success rate of endodontic treatment performed by Brazilian undergraduate students

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ABSTRACT

Objective: The aim of this paper was to evaluate the success rate of endodontic treatments performed by undergraduate students at São Paulo State University (Brazil). **Methods:** A random sample of 94 records from 85 patients who received endodontic treatment was analyzed. All evaluated cases undergone endodontic treatment using the same standardized irrigation solutions and intracanal medication. The criteria used to characterize the clinical successful were: no pain, swelling or fistula and complete or partial regression of apical injury, radiographically observed. Kappa's test was used to verify the statistic correlation between treatment, clinical and radiographic success. **Results:** According to clinical and radiographic evaluation, the success rates were 89.36% and 88.29%, respectively. The statistical analysis indicated that the success of endodontic treatment occurred in 79 (84.04%) of 94 cases (p=0.14). **Conclusion:** The treatment of teeth with pulp necrosis performed by Brazilian undergraduate students showed, clinically and radiographically, a high level of success.

Keywords: Endodontics. Treatment failure. Dentistry students.

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Introduction

In order to obtain success in the endodontic treatment, the elimination of the microorganisms in root canal system and the respect of the periapical tissue are considered an important step.[1]

Therefore, the outcome of treatment should be classified in terms of success, when upon follow-up examination there are no clinical signs and symptoms (absence of pain and swelling, normal function of the tooth) or periapical radiolucency (disappearance or reduction of the lesion or the periapical bone rarefaction).[2]

The most common failures observed after endodontic treatment can be related directly with errors in surgical technique, such as inadequate aseptic control, poor cavity access design, missed canals, inadequate instrumentation, use of inadequate root filling material, inadequate root filling, lack of debridement, fractured instruments in root canal, perforations and leaking temporary or permanent restorations.[3]

However, the appearance or persistence of pain or other signs and symptoms after endodontic treatment is an important clinical feature, suggesting endodontic failure[4]. Undoubtedly, the major factors associated with endodontic failure are the persistence of microbial infection in the root canal system and/or the apical area.[5,6]

The correct evaluation of all factors responsible for failure is very important and determines the appropriate treatment plan, beginning with an anamnesis and thorough clinical examination.

The completion of clinical studies that evaluate the success of endodontic treatment based on signs and symptoms is a fundamental point and should guide the basic research to propose new techniques, medicines and medical procedures. Therefore, the purpose of this study was to evaluate the rate of clinical and radiographic success of endodontic treatment performed by undergraduate students.

Material and Methods

It were analyzed 94 teeth of 85 patients who received endodontic treatment performed by undergraduate students of the Faculty of Dentistry of São José dos Campos, São Paulo State University (UNESP). All selected teeth had pulp necrosis and periapical injury radiographically visible.

To each patient was assigned a dental clinical record with a card for each element to be evaluated. In each card, clinical and radiographic signs and symptoms were documented to assess the actual condition of the teeth before, during and after treatment.

The selected teeth were treated using the same irrigant solution, intracanal medication and the same root canal preparation, material and root canal filling technique. During biomechanical preparation the canals were instrumented by serial instrumentation technique, associated with the anatomical step by step preparation and irrigated with 1% sodium hypochlorite (Biodinâmica Ltda. Ibiporã, Brazil). The intracanal medication used for 14 days was calcium hydroxide with CMCP (Calen-PMCC, SS White Ltda. Rio de Janeiro, Brazil) and the filling was performed by the active lateral condensation technique using gutta-percha and Grosmann Sealer - Fill Canal (Ligas Odontológicas Ltda. Catumbi, Brazil).

The cases were followed-up for a period ranging from 5 to 36 months with clinical and radiographic monitoring in order to establish the success rate of these treatments. The criteria adopted to characterize the case as a success were:

» Absence of any painful symptoms: pain to vertical and horizontal percussion, chewing, apical or spontaneous palpation.

» Absence of intra and extraoral edema.

» Absence of fistula.

» Absence of tooth mobility.

» Complete or partial regression of the radiographic image lesion.

When any of the criteria was not filled out, the case was regarded as failure. The results were submitted to the Kappa's test to verify the correlation between clinical and radiographic success.

Results

The results have shown, clinically, success rate of 89.36% (84 cases had clinical success) and of 88.29% radiographically (83 cases showed radiographic success). Statistical analysis indicates that there was correlation between success and treatment in 79 (84.04%) of a total of 94 cases (p= 0.52).

Discussion

With technological and scientific advances through the large number of studies in endodontics, there is a clear expectation for increasing success rate in endodontic treatments. Recent studies based on radiographic

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Table 1. Percentiles of success and failure.

	CLINICAL	RADIOGRAPHIC	CLINICAL + RADIOGRAPHIC
Success	84 (89.36%)	83 (88.29%)	79 (84.04%)
Failure	10 (10.64%)	11 (11.71%)	6 (6.38%)

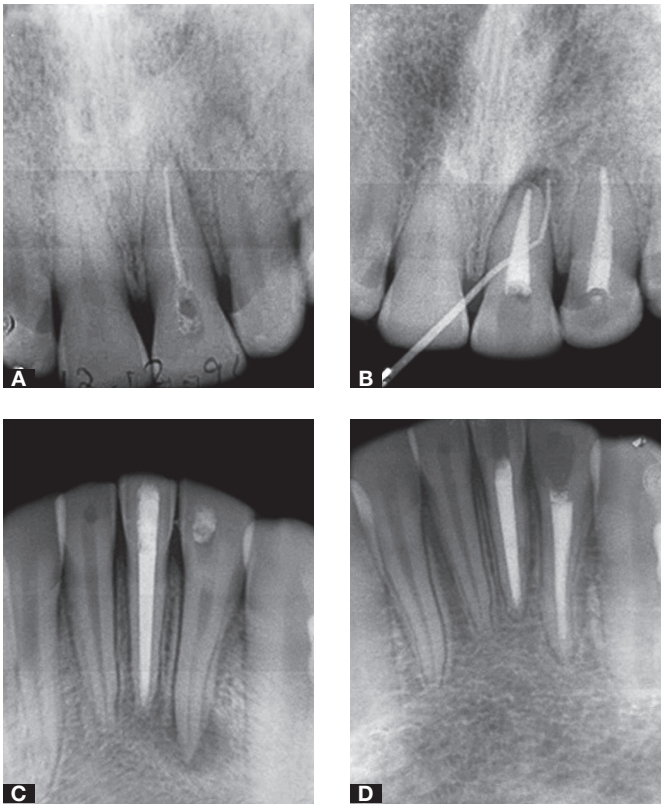


Figure 1. Case 1: **A**) initial radiograph, **B**) failure. Case 2: **C**) initial radiograph, **D**) success.

evaluation of success/failure in endodontic treatment conducted by specialists, showed a mean failure rate of 21.09%.[7] In data collected for certain populations at the same period,[8] it was found in endodontic treatment performed by no specialists in teeth with pulp necrosis, failure around 39.9%. In this study, the treatments were performed by undergraduate students and the rate of failure based on radiographic examination was 11.70%, lower than the rate found by Kojima et al[7] when treatment was performed by specialists.

The results show that 6.38% of the cases presented as clinical and radiographic failures simultaneously and that 54.54% of cases of radiographic failures are related to problems in the filling of root canals (incomplete filling, and overfilling of 2 mm below the apical limit). Especially the cases of extrusion are in agreement with the findings of Nair et al,[9] which reported that the presence of gutta-percha through the apical foramen during the root canal filling is associated with delay in the periapical healing process. The studies of Moussa-Badran et al[10] showed that from 304 endodontic treatments performed by undergraduate students at a dental teaching centre, 69.7% had inadequate root fillings. These results are consistent with those from the present study and indicate that the correct root canal filling is important for the endodontic success.

However, according to Lin et al[11] and Sjögren et al,[5] it is unlikely that most contemporary endodontic materials can maintain a periapical inflammation in the absence of endodontic infection. They claim that this statement is reinforced by the high rate of treatment success in teeth without apical lesions even in cases of overfilling.

Moreover, Siqueira[6] concluded that although it has been suggested that non-microbial factors may be implicated in endodontic treatment failure, the literature suggests that persistent infections inside the root canal system or secondary infections, and in some cases apical infections, are the major causes of failure. Also, in most cases, the apical seal is inadequate in overfilled root canal.

Anyway, Chugal et al[12] observed that the prognosis of teeth with periapical disease is increased if they are instrumented closer to the radiographic apex. In this study, 13 cases were closed in the apical limit and the rate of success in these cases was 76.92%. Probably fillings in the apical radiographic limit may in fact be overfilled. Fillings slightly below this limit could have better results.

As for the risk of reinfection, Saunders and Saunders[13] argue that it is dependent on the quality of filling and restoration and that in all cases where the bacteria remain in the canal system there is a constant risk of perpetuation of apical inflammation. For these authors, the coronary infiltration may be an important cause of failure in endodontic treatment, as well that in some situations the root canals can be contaminated through the oral cavity.

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Kirkevang et al[14] also reported that teeth with inadequate crowns and restorations have a greater risk of developing apical changes, and this risk is 1.7 higher for teeth with inadequate than for those with adequate restorations.

In this study, 32.35% of teeth showed up without sealing or with provisional sealing in the control clinical visit. This result is disturbing because endodontically treated teeth should be restored in the shortest time as possible, to ensure the success of endodontic treatment. Despite this, the success rate was higher than the related in literature regarding this evaluation.

For the data of clinical failures, the most frequent symptom reported by patients was pain (93.75% of cases), and in 43.75% of these cases the pain was associated with the presence of fistula and/or edema. In this study the fillings were performed using gutta-percha and cement based on zinc oxide and eugenol. Sealers based on zinc oxide and eugenol do not offer satisfactory biocompatibility. Many studies have shown that the use of these sealers leads to a chronic and persistent inflammatory process in the periapical area.[15,16] Thus, nowadays, studies being conducted indicate the preferential use of cements based on less toxic materials.[16,17]

It should be noted that currently, chlorhexidine associated with calcium hydroxide is recommended as intracanal medication, due to CMCP toxicity, which is considered carcinogenic.[18,19] The association of chlorhexidine with calcium hydroxide as intracanal medication is the best option for treatment of pulp necrosis with periapical lesion, showing excellent results.[20]

It is believed that with developments in the protocol of endodontic treatment of teeth with pulp necrosis, futures researches may show even higher rates indicative of success. However, despite the high rate of success found in this study, the development of techniques, tools and materials led to the perception of the need for changes in the protocol of endodontic treatment of teeth with pulp necrosis.

Conclusion

The treatment of teeth with pulp necrosis performed by graduate students of the Dental School of São José dos Campos/UNESP showed a high rate of clinical and radiographic success.

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Assessment of coronary microleakage marker capacity of three dyes

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ABSTRACT

Objective: The aim of this study was to assess the marker capacity of 2% methylene blue, 2% Rhodamine B and 5% nickel sulfate. **Methods:** After biomechanical canal preparation in 84 single-root pre-molar teeth extracted from human beings, the access cavities were sealed with Coltosol® and the specimens were made impermeable, except for 1 mm adjacent to the temporary sealing. The samples were immersed in the staining solutions and kept in an oven at 37 °C for 3 and 7 days, and were submitted to thermal cycling. During this period, 300 cycles (5 °C and 55 °C) of 30 seconds each were performed in a specific appliance with a digital programmer for temperature, time and number of cycles. Longitudinal sections of the specimens were obtained and observed under stereomicroscopic lens. **Results:** The statistical results by the Analysis of Variance showed a significant difference ($p<0.05$) among the groups and between the two time intervals assessed. **Conclusion:** There was greater leakage in the 7 day interval in all groups, and the Rhodamine B dye exhibited the higher mean leakage depth values in the two time intervals, followed by methylene blue and nickel sulfate.

Keywords: Dyes. Microleakage.

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Introduction

The efficacy of coronary access, disinfection, modeling, and hermetic and three-dimensional filling of the entire root canal system, to provide the return of the tooth to its physiological function in the stomatognathic system as soon as possible, determines the success of endodontic therapy. Thus, in order to achieve success, it is imperative for the root canal to be adequately sealed. It is known that in the course of time, the success of endodontic treatment is safeguarded by this sealing that preserves the root canal from possible recurrent contaminations.

It has been difficult to find a temporary restorative material that resists the more accentuated imbalances, such as those that occur in the oral cavity as a result of the ingestion of liquid or solid foods with different temperatures. Marginal microleakage basically depends on two factors: Material/enamel-dentin interface and physical-chemical properties of the temporary sealing materials.[1] Thus, the solubility, capacity to disintegrate and dimensional instability (contraction and expansion) of the material prejudice the maintenance of coronary sealing, contributing to the increased passage of microorganisms, toxins and chemical substances from the oral cavity to inside the dentin tubules.

To identify marginal microleakage in temporary sealing materials, different methods can be used, among them the following are mentioned: dyes,[2,3,4] radioisotopes,[5,6] microorganisms,[7,8] fluid filtration,[9] the identification of ions and histochemical[10,11] and electrochemical processes.[12,13]

In vitro studies using dyes to assess microleakage have been conducted because they are easy to use and allow safe interpretation of results. Various marker substances have been used, and the following were mentioned: methylene blue, fluorescein, basic fuchsin, silver nitrate, Rhodamine B, nickel sulfate and Indian ink. A huge discrepancy has been observed with respect to the concentrations of these dyes and also in relation to the immersion time of specimens in them.

Methylene blue is an organic acid dye in the phenothiazine class, with molecular formula $C_{16}H_{18}N_3SCl$, widely used as a marker solution. This dye is highly soluble in water and easily penetrates the tooth structures, without undergoing adsorption by the mineral matrix, in addition to having low molecular weight, similar to the size of the nutrient molecules of microorganisms.[14]

Rhodamine B is a basic intense organic dye, with molecular formula $C_{28}H_{31}ClN_2$, soluble in water at ambient temperature, also solvent in alcohols and common organic solvents, in addition to being highly stable. It presents with red coloring, and when it reacts with Sb, Hg, Au and Bi chlorides, it turns purple. When this substance is diluted, it is capable of producing fluorescence.[15,16] Due to this property, Rhodamine B has also been used as marker to assess marginal coronal microleakage in temporary sealing materials.

Nickel sulfate is an inorganic compound with molecular formula $NiSO_4$, and is obtained by dissolution of the metal, oxide or nickel carbonate in a diluted sulfuric acid solution.[15] The metal nickel in minimally alkaline solutions with ammonia or in acid solutions buffered with sodium acetate, form a precipitated red reagent, almost insoluble in water, called nickel-dimethylglyoxime.[16] The nickel is revealed by means of an alcohol solution of dimethylglyoxime. The reaction of the nickel with dimethylglyoxime produces the formation of the Ni-dimethylglyoxime complex that presents a red color. The identification limit is 0.16 µg of nickel.

The chemical method to assess the dimensional stability of temporary sealing materials involves nickel ion leakage through the access cavity walls in the direction of the root canal.[1] The contact of the nickel ions with the revealing solution (dimethylglyoxime) impregnated in the cotton ball and paper cone lead to the formation of a red coloring substance, the nickel-dimethylglyoxime complex ($C_8H_{14}N_4NiO_4$). The presence of this substance inside the root canal allows the occurrence of microleakage to be detected.

Although there have been many researches to find a sealing material close to the ideal characteristics, little attention has been given to identifying the best marker solution for evaluating coronary marginal microleakage. Furthermore, the pertinent results found in the literature are discrepant due to the use of several dyes and the different immersion times applied to them. The use of staining solutions as leakage markers on materials being tested is an aspect that has been much questioned in recent years. Therefore, there is clearly a need to assess leakage levels revealed by different dyes and compare them, under the same experimental conditions, analyzing their influence on marking leakage in relation to a material used for temporary sealing.

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This study assessed coronary microleakage marker capacity of 2% methylene blue, 2% Rhodamine B and 5% nickel sulfate, associated with 1% dimethylglyoxime at intervals of three and seven days, using Coltosol™ cement as temporary sealer.

Material and Methods

It was made a selection of 84 single-rooted human pre-molars with completely formed apexes, extracted by orthodontic indication or periodontal involvement, either healthy or with incipient caries, without detectable cracks after been inspected by a lens with enlargement of four times.

The coronary preparation performed presented proportions of 2.5 mm diameter in the mesiodistal direction and 3.5 mm in the buccolingual direction, carried out with a carbide steel bur #1557, operated in the vertical direction parallel to the long axis of the tooth, coupled to a high speed pen. The measurements were confirmed with a digital caliper. All the internal cavity walls were flatted after removing the roof of the pulp chamber with an Endo-Z bur and an endodontic probe was used to locate the entry to the root canal. After biomechanical preparation of the root canals using oscillatory technique[17] the #25 file was preserved as a memory file.

The teeth were randomly divided into 6 groups of 14 specimens each, and 2 teeth were used as control. The positive control teeth did not have temporary sealing and only the root surface was made impermeable, whereas the negative control was sealed and made completely impermeable. Both were immersed on the methylene blue, Rhodamine B and nickel sulfate dyes (Table 1).

Cotton balls were compacted in the pulp chamber of each specimen, leaving a distance of 4 mm from the cavitary surface angle, measured with a millimetric probe. For the specimens immersed in nickel sulfate solution, #25 absorbent paper cones and cotton balls to be introduced into the pulp chamber were previously treated with an alcohol solution of dimethylglyoxime, respecting the previously determined 4 mm.

Then Coltosol® was inserted in the cavity with the aid of a Hollenback 3S, and was smoothly adapted to the cavity walls by means of a condenser. Its adaptation was observed with a lens with enlargement of four times.

The entire external surface of the teeth was made impermeable, except in the coronary sealing region, respecting the cervical margin of the restoration and about 1 mm around it. The surfaces were covered with two layers of epoxy resin (Araldite™ Hobby) observing an interval of 24 hours for drying between the two applications. When this time had elapsed, the samples were covered with a layer of red colored nail polish (Niasi S/A) to make them completely impermeable, waiting 40 minutes for it to dry.[18] After sealing and respecting the drying times of the materials used to make them impermeable, the teeth were immersed in 6 standardized glass recipients, previously identified in accordance with the group, the dye used and the time interval. The volume of staining solution used in each recipient was standardized at 10 ml, measured by means of a hypodermic syringe, and these substances were prepared by a specific laboratory (Lenza Farmacêutica, Brazil).

The recipients containing the samples immersed in the dyes were placed in a bacteriologic oven where they stayed for a minimum of 8 hours at a temperature of 37 °C and 100% humidity.

The samples were removed from the oven and taken to a duly calibrated and electronically programmed thermal cycling appliance. Two (2) rounds of thermal cycling were conducted at temperatures of 5 °C and 55 °C, totaling 300 cycles of 30 seconds each. Firstly, the uneven numbered groups, that were immersed for 3 days in the staining solution, were thermal cycled and next, the even numbered groups, that remained in the staining solutions for 7 days.

Immediately after the thermal cycling process, the samples were put back into the bacteriologic oven at 37 °C, where they stayed, respecting the immersion time in the dyes, until the time they were sectioned.

After the time intervals had been completed, the teeth were removed from the staining solutions and washed under running water for 5 minutes and dried at ambient temperature for 24 hours to allow the dyes to fix.[18]

Table 1. Division of samples into groups.

GROUPS	DYE USED	TIME INTERVAL
I	2% methylene blue	3 days
II	2% methylene blue	7 days
III	2% Rhodamine B	3 days
IV	2% Rhodamine B	7 days
V	5% nickel sulfate	3 days
VI	5% nickel sulfate	7 days

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After drying, longitudinal sectioning of the samples was done in the bucco-lingual direction, using a flexible double-faced diamond disk coupled to a micromotor.

Macroscopic observations of the longitudinal sections obtained were performed and the linear measurement of dye leakage was taken using a Wild M-8 stereomicroscopic lens under an enlargement of 4 times, with a digital camera coupled to a computerized quantitative analysis system, by means of Image Pro-Plus software calibrated to a 1-mm scale. The images were captured with this program from the most coronal distance on the cotton ball up to the maximum leakage point marked on it, measured in millimeters. The data found were put into tables and submitted to the Analysis of Variance and the *t* test, considering the level of significance of 5% ($p<0.05$), to check the differences among the studied groups.

Results

Table 2 shows that there was significant difference ($p<0.05$) among the three staining solutions used for each one of the evaluated periods.

In the two time intervals, three and seven days, Rhodamine B exhibited a significantly higher leakage depth than methylene blue and nickel sulfate. Furthermore, in relation to the latter two dyes, methylene blue showed greater penetration efficacy compared with nickel sulfate in the two time intervals assessed. On the other hand, no presence of leakage was observed in any of the specimens immersed in the three staining solutions in the negative control in the two time intervals assessed. In the positive control all the samples showed leakage of the dyes.

When the time periods were compared, it was observed a significant difference ($p<0.05$) between the two intervals, in which the leakage depth found in the seven day interval was significantly higher to the one found in the three day interval, in all the analyzed groups (Fig 1).

Table 2. Mean values of the leakage depth measurement (in mm).

GROUP	TIME INTERVALS		
	3 days	7 days	
2% Methylene Blue	1.524	2.597	2.061[b]
2% Rhodamine B	2.760	3.380	3.070[a]
5% Nickel Sulfate	1.191	1.910	1.551[c]
	1.825[b]	2.629[a]	

Means followed by different letters differ among them ($p<0.05$) by the t test.

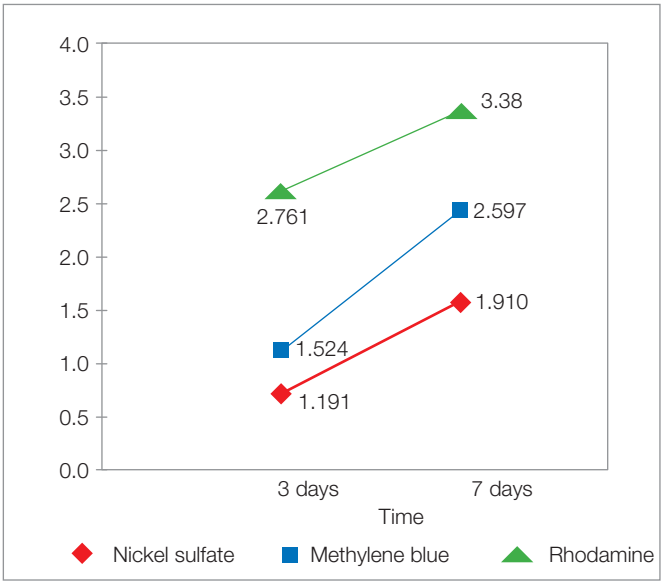


Figure 1. Leakage depth measurement in the two time intervals (in mm).

Discussion

Methylene blue, Rhodamine B and dimethylglyoxime are organic solutions used as indicators for qualitative inorganic determination.[19] The indicators must comply with two conditions: The associated or the ionized forms must present different colors, and the change in coloring must be fast.[20] Any dye with intense color that behaves by presenting a double redox reaction (reduction-oxidation) may be used as an indicator, if its oxidized or reduced forms have different colors.[21] The colors must be sufficiently intense to allow the dye to be used in such small concentrations that its addition to the test mixture insignificantly changes the redox potential. Methylene blue dye is a redox indicator and has an intense blue when oxidized, but it is colorless when reduced. Thus the color stability of organic dyes is an important factor that must be observed in microleakage studies and is related to the hydrogen potential (pH) that should also be assessed. It is known that methylene blue dye presents an acid character and Rhodamine B, a basic one. In contrast, methylene blue has less chemical stability

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than the latter, thus it may change color over time or when coming into contact with a basic substance and can be transformed. By changing the pH, methylene blue is converted into leucomethylene (which is its non-visible form) and may change the accuracy of the reading.[22] A staining solution with acid or alkaline characteristics may alter the dentin itself during the period of experimental material setting, thus facilitating the creation of spaces and increasing the possibility of the dye leakage. Thus, an attempt was made in this study to implement a standardization of the pH of staining solutions around 7.0, the same precaution that is recommended in the literature.[23]

Results of microleakage studies are more reliable since the use of a phosphate ion buffer solution maintains the pH practically constant, even when an acid or a strong base is added. Thus it is important for the marker solution to be buffered and have its pH adjusted to 7.0 or close to it.[20] To evaluate sealer materials, one perceives that in the choice of the dye, little attention has been given to the various molecule sizes and their specific behavior in the face of certain circumstances. The choice is based more on the appearance of the dye.[24] From this aspect, it is important to emphasize that the organic dyes methylene blue and Rhodamine B are molecules that present high molecular weight and little capacity for ionic dissociation, in relation to the nickel sulfate, an inorganic salt. This substance has a low molecular weight compared with the macromolecules of the organic dyes and a high capacity of ionic dissociation when in the form of a solution. Thus, when staining solutions are studied comparatively, their concentrations must be adjusted in order to allow an equal competitiveness of the ions for penetrating the tooth/sealing material interface, when they are kept in the same volume of solution. Thus the relation between the mass and the volume of the staining solution determined for methylene blue and Rhodamine B was 2% and for the nickel sulfate solution, it was 5%.

The penetration depth of staining solutions into the dental structure varies in accordance with the amount of air trapped inside the root canal.[25] In our study, no vacuum was used and dye penetration occurred in a passive manner with a view to similar conditions closer to clinical reality. The elimination of air may induce an overestimate of the extent of microleakage in vivo,[23] although some authors have demonstrated that the use of vacuum gives a more precise assessment of dye penetration through the space existent between the sealing material and the canal walls.[25,26]

In the methodology used in this study, it was opted to use a 4 mm thickness of the sealing material on each one of the specimens. This is in agreement with some studies that proposed a thickness ranging between 3 and 5 mm, sufficient to assure marginal sealing.[2,27]

The option for the Coltosol sealing material, used in this study to assess the dye leakage marker capacity, was due to this cement has properties of expansion during its setting, providing a very dense filling and good marginal sealing.[28,29]

In the present study it was opted to use thermal cycling in an electronically programmed appliance, similarly to another study,[4] because it reproduced extreme temperatures more faithfully, although many authors prefer to simulate these variations by means of manual thermal baths on ice and in an oven[26,30] and by means of a humidifier chamber.[31]

In the literature there is still no standardization of the immersion time of specimens in the dyes.[10,11,26,29,30,32] In this study, periods of three and seven days of immersion time in the dyes were adopted to simulate the usual time between consultations.

The dye penetration depth was measured linearly, using a Wild M-8 stereomicroscope lens at a 4 times enlargement, with a digital camera coupled to a computerized quantitative analysis system, using Image Pro-Plus software. The use of a stereomicroscopic lens was found in other studies[30,33] and these measurements allowed more precise results to be obtained, although literature showed a large number of studies that analyzed marginal microleakage by means of scores.[1,4,32]

The Rhodamine B dye molecule is smaller than that of methylene blue and it is less tensoactive than the latter, as its penetration is greater, which is in agreement with the results of the present study.[22] Other studies also observed that Rhodamine B exhibited the highest intradentinal penetration indexes and allowed an adequate visualization.[34,35] On the other hand, some authors believed that methylene blue favored the reading of marginal microleakage when compared with Rhodamine B and fluorescein.[3]

The specimens immersed in the nickel sulfate staining solution exhibited the lowest penetration indexes among the dyes assessed. No studies making a

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comparative assessment of this dye were found, so that the information in this study could be compared

The type of methodology used is an important factor for microleakage study, because the dyes, ions and isotopes present smaller molecular sizes than the bacteria and their by-products. However, if the dye identifies the microleakage, it means that there is a passageway and that the bacterias or its by-products could pass in a shorter or longer period of time. Thus, a tooth submitted to endodontic treatment should receive a well performed definitive restoration as quickly as possible, as defects in marginal adaptation that allow microleakage of saliva may place the entire endodontic treatment at risk.

Conclusion

Within the experimental conditions of this study, and considering the results obtained, it was concluded that:

1. The leakage magnitudes determined by 2% Rhodamine B, when the sealing material Coltosol® was used, were significantly higher than those found for 2% methylene blue and for 5% Nnickel sulfate, indicating the greater coronary microleakage marker capacity of Rhodamine B in relation to the other dyes, both at the interval of three and seven days ($p<0.05$).
2. The 2% methylene blue exhibited greater penetration depth in relation to 5% nickel sulfate in the two time intervals assessed ($p<0.05$).

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Marginal leakage evaluation of three endodontic sealers according to the moment of post preparation

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ABSTRACT

Objectives: The purpose of this in vitro study was to evaluate the coronal marginal leakage after post space preparation in teeth filled with three different sealers, according to the period between the root canal filling and preparation of post space. **Methods:** Ninety human teeth recently extracted were cleaned and shaped and then filled with Sealapex, Endométhazone or TopSeal. Gates Glidden drills were used for immediate post space preparation of 10 teeth with each sealer until 5 mm of filling were left. Sixty filled roots were incubated at 37 °C in wet environment during 30 and 60 days to have, then, the post space prepared as previously. The external surface of each root was covered with Araldite™. The specimens were immersed in 2% methylene blue dyer under vacuum for 24h; so they could be analyzed. The infiltration was measured by the Sigma Scan (Jandel Scientific) software from the upper part of the obturation to the most apical point reached by the dyer. **Results:** Sealapex and TopSeal showed smaller infiltration after post space preparation than Endométhazone; Immediate post space preparation showed smaller infiltration than post space preparation after 30 and 60 days of the root canal filling.

Keywords: Marginal microleakage. Post preparation.

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Introduction

One of the major aims of the endodontic treatment is to perform a root canal filling that respects the well-known physical and biological properties. Among the physical requirements, the root canal must be properly sealed, avoiding gaps between the sealing material and the dentinal walls. As the occurrence of such gaps might compromise the endodontic treatment, many obturation techniques and different materials have been investigated through the marginal leakage method.

The majority of reports concerning obturation focus on apical leakage. However, in the last decades, the importance of coronal sealing after root canal filling has also being emphasized. According to Swanson and Madison,[8] the amount of coronal microleakage that can occur in a short period should be considered a potential etiological factor for root canal failure.

On the same hand, it is necessary to concern about the coronal microleakage after space preparation for the installation of a prosthetic post for two reasons: a) There are evidences that coronal leakage might reach the periapical tissues of teeth with fully filled root canals; b) in theory, this possibility increases after post space preparation, as a shorter extension of filling material remains.

When a root canal filling will be partially removed it must be taken into account that the dentinal walls have already been under instrumentation and irrigation with bactericidal solutions. Normally, after root canal filling, the access cavity has to be temporally and adequately sealed, because the treatment might be affected when this procedure is not performed correctly. Moreover, clinical procedures from modelling to final cementation of the post allows for the contamination of the portion of the canal prepared to receive the post.

The installation of an intraradicular post to support a prosthetic crown, in a root canal previously filled, brings another dilemma. Should this root canal be retreated or not? Hence, the purpose of this in vitro study was to evaluate the coronal marginal leakage after post space preparation in teeth filled with three different sealers, according to the period between the root canal filling and preparation for the post.

Material and Methods

Ninety human single rooted teeth were collected from the teeth bank of the Unesp-Araçatuba Dental School. The coronal portion of the tooth was removed at the enamel-cement junction using a low speed diamond wheel. Conventional debridement and mechanical preparation were made until #60 Kerr file. During biomechanical preparation the canals were irrigated with distilled water. After cleaning and shaping, the canals were filled with EDTA for 3 minutes, and then washed with distilled water and dried with paper points.

Canals were obturated by lateral condensation technique with gutta-percha cones and 3 sealers: Sealapex (Kerr) (30 specimens), Endométhazone, (Specialités Septodont, Paris, France), 500 mg powder and 0,2 ml Eugenol (30 specimens) or Topseal (Dentsply/Maillefer) (30 specimens).

After obturation, 30 specimens (10 of each sealer) received immediate post space preparation; 30 specimens (10 of each sealer) were incubated at 37 °C during 30 days in 100% humidity, and then received post space preparation; the remaining 30 specimens (10 of each sealer) were incubated in the same conditions mentioned above during 60 days to receive post space preparation. Therefore, 9 groups were established (Table 1).

Post spaces were prepared using Gates-Glidden drills plus manual condensers slightly warmed up. The root canal filling was removed until 5 mm of the obturation remained in each specimen, and then, a hermetic vertical condensation was made with manual condensers.

After post preparation, the teeth were externally air-dried and the external surface or the root made impermeable with Araldite adhesive (Brascola, Brazil). Then, all specimens were placed in 2% methylene blue solution (pH 7,0) under vacuum during 15 minutes,[1] and incubated in the marker solution for 24 hours. The specimens were then washed under running water and dried.

Table 1. Summary of the treatment performed in each group according to the sealer used and period after obturation that the post space preparation was realized.

Sealer	Period after root canal filling		
	Immediately	30 days	60 days
Sealapex	Group I	Group II	Group III
Endomethazone	Group IV	Group V	Group VI
TopSeal	Group VII	Group VIII	Group IX

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A 169 drill was used to open 1-mm depth longitudinal cavities in both vestibular and lingual faces, without, however, reaching the root canal. Chisel acting directly in these cavities was used to split each root in two pieces.

Both pieces were then disposed in wax sheets so that the full length of the obturation could be observed and these images could be recorded. Finally the marginal leakage was measured by the Sigma Scan (Jandel Scientific) software from the upper part of the obturation to the most apical point reached by the dyer. The data were statistically analyzed.

Results

A global analysis of the results showed that the lowest dyer infiltration happened when the post space was prepared immediately after root canal filling. Besides, among the sealers studied, Sealapex in the immediate and 30 days preparation and Topseal in the immediate preparation showed the smallest infiltration. Endométhazone, in the 3 periods of the study, Topseal, in 30 and 60 days, and Sealapex in 60 days showed the bigger infiltration and there were no statistical differences in these results (Fig 1).

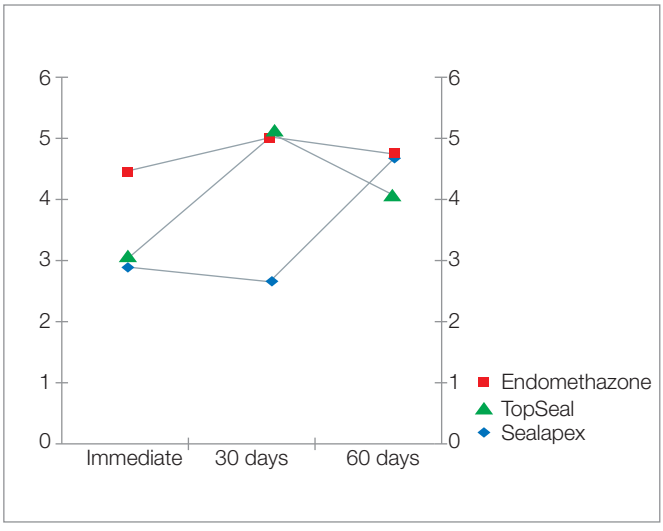


Figure 1. Marginal leakage medians of the root canal sealers X time of post space preparation.

Discussion

The post space preparation of the root canal that will receive a prosthetic post is generally performed with Gates-Glidden, Largo or Peeso drills, warm condensers or chemical substances such as chloroform, eucalyptol or xylene. Although Kwan and Harrington[4] reported better results when Gates-Glidden drills were used in comparison to warm condensers, Madison and Zakariansen[5] did not find significant differences between the utilization of Gates-Glidden drills, warm condensers or chloroform. As a consequence of such contradictions, some alternatives have been suggested. Zuolo et al[11] advise the partial removal of the filling with warm condensers and Largo drills, followed by a condensation of the remaining filling with cold condensers. Valera et al[10] performed immediate preparation with warm condensers and late preparation with Peeso drills. According to Romeiro et al,[7] during the late preparation a complete displacement of the filling may occur as a consequence of the sectioning procedure itself, of the vibration and of the traction action of the drill.

In the present study the post space preparation was performed with Gates-Glidden drills, followed by vertical condensation with cold condensers, in all groups evaluated.

Regarding the time of post space preparation it was possible to verify that the post preparation performed immediately after obturation provided a coronary infiltration significantly smaller than when it was performed after 30 and 60 days of post preparation, and the canals filled with Sealapex showed similar infiltration among the groups where the post space preparation was performed immediately and after 30 days of root canal filling.

The results of the present study regarding the moment of post space preparation agreed with the ones of Romeiro et al,[7] who obtained better results with N-Rickert sealer in the immediate post space preparation and with the sealers Alpha Canal and Fill Canal after 72 hours and one week of root canal filling. The infiltration increased over the time of post space preparation for all sealers.

The results obtained by Valera et al[10] can be compared to the present report, as in both studies the dyer immersion was performed under vacuum condition. These authors did not find significant differences

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between the post space preparation immediately and 7 days after filling with Sealapex. Similarly, the present study verified that immediate post space preparation or after 30 days of root canal filling with Sealapex did not differ in the amount of infiltration measured.

We agree with Valera et al,[10] who stated that the endodontist should perform the post space preparation, mainly because the endodontist often faces the morphological variations of the root canals, its inclination or incorrect teeth placement in the dental arcade. Such factors may influence in the direction of the post space preparation. Besides, it would reduce the risks of accidents related to the post space preparation.

It should also be taken into account that generally after post space preparation, a period of time is required until the post can finally be installed, when the contamination of the prepared portion can occur. The empty space created by the post space preparation might became a saliva and bacteria reservoir. Moreover, the shorter length of the root canal filling and the presence of ramifications in this part of the canal will contribute to the microorganisms diffusion towards the periodontal ligament, which highlights the importance of the coronal marginal leakage in relation to the apical leakage.

The recontamination of the root canal after its filling might be enhanced in the following situations: a) when the patient takes to long to make the final restoration; b) when the temporary sealing is lost; c) when there is a dental fracture.[9] Recontamination may also occur during cementation of provisory crowns during appointments of a prosthetic job.[31]

According to Swanson and Madison,[8] when filled root canals were exposed to artificial saliva coronary infiltration could be observed either after 3 or 8 weeks of exposition. Torabinejad et al[9] verified that root canals completely filled were totally infiltrated by *P. vulgaris* in 10 to 73 days and by *S. epidermidis* in 15 to 51 days. Gish et al[2] observed full bacterial penetrations in filled teeth with post space preparation in 66 and 90 days. Therefore, Magura et al[6] recommend retreatment of root canals if the filling materials were exposed to the oral environment during at least 3 months, as it is considered that not only bacterial infiltration can occur, but also infiltration of its by-products and toxins, which are smaller molecules and infiltrate faster.

This research evaluated the coronal leakage after partial removal of the filling material immediately or after 30 and 60 days of obturation. As it is an in vitro study, many variables of an in vivo situation were not eliminated, such as temperature alteration, pH, masticatory forces, etc. However, infiltration was observed in all specimens. These results agree with previous reports and are an alert to the clinician. Coronary infiltration happens in such a frequency that special cares, from the post space preparation to the post cementation, must be taken to prevent contamination or recontamination of the root canal already filled. Therefore, asepsis of the space prepared for post must be kept during all clinical procedures.

Regarding the special care mentioned above we would like to add to the observation of Valera et al[10] that not only the endodontist should be the one to prepare the post space, but also it would be ideal an post cementation in the same appointment, under rubber dam isolation, which will, therefore, reduce the chances of contamination of the space created in the root canal. Nevertheless, it would only be possible if pre-fabricated posts were used.

Conclusion

According to the experimental conditions and the results of the present research it was possible to conclude that the immediate post space preparation resulted in smaller marginal infiltration than the preparation after 30 and 60 days of the root canal filling. Root canals filled with Sealapex and Topseal sealers showed smaller marginal coronary infiltration than the root canals filled with Endométhazone when the post space was prepared immediately after obturation. Post space preparation performed 30 and 60 days after root canal filling compromised the marginal coronary sealing when Endométhazone and Topseal were used and after 60 days when Sealapex was used.

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Evaluation of Cell Pack paper points: A microbiological study

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ABSTRACT

Introduction: The presence of humidity inside the root canal system after instrumentation and removal of the septic content can influence the apex sealing and, consequently, the success of the endodontic treatment. **Objective:** To evaluate the efficacy of the sterilization method used in these cell-packed paper points; to evaluate whether paper points were contaminated after opening, to evaluate the effectiveness of the autoclave to sterilize the contaminated paper points in their cell packs, and to evaluate whether the calibration of these paper points can contaminate them. **Methods:** Dentsply, EndoPoints, Precise, Protaper, SybronEndo, VDW and Tanari cell-packed paper points were used. Evaluations were made according to propositions. In all steps, the paper points were placed onto brain heart infusion broth and incubated at 37° C in a CO_2 atmosphere for 7 days. The BHI broth was checked daily for appearance of turbidity. **Results:** No paper point removed directly from the cell pack presented contamination; however, contamination was observed when the cell packs were violated; after sterilization, the contaminated paper points were decontaminated; and finally, no contamination was found in paper points seized with sterilized tweezers and placed on a sterilized millimeter ruler. **Conclusion:** The cell-packed paper points are sterilized and when violated during handling, autoclaving is necessary and effective.

Keywords: Endodontics. Dental sealers. Sterilization.

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Introduction

The aim of the root canal filling is to fill the space previously occupied by the pulp, making this space impermeable and blocking its communication with the periapical tissues.[1] Drying the root canal is therefore an important aspect for a successful hermetic sealing as both adhesion and physicochemical properties of the filling materials are altered by moisture.[2,3]

The use of absorbent paper points after root canal aspiration is the most used method to obtain dry walls before filling.[4,5,6] Although these paper points are manufactured under aseptic conditions and present some antibacterial action,[7] they can be contaminated by physical sources during storage process, handling or aerosols after opening.

Leonardo et al[8] have evaluated the sterility of 96 absorbent paper points (Tanari, Conne and Odahcan) immediately after being removed from their sealed packages, and it was found that all brands showed some degree of contamination. Tartarotti et al[9,10] have also evaluated such a contamination in the box of absorbent paper points used in endodontic practice by the students of dentistry and observed that all paper points were contaminated. Due to the contamination of paper points in single-box, manufacturers began to make cell-pack boxes, where the paper points are sterilized and packed individually in small numbers to prevent this type of contamination.

Based on the findings above, the purposes of this study were: 1) to evaluate the efficacy of the sterilization method used in these cell-packed paper points; 2) to evaluate whether the paper points were contaminated after the cell packs were opened, 3) to evaluate the effectiveness of the autoclave to sterilize the contaminated paper points in their cell packs and, 4) to evaluate whether the calibration of these paper points can contaminate them.

Material and Methods

The brands of cell-pack boxes evaluated in this work were: Dentsply (Dentsply Maillefer, Petrópolis, Brazil), EndoPoints (EndoPoints, Paraíba do Sul, Brazil), Precise (Precise Dental Products, Canoga Park, CA, USA), Protaper (Dentsply Tulsa Dental, Tulsa, Ok, USA), SybronEndo (SybronEndo, Orange, CA, USA), Tanari (Tanarinan Industrial, Manaus, Brazil), and VDW (VDW, GmbH, Munich, Germany).

Analysis of the sterilization process in cell-packed paper points

To evaluate the sterilization of the cell-packed paper points, ten cells of each brand were used. The groups evaluated were the following:

» Positive controls: Groups 1 to 7 represent positive controls of Dentsply (G1), EndoPoints (G2), Precise (G3), Protaper (G4), SybronEndo (G5), Tanari (G6) and VDW (G7), respectively. The paper points were removed from their cell packs, manipulated with gloves, contaminated in the clinical setting, and placed onto brain heart infusion broth (BHI, Hi-Media, Mumbai, India).

» Negative controls: Groups 8 to 14 correspond to negative controls of Dentsply (G8), EndoPoints (G9), Precise (G10), Protaper (G11), SybronEndo (G12), Tanari (G13) and, VDW (G14), respectively. In this case, the paper points were removed from their cell packs, placed on appropriate paper, autoclaved and placed onto BHI medium.

» Experimental groups: The paper points were removed from their cell packs and immediately placed onto BHI medium as follows: Dentsply (G15), EndoPoints (G16), Precise (G17), Protaper (G18), SybronEndo (G19), Tanari (G20) and VDW (G21), respectively.

Analysis of paper points after opening the cell packs

In this step, ten cell packs were randomly selected for study. The cells remained partially opened in clinical setting during one month. Then, they were removed from their cells and placed onto BHI culture medium.

Analysis of the sterilization of the contaminated paper points

Ten cell packs of each brand were selected and used. The cells remained partially opened in clinical setting during one month. To ensure the total contamination of the paper points, they were manipulated with contaminated gloves. Then the cell packs with their contaminated points were placed on appropriate paper and autoclaved. Then, the points were removed from the cell packs and placed onto BHI.

Simulating clinical use

Again, ten cell packs were randomly selected for study. This step was carried out in clinical setting.

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The paper points were seized with sterilized tweezers and placed on a sterilized millimeter ruler to simulate the contact, which occurred during endodontic therapy. Next, the points were placed in sterilized Eppendorf tubes to simulate the placement into the root canal. After that, the samples were removed from the tubes and placed onto BHI.

In all steps, the tubes were vortexed and incubated at 37° C in a CO_2 atmosphere for 7 days, with BHI broth being checked daily for appearance of turbidity. After this period, blood agar plates were inoculated with 10 mL from each tube and left at 37° C for 24-48 days in appropriate gaseous conditions in order to investigate all possible bacterial growth.[10] The data were evaluated qualitatively by the presence of turbidity broth (Fig 1).

Results

Regarding the analyses of the sterilization process in cell-packed paper points, in all samples, excluding positive controls (groups 1 to 7), the point cells were free of contamination.

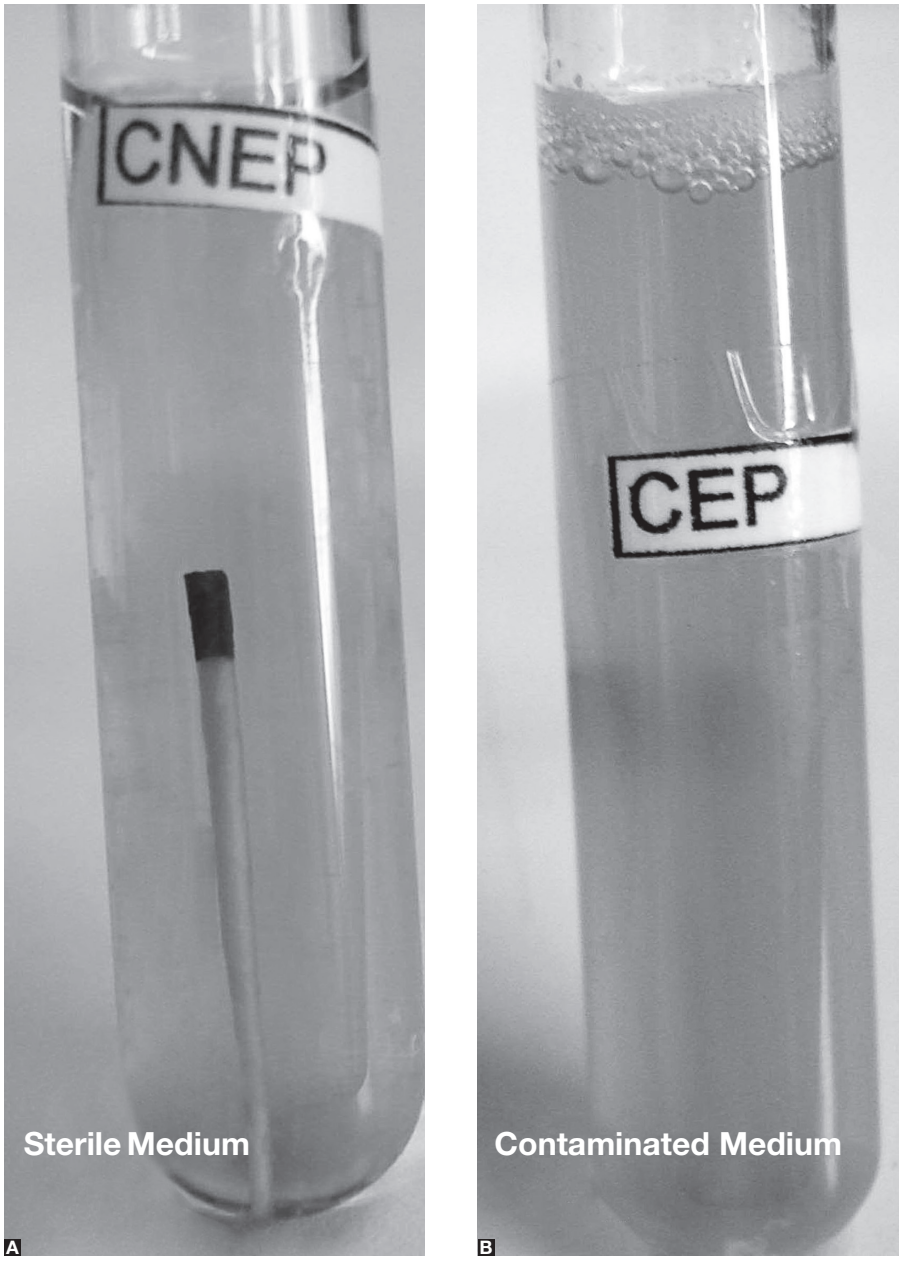


Figure 1. Broth appearance.

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After exposure to clinical setting during one month, all cell packs were shown to be contaminated, and following the process of sterilization, no degree of contamination was found in these point cells.

With regard to the clinical application, the paper points seized with sterilized tweezers and placed on a millimeter ruler showed no contamination

Discussion

Endodontic therapy is associated with infection control and the maintenance of the aseptic chain is crucial to provide a better prognosis.[11] Thus, the use of sterilized paper point is essential during this therapy.

Although the literature presents many studies evaluating the absorbing capacity of paper points,[12-16] which is directly associated with the seal of the filling[2,3] few studies on microbiological evaluation are available.

In the present study, it was initially evaluated whether the cell-packed paper points were sterilized. According to the results, the point cells from all brands evaluated were sterilized. In this aspect, all manufactures, excluding the EndoPoints brand, affirm that cell packs were sterilized. Almeida et al[17] observed contamination in EndoPoints brand in their study. They also reported that these cells were easily violated during the handling.

These are packed side by side and during the removal of one cell, violation of the adjacent cell can occur. Thus, this study also evaluated whether the paper points were contaminated after the cell packs were violated and remained in clinical setting. The results showed that after violation the contamination occurred in all points.

As this point cells can be violated and the EndoPoints manufacture did not report the effectiveness of its sterilization process, the present study evaluated the efficacy of autoclaving process to sterilize the point in their cells and observed that this process was effectiveness. So, when the cell packs are violated, autoclaving of these points is necessary. With regard to this aspect, the literature shows that the sterilizing process does not affect the absorption capacity of paper point when submitted to fewer cycles.[18]

Finally, the present study has evaluated the clinical environment associated with the interactions between these paper points and endodontic instruments. This step of the endodontic therapy is critical because paper points are not like gutta-percha and Resilon points as the latter can be disinfected by using substances such as sodium hypochlorite and chlorhexidine.[19,20,21] Thus, care with sterilized paper points is crucial to avoid contamination of the root canal through them. The use of a sterilized millimeter ruler is essential at the moment of calibration and measurement of the paper point so that the root canal is not contaminated.

Conclusions

The cell-packed paper points are sterilized and when these cells were violated during handling, autoclaving this package is necessary and effective

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Subcutaneous tissue reaction to modified Portland cement (CPM)

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ABSTRACT

Introduction: The aim of this study was to evaluate the rat subcutaneous tissue response to implanted polyethylene tubes filled with Modified Portland Cement (CPM®) (Egeo S.R.L., Buenos Aires, Argentina) compared with Angelus MTA® (Angelus, Londrina, Brazil). **Methods:** These materials were placed in polyethylene tubes and implanted into dorsal connective tissue of Wistar rats for 7, 15, 30, 60, and 90 days. The specimens were prepared and stained with hematoxylin and eosin or Von Kossa or not stained for polarized light. Qualitative and quantitative evaluations of the reaction were performed. **Results:** Both materials caused moderate reactions at 7 days that decreased with time. Angelus MTA® caused mild reactions at 15 days that decreased with time. The response was similar to the control on 30, 60 and 90 days with CPM® and Angelus MTA®. Mineralization and birefringent to the polarized light granulations were observed with both materials. **Conclusions:** It was possible to conclude that CPM® and Angelus MTA® were biocompatible in the rat model, and that they stimulated mineralization.

Keywords: Biocompatibility. Connective tissue. Mineral trioxide aggregate.

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Introduction

Endodontic repair materials should adhere to tooth structure, maintain a sufficient seal, be insoluble in tissue fluids, dimensionally stable, non-resorbable, radiopaque, and exhibit biocompatibility if not bioactivity. A number of materials have historically been used in apical surgeries and perforation but none has been able to satisfy the total requirements of an ideal material.[1]

Mineral trioxide aggregate (MTA) is a biomaterial that has been investigated for endodontic applications since the early 1990`s and was first described in the dental scientific literature in 1993.[2] It received approval for endodontic use by the U.S. Food and Drug Administration in 1998.[3] Studies have shown that MTA promotes favorable tissue reactions characterized by the absence of severe inflammatory responses, the presence of a fibrous capsule, and the induction of mineralized repair tissue.[4,5,6] MTA has been used for both surgical and nonsurgical applications, such as root-end filling, perforation repair, direct pulp capping, and apexification.[4-8] Compared with other filling materials, MTA has good sealing ability, superior biocompatibility and antimicrobial activity.[9-12]

The MTA powder is mixed with sterile water and it is recommended that a moist cotton ball be temporarily placed in direct contact with the material and left until a follow-up appointment. Upon hydration, MTA materials form a colloidal gel that solidifies to a hard structure in approximately 3-4 h,[1,13,14] with moisture from the surrounding tissues supposedly assisting the setting reaction.[5] Hydrated MTA products have an initial pH of 10.2, which rises to 12.5 three hours after mixing.[1,14,15] The setting process is described as a hydration reaction of tricalcium silicate ($3CaO.SiO_2$) and dicalcium silicate ($2CaO.SiO_2$), which is the latter said to be responsible for the development of material strength.[1,13] Although weaker than other materials used for similar purposes, MTA compressive strength has been reported to increase in the presence of moisture for up to 21 days.[1,14] In 2001, the company Angelus Soluções Odontológicas introduced a MTA developed in Brazil, which is similar to the Modified Portland Cement. The powder also consists of fine hydrophilic particles that form a colloidal gel in presence of moisture, solidifying to form a hard sealer in one hour.[16,18] The presence of calcium carbonate is the most significant difference, which has been reported to increase the release of calcium ions, but maintaining the sealability, adhesion, flow rate, and biocompatibility.[19,20] Its package insert states that the chemical composition is similar to the MTA, except for the addition of calcium carbonate to reduce the pH from 12.5 to 10.0 after setting and to restrict the surface necrosis in contact with the material.

Although CPM® apparently presents positive characteristics, there is no study evaluating the reaction after its implantation. Thus, the aim of this study was to evaluate the tissue response of CPM® (Egeo S.R.L., Buenos Aires, Argentina), compared to Angelus MTA® (Angelus, Londrina, Brazil) in a rat model.

Material and methods

Thirty male 4- to 6-month-old *Wistar albino* rats, weighing 250 to 280 g, were used. The animals were housed in temperature-controlled rooms and received water and food *ad libitum*. The care of the animals was performed according to the Araçatuba Dental School/UNESP ethical committee, which approved the project before the beginning of the experiments.

Sixty polyethylene tubes (Abbott Lab of Brazil, São Paulo, SP, Brazil) with 1.0-mm internal diameter, 1.6-mm external diameter, and 10.0-mm length were filled with the tested materials. The Angelus MTA® and the CPM® were prepared according to the manufacturer's recommendations and inserted into the tubes (30 tubes for each material) with a Lentulo spiral (Maillefer Dentsply, Tulsa, USA). Thirty extra polyethylene tubes remained empty and were used as control.

The animals received antisepsis with 5% iodine solution and then shaved under xylazine (10 mg/kg) and ketamine (25 mg/kg) anesthesia. The shaved backs received a 2-cm incision in a head-to-tail orientation with a #15 Bard-Parker™ blade (Franklin Lakes, NJ). The skin was reflected, creating two pockets in one side of the incision, one in the cranial portion and other in the caudal portion distant 6 cm from each other, and another pocket in the opposite side of the incision. The tubes were implanted into the spaces created with blunt dissection, and the skin was closed with 4/0 silk suture.

After 7, 15, 30, 60, and 90 days from the implantation time, six animals were killed by overdose of anesthetic solution, and the tubes with surrounding tissues were removed and fixed in 10% buffered formalin at pH 7.0.[21,22] The tubes were bisected transversely and both halves were cut again longitudinally with the use of a sharp blade. This procedure was done to allow the

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surfaces to be readily kept in contact with the processing solutions. The specimens were processed for glycol methacrylate embedding, serially sectioned into 3-µm cuts, and stained with hematoxylin-eosin.[23] The 10-µm cuts were stained according to the Von Kossa technique or remained without staining to be observed under polarized light. The Von Kossa technique was used to observe mineralized structures in the tissue, which were dark stained. Polarized light technique allows the observation of birefringent structures related to calcium carbonate crystals generated from the combination of calcium ions from the material and carbonic gas from the tissue.[24]

Reactions in the tissue in contact with the material on the opening of the tube were scored according to previous studies[22,25,26] as: 0= none or few inflammatory cells and no reaction; 1= less than 25 cells and mild reaction; 2= between 25 and 125 cells and moderate reaction; and 3= 125 or more cells and severe reaction. Fibrous capsules were considered to be thin when thickness was <150 µm and thick at >150 µm. Necrosis and calcification were recorded as present or absent. The observer was blinded to treatment allocation. An average of the number of cells for each group was obtained from 10 separate areas. Results were statistically analyzed by analysis of variance (one-way ANOVA) and Kruskal-Wallis tests at 5% of significance.

Results

Angelus MTA®

On the 7th day, a moderate inflammatory cell infiltration was present in a fibrous capsule (Fig 1A). The intensity of the inflammation was reduced on days 15, 30, 60, and 90 with a thin fibrous capsule near the tube and small number of inflammatory cells (Figs 1B, C and D). Granulations birefringent to polarized light and Von Kossa positive structures were observed near the tube opening (Figs 2A, B, C and D).

CPM

On days 7 and 15, a moderate inflammatory cell infiltration consisting of lymphocytes and macrophages was present in a fibrous capsule (Fig 1E). The intensity of the inflammation and the thickness of the fibrous capsule were reduced on day 30, similar to the control group (Fig 1F). On days 60 and 90, few chronic inflammatory cells were present in a thin fibrous capsule similar to the Angelus MTA® and the control group (Figs 1G and H). Granulations birefringent to polarized light and Von Kossa positive structures were observed in close contact with the CPM® (Figs 2E, F, G and H). It was observed that there was tissue growing into the tubes for many of the examined specimens.

Control (empty tubes)

On days 7 and 15, a moderate chronic inflammatory cell infiltration consisting of lymphocytes and macrophages was present in a fibrous capsule (Fig 1I). The fibrous capsule surrounding the tube was thin with few chronic inflammatory cells present at 30, 60, and 90 days (Figs 1J, K and L). The empty tubes were not positive to Von Kossa stain, and no birefringent structures were observed under the polarized light (Figs 2I, J, K and L).

Comparison among the groups

The data were compared in each period of time and are present in Table 1.

Day 7

There was no statistically significant difference among the scores of the different groups (median = score 2).

Day 15

There was a statistically difference ($p<0.05$) between inflammatory cell numbers with Angelus MTA® and the other groups ($p<0.05$). The median inflammatory cell score for MTA (median score = 1) was lower than the other groups (median score = 2) ($p <0.05$).

Days 30, 60 and 90

There was no statistically significant difference ($p>0.05$) among the scores of the different groups (median score = 1).

Discussion

The physical properties, sealing ability, biocompatibility, and clinical performance of MTA materials have been reported. MTA materials appear not only to demonstrate acceptable biocompatibility behavior but also exhibit acceptable in vivo biologic performance when used for root-end fillings, perforation repairs, pulp-capping and pulpotomy, and apexification treatment.[27]

The response to empty tubes in this study showed minor reactions in subcutaneous connective tissues, consistent with results previously reported.[22-26,28]

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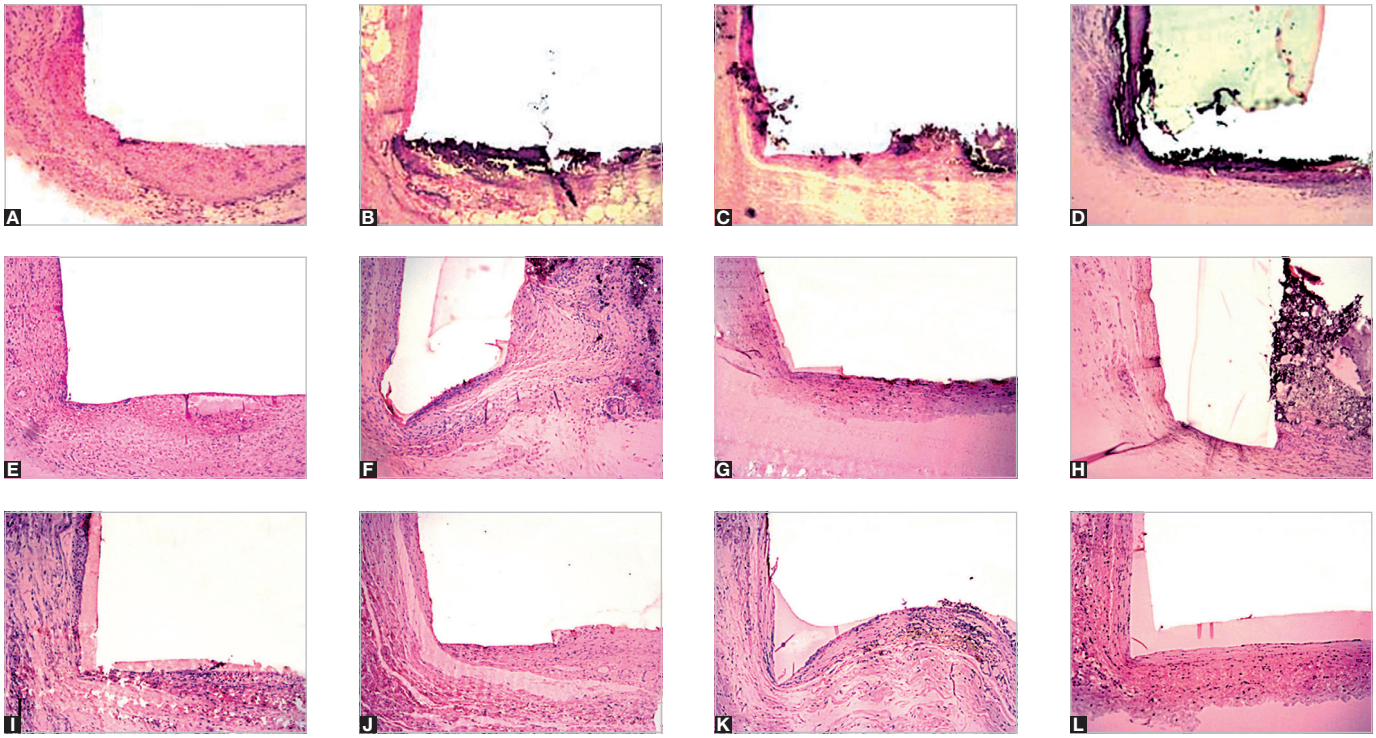


Figure 1. Angelus MTA: **A**) thick fibrous capsule and moderate inflammatory cell infiltration consisting of lymphocytes and macrophages (7 days, hematoxylin and eosin, 10x), and **B**-**D**) the fibrous capsule surrounding the tube was thin with few chronic inflammatory cells (30, 60, and 90 days, respectively; hematoxylin and eosin, 10x). CPM: **E**) thick fibrous capsule formation and moderate inflammatory cell infiltration (7 days, hematoxylin and eosin, 10x), and **F**-**H**) mild chronic inflammatory cell infiltration in a thin fibrous capsule (30, 60, and 90 days, respectively; hematoxylin and eosin, 10x). Control: **I**) moderate chronic inflammatory cell infiltration and thick fibrous capsule formation (7 days, hematoxylin and eosin, 10x), **J**-**L**) reduction in thickness of fibrous capsule and inflammatory cell near tube infiltration (30, 60, and 90 days, respectively; hematoxylin and eosin, 10x).

Table 1. Percentage of samples in each group classified according to the index of inflammation, presence of necrosis and fibrous capsule thickness.

MATERIAL/ TIME	SCORE				Calcification
	0	1	2	3	
7 days					
MTA	0	0	100	0	100
CPM	0	0	100	0	100
Control	0	0	100	0	0
15 days					
MTA	0	100	0	0	100
CPM	0	0	100	0	100
Control	0	0	100	0	0
30 days					
MTA	0	100	0	0	100
CPM	0	100	0	0	100
Control	0	100	0	0	0
60 days					
MTA	0	100	0	0	100
CPM	0	100	0	0	100
Control	0	100	0	0	0
90 days					
MTA	0	100	0	0	100
CPM	0	100	0	0	100
Control	0	100	0	0	0

An adequate tissue repair was found in the connective tissue around the tubes containing Ângelus® MTA and it showed a mild chronic inflammatory response at 30 days, characterized by organized connective tissue, with presence of some macrophages and inflammatory multinuclear cells, similar to other studies.[11,24,25,29] In all periods of time, Von Kossa areas positive for calcium and structures birefringent to polarized light were observed for all specimen, which were similar to the results previously reported.[24] It was possible to observe at 30 days that Ângelus® MTA also promoted a tissue response characterized by a fibrous capsule, a mild inflammatory infiltrate, in accordance with other studies.[24,25] At 60 and 90 days, it was possible to notice a decrease of the inflammatory cells number and thickness of fibrous capsule adjacent to the tube opening, similar to that described in the literature.[24,25]

Moreover, the results of the present study taken together with those previously reported corroborate with the promotion of dystrophic calcifications by MTA in contact with connective tissues. These calcifications can be originated from the calcium oxide present in MTA.[24]

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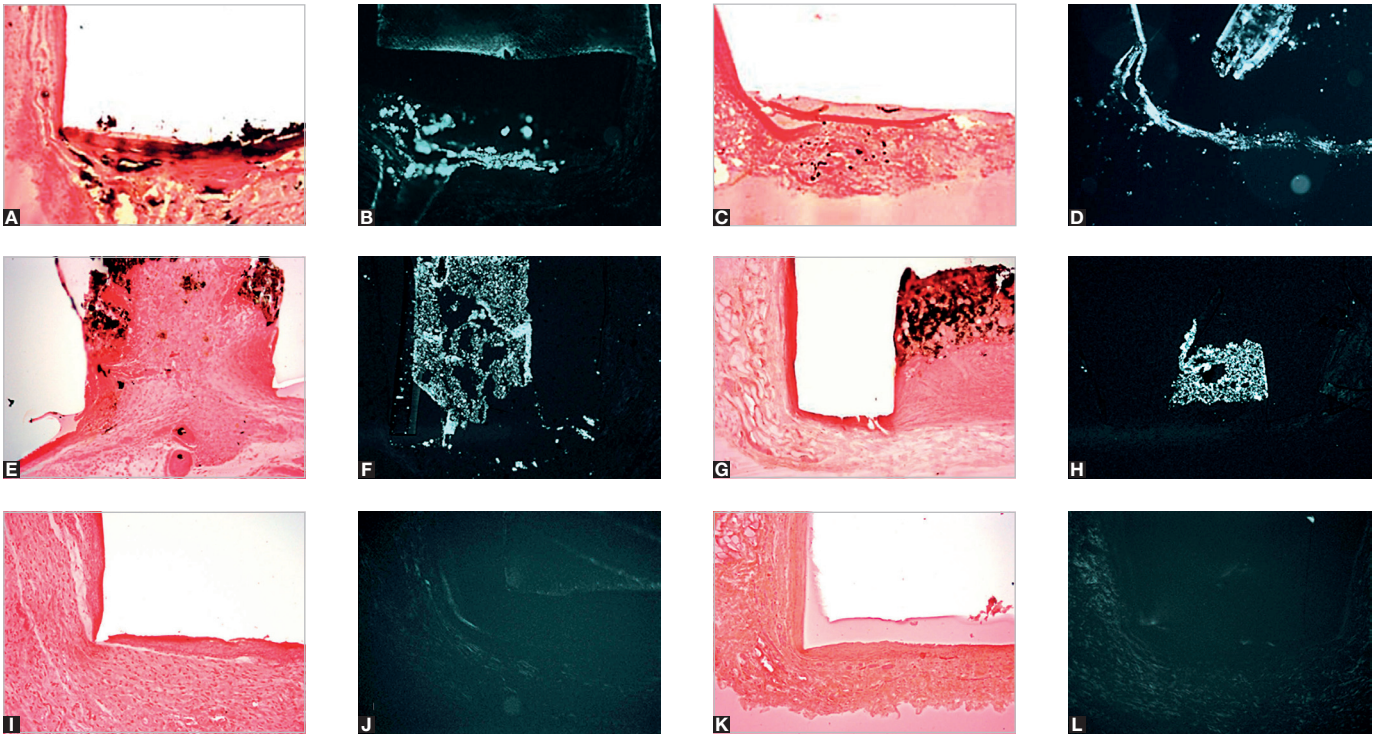


Figure 2. Angelus MTA: **A** and **C**) presence of dystrophic calcification on the tube opening (30 and 90 days, respectively; Von Kossa, 10x); **B** and **D**) presence of structures birefringent to polarized light (30 and 90 days, respectively; polarized light, 10x). CPM: **E** and **G**) presence of dystrophic calcification on the tube opening (30 and 90 days, respectively; Von Kossa, 10x); **F** and **H**) presence of structures birefringent to polarized light (30 and 90 days, respectively; polarized light, 10x). Control: **I** and **K**) absence of dystrophic calcification on the tube opening (30 and 90 days, respectively; Von Kossa, 10x); **J** and **L**) absence of structures birefringent to polarized light (30 and 90 days, respectively; polarized light, 10x).

This component, when in contact with water can be converted into calcium hydroxide and dissociated in Ca_2^+ and OH^-. The diffusion of hydroxyl ions from the root canal raises the pH at the surface of root adjacent to the periodontal tissues, thereby possibly interfering with osteoclastic activity, and promotes an alkalinization in the adjacent tissues favoring the healing process.[30] Calcium ions are important due to their participation in the activation of calcium-dependant adenosine triphosphatase.[31] Calcium reacts with carbonic gas to form calcium carbonate crystals (birefringent to polarized light), which serve as a nucleus for calcification, and favors mineralization.[31] A rich extra-cellular fibronectin network in close contact with these crystals strongly support the role of calcite crystals and fibronectin as an initiating step in the formation of a hard tissue.[31] Calcium is also needed for cell migration and differentiation.[32]

According to the manufacturer, CPM® has similar properties compared to MTA and the same clinical indications.[2] In this study, the results observed with CPM® were similar to the control and MTA groups, showing a moderate chronic inflammatory response in the initial periods that reduced with time. Positive Von Kossa areas and structures birefringent under polarized light were also observed showing that this material stimulated the formation of mineralized tissue in subcutaneous tissue of rats similarly to that observed with MTA.[24] Although the manufacturer stated that calcium carbonate had been added to the material to reduce the pH from 12.5 to 10.0, it was not observed changes in the biological behavior of CPM® when compared to the MTA.

An interesting fact observed with CPM® was the tissue ingrown into the tube for many of the specimens examined, that showed a high solubility of the material. A material with high solubility is not suitable for the endodontic use since it may not promote an adequate sealing, thereby increasing the probability of failure in endodontic treatment. Previous results showed poorer sealability of CPM® in comparison to Angelus MTA® when used as apical plug.[16]

In conclusion, the tissue reactions observed with CPM® were similar to those observed and reported for Angelus MTA®. Other studies are necessary to better analyze the behavior of this material.

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Antibacterial capacity of different intracanal medications on *Enterococcus faecalis*

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ABSTRACT

Objective: To evaluate the antimicrobial activity of 2% chlorhexidine gel, copaiba oil, propolis extract and calcium hydroxide associated to propylene glycol on *Enterococcus faecalis*. **Methods:** Fifty single-rooted human teeth were prepared until K-file #50 and distributed into groups according to the intracanal substances. The positive control group was only propylene glycol. Subsequently, 100µL of microorganism broth was inoculated into the roots, except in the negative control group. Then the roots were placed in individual test tubes immersed in BHI and put into incubator at 37° C for 48 hours. After the turbidity of the medium, the roots were irrigated with sodium thiosulfate, filled with one of the test substances and immersed in BHI for 7 days at 37° C. Thereafter, the drugs were removed from the roots with help of K-files and abundant irrigation with sodium thiosulfate. The roots were immersed in BHI for 24 hours. After this, the tubes were analyzed by single trained examiner to the categorization of the culture medium turbidity. **Conclusion:** According to this methodology, it was possible to conclude that none substance was effective against *Enterocossus faecalis*.

Keywords: Calcium hydroxide. Chlorhexidine. Enterococcus faecalis. Intracanal dressing. Propolis.

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Introduction

The success of the endodontic treatment is closely related to the disinfection of the root canal system, obtained by the emptying, enlargement, action of the irrigant solution and intracanal dressing.[1,2,3] The chemico-mechanical preparation is effective to reduce the microbiota in the root canal lumen, however the microorganisms located in some areas of the canal, such as the non-mechanically prepared walls by the endodontic instruments, dentin tubules, isthmus, lateral canals and apical ramifications can show treatment-resistant due to the biofilm organization.[5,6] Persistent apical lesions that not reduced after the appropriate endodontic treatment are caused by the permanence of the pathogenic bacteria in the inner of the root canal system.[1]

Approximately 700 specimens of microorganisms compose the oral microflora.[7,8] *Enterococcus faecalis* are facultatively anaerobic bacteria, gram positive; forming a part of the intestinal microflora and commonly present in infections, for example, urinary tract and endocardium.[9,10] Rarely is found in cases of primary endodontic infections, but in cases of retreatment, represents between 38 to 70% of the microbiota.[9,10] Plays important role in the etiology of the endodontic infection in function of the particular strategy in the biofilm formation, virulence factors, adhesion to dentin collagen, survival to critic medium and resistance to endodontic therapy.[11]

Endodontic irrigants and intracanal dressing with antimicrobial action develop an important role in the way of reducing or completely eliminating the niches of bacterial colonization.[12] In this context, the calcium hydroxide, used in Dentistry since the beginning of twentieth century, presents an excellent ability to contribute in the repair of periapical lesions, anti-exsudative action and activity of induction the mineralization.[13] Chlorhexidine gluconate solution, in function of its substantivity and large antimicrobial spectrum has been proposed for this purpose, however due to its concentration of 2% (toxic to the most living tissues), inability of the inactivation of bacterial LPS and pulpal tissue dissolution, needs more rationale to be duly indicated.[2,14] However, the complete eradication of the microbiota in the root canal is not guaranteed with the use of these substances, needing the search for new medications for this purpose.[15]

Recently, products with natural origin are gaining space in the dentistry,[16,17] most of this related to undesirable properties of the classic solutions used with this objective.[18] One of these substances, the extract of propolis is the major substance in healthcare because of its therapeutic properties.[15,16,17] The antimicrobial characteristic is related to the presence of flavonoids and esters in its composition, in addition to being approximately 10 times less cytotoxic than the calcium hydroxide.[19] The copaiba oil, used a long time ago in empirical way by the indigenous people, spurred the interest of the scientific community, resulting in studies that confirmed its antibacterial activity.[20,21] Considering the importance of searching new medicaments for the endodontic therapy, the present study aims to evaluate the antibacterial effect of the extract of propolis and copaiba oil against to *Enterococcus faecalis*.

Material and methods

Fifty single-rooted human teeth, extracted by orthodontic reasons, were selected and prepared by manual technique until to K-file #50 (Maillefer Dentsply, Switzerland). The working length was determined to one millimeter before the radicular vertice. The root canal were irrigated with sodium hypochlorite 1% (Biodinâmica, Ibiporã, Brazil). After the biomechanical preparation, the crowns were removed and the root autoclaved to 121° C for 15 minutes. The specimens were randomly distributed in groups, with 10 units each,



Figure 1. Test substances: 1) Calcium hydroxide, 2) Extract of propolis, 3) Copaiba oil, 4) Chlorhexidine gluconate gel 2%, 5) Propylene glycol.

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in accordance to the test substance (Fig 1), described in Table 1. Ten teeth, distributed equally, formed the positive control group, with propylene glycol, and the negative control group, without microorganism and intracanal dressing. The study was approved by the Ethics and Research Comitee of the University of Cuiabá with protocol number 2011-066.

In sequence, the roots, in the test groups, were inoculated with the *Enterococcus faecalis* (ATCC 29212) broth, with the pipette (Kacil Indústria e Comércio Ltda, Recife, Brazil) of fixed volume patterned with 100μL (Fig 2). All of roots were put in the 8 cm test tubes containing 5 mL of BHI culture medium (Newprov, Pinhais, Brazil) (Fig 3A) and taken to incubator, at 37° C for 48 hours, for the contamination of the dentin tubules. In the end of the period, the tubes were removed for the verification of the medium turbidity, parameter to confirm the microorganism growth in the culture medium (Fig 3B). In the next step, the samples were removed from the culture medium and washed with 10 mL of sodium thiosulfate 10% (Quimesp Química Ltda, Guarulhos, Brazil) (Fig 3C). The external drying was proceeded with a gauze and sterilized absorbent paper cones #50 (Dentsply, Petrópolis, Brazil) (Fig 3D) used for inner purpose.

The calcium hydroxide paste (Biodinâmica, Ibiporã, Brazil) was prepared in a toothpaste consistence and inserted in the roots of the CH group, by a K-file #25 (Maillefer Dentsply, Switzerland) (Fig 4A).

Table 1. Test substances and manufacturers.

Substance	Manufacturers
Copaiba Oil (CO)	Flores e Ervas Produtos Naturais Ltda.
Extract of Propolis (EP)	Flores e Ervas Produtos Naturais Ltda.
Calcium Hydroxide (CH)	Biodinâmica Química e Farmacêutica Ltda.
Chlrohexidine gluconate 2% (C)	Biológica Comércio e Manipulação de Medicamentos Ltda.
Propylene glycol (P)	Biológica Comércio e Manipulação de Medicamentos Ltda.



Figure 2. Inoculation of root canals in test groups with the broth of *Enterococcus faecalis* (ATCC 29212) through the pipette.

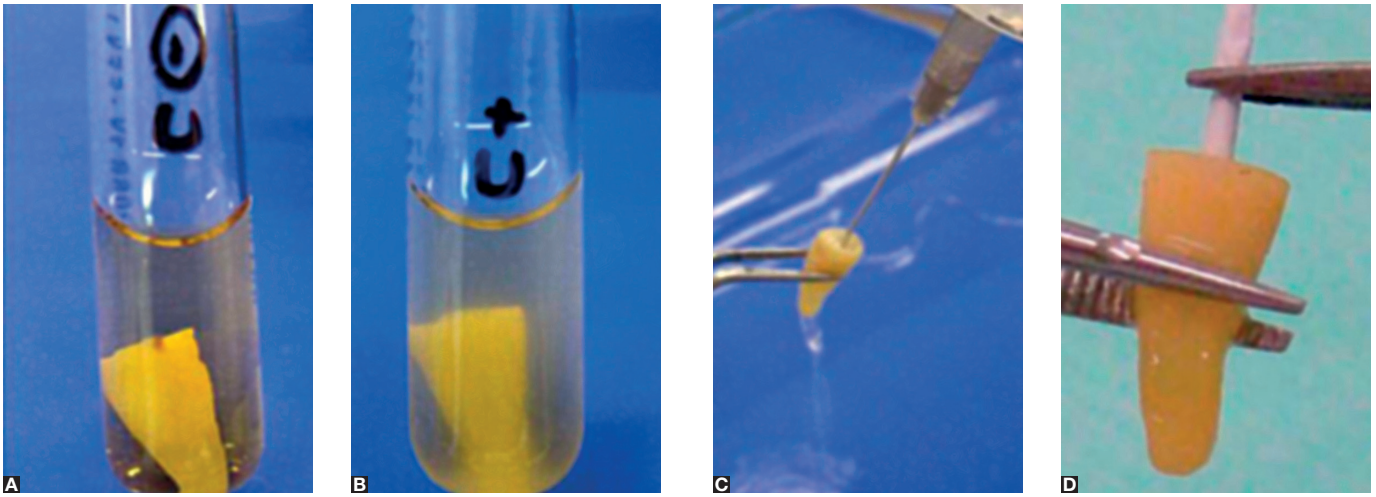


Figure 3. A) Roots in test tubes, containing 5 mL of BHI culture medium. **B**) Verification of medium turbidity, parameter to confirm microorganism growth of the culture medium. **C**) Samples washed with 10 mL of sodium thiosulfate 10% **D**) Drying of the roots with absorbent sterilized paper cones # 50.

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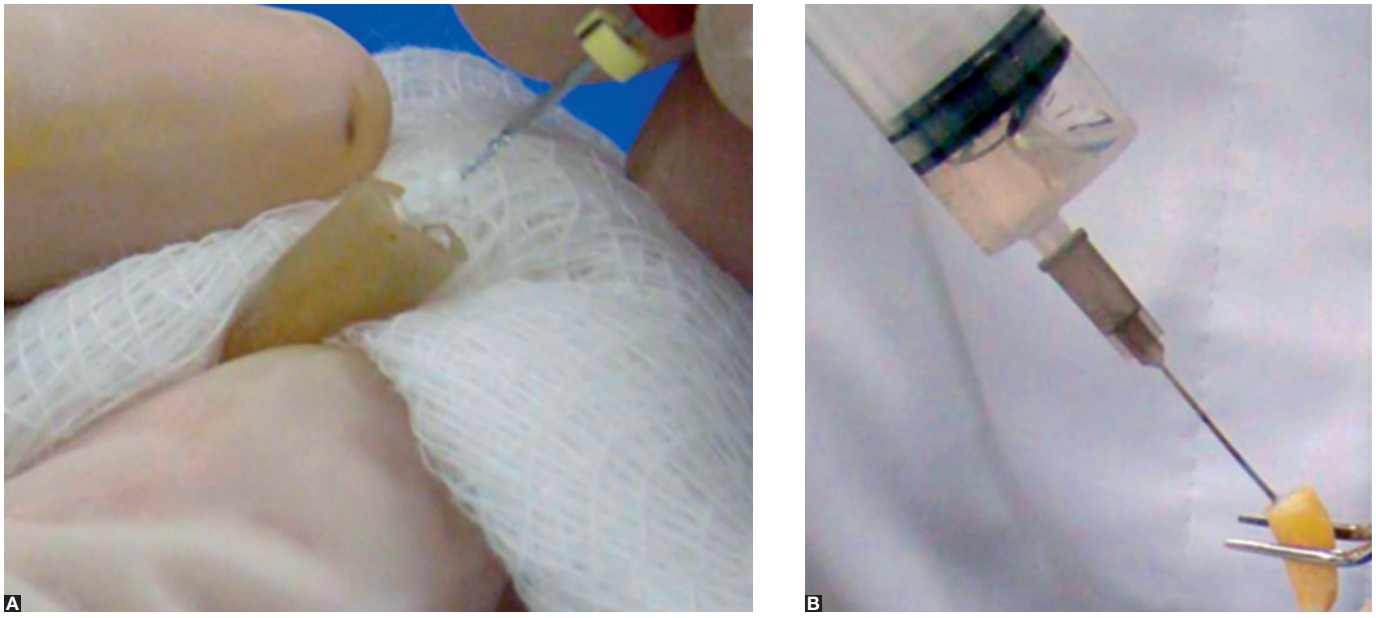


Figure 4. A) Insertion of calcium hydroxide paste, in toothpaste consistence, in roots by K-files #25. **B**) Insertion of others test substances in root canals according to each group.

The propylene glycol (Biológica Comércio e Manipulação de Medicamentos Ltda., Cuiabá, Brazil), copaiba oil (Flores e Ervas Produtos Naturais Ltda., Piracicaba, Brazil), extract of propolis (Flores e Ervas Produtos Naturais Ltda., Piracicaba, Brazil) and chlorhexidine gel 2% (Biológica Comércio e Manipulação de Medicamentos Ltda., Cuiabá, Brazil) were removed from the respective recipients by disposable syringes and inserted in the root canals of each test group (Fig 4B). The roots in the control groups did not receive microorganisms inoculation and no intracanal dressing.

A small sterile cotton pellet was inserted in the root canal entrance and again immersed in the BHI broth, taken to the incubator at 37° C for 7 days. After this period, the medications were removed from the root canals by K-files #25 (Maillefer Dentsply, Switzerland) and abundantly irrigated with 10 mL of sodium thiosulfate 10%. In continuous act, the roots were externally dried with gauze, internally with sterilized absorbent paper cones #50 (Dentsply, Petrópolis, Brazil) and taken in BHI broth at 37° C for 24 hours. The test tubes were analyzed by a unique calibrated examinator for the culture medium ranking, indicating the contamination presence.

Results

The results obtained in relation to the effectiveness of all of the substances were not efficient to the bacterial control, described in the Table 2.

Table 2. Values of presence or absence of culture medium turbidity.

Groups	
Copaiba oil	+ + +
Chlorhexidine gluconate gel 2%	+ + +
Extract of propolis	+ + +
Calcium hydroxide + propylene glycol	+ + +
Positive control (propylene glycol)	+ + +
Negative control	– – –

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Discussion

Rests of pulpar tissues, bacteria and dentinal debris can persist the irregularities of the root canal system, even after the use of intracanal dressing, avoiding the endodontic treatment success.[1,2,3,12] For this study, the *Enterococcus faecalis* was selected in function of its microbiological role in the persistent infection processes in endodontic treatments.[11] Many methods are used to evaluate the antimicrobial activity of the intracanal dressing, and so *in vitro* test has the advantage of easy execution and fast in collecting the results, free of factors that can influence the results, inherent to *in vivo* studies.[4-8,11,13] The BHI culture medium was used for the evaluation because of being rich in appropriated nutrients to cultivate the *E. faecalis*.[22]

Classically, the calcium hydroxide was used to compare the new intracanal dressing substances in reason of been a consensus in its purpose in Endodontics.[12,13] This medication acts in the cellular wall of bacteria, causing damage to bacterial cytoplasmic membrane, protein denature and damage to DNA.[13,23] At same time, by the methodology used in this experiment, should be considered the buffer action of dentin that interferes particularly in the calcium hydroxide antibacterial ability.[24]

Comparatively, in this study, chlrohexidine 2% presented similar antibacterial activity to calcium hydroxide. The antibacterial effect of calcium hydroxide increases with the time, possibly as a result of the slow dissolution of the paste and respective effective diffusion of the hydroxyl ions through the dentin.[12,13] These findings are in agreement to others studies.[12,13,15]

The antimicrobial activity of propolis has been investigate in others studies by the dilution in broth and diffusion tests in agar; however, few are done in the inner of the root canal, environment that neutralize the antibacterial effects of the disinfectants.[7,15,24] In this study, the antibacterial effect of the propolis was similar to the others test substances. The mechanism of action that happen the antibacterial effect of this substance is related to the presence of flavonoids, many esters of caffeic acid and galagine (3,5,7-trihidroxiflavone) and its bioautograme components.[25] These findings are consonant to Oncang et al,[26] however different to others studies in that the extract of propolis presented greater antibacterial effect than the calcium hydroxide.[25,27,28]

Copaiba oil, as related in the literature, presents antibacterial activity against to gram positive bacteria, as it is *E. faecalis*.[21] The substance acts directly in the cellular wall of the bacteria, breaking and releasing cytoplasmic components, with consequent reduction of cell volume, thus proving its effectiveness.[21]

Conclusion

Within the limitations of the method proposed in this study, it was concluded that the tested drug substances were not effective against *E. faecalis*.

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Quality of reminiscent root in endodontically treated teeth with intraradicular retainers

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Mariana de Lima **DIAS**[4]

ABSTRACT

Objective: The objective was to evaluate the quality of root reminiscent of endodontically treated teeth with intraradicular posts. **Methods:** This retrospective study assessed the records from every patient treated in the Integrated Clinic of the Dentistry School of Pernambuco University, from 2006 to 2007, which recorded the presence of an intraradicular post, totalizing 78 patients. Two professionals graduated in dentistry evaluated the intraradicular post and root reminiscent data. **Results:** For 99 of the evaluated teeth, the post length was lower than 2/3 of the root length in 86,87%, and the post diameter was inadequate in 31.31%, 20.20% and 11.11% in the coronal, middle and apical thirds, respectively. In 51.51% there was a void between the root canal filling and the intraradicular post, and the reminiscent of root canal filling was classified as satisfactory in most cases, mean 6.1 mm. Eight cases showed deviation in the root canal shaping for the post. **Conclusion:** The endodontically treated teeth rehabilitated by intraradicular posts did not follow the recommended standards.

Keywords: Post and core technique. Root canal obturation. Mouth rehabilitation.

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Introduction

The rehabilitation of endodontically treated teeth is based in esthetical and physical principles.[1] They are considered less resistant and more leaning to fractures compared to vital teeth.[2] This frailty could be determined by collagen and water loss.[3] However, the main factor to this frailty is structural loss due to tissue damage by carious lesion.[4]

Due to the extensive coronal destruction, usually associated to teeth indicated for the endodontic treatment, these teeth rehabilitation normally needs the use of an intraradicular post to reinforce the restorative materials retention.[5,6]

Despite the efforts to reinforce the endodontically treated teeth, fractures can happen.[1,7] Studies showed that the use of an intraradicular post did not enhance the tooth resistance. This is mainly related to the coronal reminiscent.[5,8,9] In addition, the correct diagnosis of reminiscent structures, root anatomy, periodontium and root canal filling is crucial for a teeth to receive an intraradicular post.[6]

The retention is affected mainly by length, diameter and taper of the post.[10] Even though retention may be enhanced significantly by increasing the post diameter, the loss of structure weakens the tooth. Thus, the root canal shaping must follow principles that aim to maintain the most tissue from the tooth, and the intraradicular post might distribute uniformly the mastication tensions.[2]

Considering the importance to preserve the dental tissues to enhance resistance of endodontically treated teeth, this study aimed to evaluate the quality of root reminiscent of endodontically treated teeth with intraradicular posts.

Material and methods

This retrospective study assessed the records of 78 patients treated in the Integrated Clinic of the Dentistry School of the Pernambuco University from 2006 to 2007, which recorded the presence of an intraradicular post previously to the treatment. Poorly filled records and poorly processed radiographies were the exclusion criteria.

Many data from the records were assessed (Table 1) and recorded in previously made files. A pilot study was performed with 10 records of patient from an anterior period from the defined for the study. The data were evaluated by two examiners, graduated in Dentistry, previously trained and calibrated. When no agreement was reached by the examiners, a third examiner, specialist in Endodontics, made the final decision.

This study was approved by the Ethics Research Committee of the Pernambuco University (Process 124/03).

The data were organized in frequency tables and a descriptive analysis of the data was performed.

Results

Seventy eight patient records were assessed, mean age of 32.4 years (11-65), 54 female (69.23%) and 24 male (30.77%).

From a total of 99 teeth evaluated by the radiographs, 79.80% consisted of maxillary teeth and 20.20% were mandibular teeth. The maxillary incisors were more usually associated to rehabilitation with intraradicular posts (56.57%). Radiolucent image suggestive of periapical lesion was found associated to the teeth assessed in 71.72% of the cases.

The intraradicular post material was metallic in 88.89% and non-metallic in 11.11%.

Table 1. Evaluated data from the files of patients.

Age
Gender
Tooth category
Presence of periapical lesion
Intraradicular post category
Root length
Post length
Post/root ratio
Post diameter
Root dentin reminiscent thickness
Root canal filling reminiscent
Space between root canal filling and intraradicular post
Deviation in the root canal shaping for the intraradicular post

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The mean length of the evaluated roots was 16.1 mm. The Table 2 describes the mean length by tooth category.

The mean length of the intraradicular posts was 8.0 mm. The Table 3 describes the mean length of the posts by tooth category.

The intraradicular post was lower than 2/3 of the root reminiscent length in 86.87% of the teeth (Table 4).

The mean diameter of the intraradicular roots was 1.7 mm, 1.5 mm, 1.2 mm for the coronal, middle and apical thirds, respectively. The mean diameter of the posts by the tooth category is described in Table 5.

The Table 6 describes the thickness of the root dentin reminiscent by the different thirds of the root, assessed in the mesial and distal walls.

The intraradicular post diameter was inadequate in the coronal third in 31.31% of the teeth, in 20.20% for the middle third and 11.11% for the apical third (Table 7).

The root canal filling reminiscent had mean length 6.2 mm. The mean length of the root canal filling reminiscent by tooth category is described in Table 8.

The presence of void between the intraradicular post and the root canal filling was seen in 51 teeth (51,51%). The mean of this void is described by tooth category in the Table 9.

Eight teeth (8.08%) showed deviation in the root canal shaping for the intraradicular post: Six maxillary incisors (75.00%), one maxillary canine (12.50%), and one mandibular molar (12.50%).

Table 2. Mean length of the roots by tooth category.

Upper	Lower
Maxillary Incisors: 16.4 mm	Mandibular Incisors: 14.0 mm
Maxillary Canine: 18.3 mm	Mandibular Canine: 18.0 mm
Maxillary Premolar: 15.1 mm	Mandibular Premolar: 16.6 mm
Maxillary Molar: 14.2 mm	Mandibular Molar: 14.5 mm

Table 3. Mean length of the intraradicular post by tooth category.

Upper	Lower
Maxillary Incisors: 8.1 mm	Mandibular Incisors: 7.0 mm
Maxillary Canine: 11.0 mm	Mandibular Canine: 9.0 mm
Maxillary Premolar: 6.5 mm	Mandibular Premolar: 7.7 mm
Maxillary Molar: 6.2 mm	Mandibular Molar: 9.2 mm

Table 4. Post/root length ratio.

Lower than 2/3 of the root	86 teeth (86.87%)
Higher or equal to 2/3 of the root	13 teeth (13.13%)

Table 5. Mean diameter of the intraradicular posts by the tooth category and root third.

Tooth	Third			Tooth	Third		
	Coronal	Middle	Apical		Coronal	Middle	Apical
Maxillary incisors	1.8 mm	1.6 mm	1.3 mm	Mandibular incisors	1.5 mm	1.5 mm	1.4 mm
Maxillary canine	1.2 mm	1.2 mm	1.0 mm	Mandibular canine	1.5 mm	1.5 mm	1.4 mm
Maxillary premolar	1.5 mm	1.3 mm	1.1 mm	Mandibular premolar	1.7 mm	1.4 mm	1.2 mm
Maxillary molar	1.2 mm	1.1 mm	1.0 mm	Mandibular molar	1.3 mm	1.3 mm	1.0 mm

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Table 6. Thickness of root dentin reminiscent.

Tooth		Third			Tooth		Third		
		Coronal	Middle	Apical			Coronal	Middle	Apical
MxI	M	2.1 mm	2.1 mm	2.0 mm	MdI	M	1.0 mm	1.2 mm	1.3 mm
	D	2.1 mm	2.0 mm	1.8 mm		D	1.3 mm	1.5 mm	1.5 mm
MxC	M	1.9 mm	2.2 mm	2.0 mm	MdC	M	2.3 mm	2.4 mm	2.2 mm
	D	2.0 mm	2.2 mm	2.0 mm		D	2.5 mm	2.4 mm	2.4 mm
MxPM	M	1.8 mm	1.9 mm	1.7 mm	MdPM	M	1.8 mm	2.0 mm	1.8 mm
	D	1.7 mm	1.6 mm	1.6 mm		D	1.7 mm	1.7 mm	1.8 mm
MxM	M	2.1 mm	1.8 mm	1.5 mm	MdM	M	2.1 mm	1.5 mm	1.3 mm
	D	2.1 mm	1.8 mm	1.7 mm		D	2.1 mm	1.7 mm	1.4 mm

Table 7. Post/root thickness ratio.

Larger than 1/3	Coronal	31 teeth (31.31%)
	Middle	20 teeth (20.20%)
	Apical	11 teeth (11.11%)
Lower or equal to 1/3	Coronal	68 teeth (68.69%)
	Middle	79 teeth (79.80%)
	Apical	88 teeth (88.89%)

Table 8. Mean length of the root canal filling reminiscent by tooth category.

Maxillary incisor: 6.7 mm	Mandibular incisor: 6.5 mm
Maxillary canine: 6.3 mm	Mandibular canine: 12.0 mm
Maxillary premolar: 5.7 mm	Mandibular premolar: 6.2 mm
Maxillary molar: 4.2 mm	Mandibular molar: 3.9 mm

Table 9. Void between the intraradicular post and the root canal filling.

Tooth	Number of teeth (%)	Mean void
Maxillary incisor	29 (56.86%)	1.4 mm
Maxillary canine	2 (3.92%)	1.5 mm
Maxillary premolar	7 (13.73%)	1.4 mm
Maxillary molar	1 (1.96%)	2.4 mm
Mandibular incisor	0 (0.00%)	----
Mandibular canine	0 (0.00%)	----
Mandibular premolar	8 (15.69%)	1.5 mm
Mandibular molar	4 (7.84%)	0.5 mm

Discussion

The endodontic treatment allows the function re-establishment of a tooth affected by several pathological alterations. However, the endodontically treated teeth will only definitely recover function after the restoration concluded.[11]

Although the radiographic exam is not conclusive in evaluating the root reminiscent and intraradicular posts, this is the diagnostic tool available for the evaluation of teeth with intraradicular metallic posts, since the cone beam computerized tomography, method which allows three-dimensional visualization of the image, presents then beam-hardening as a limitation, artifact that complicate visualization of anatomical structures examined where there is high density materials near the evaluated area.[12]

The post length is the most important factor for retention of the intraradicular post. The longer the post, greater the retention it promotes.[11] Surely, two factors acts as limiting: Root canal internal anatomy and apical reminiscent of the root canal filling.[6] In this study, the post length was lesser than 2/3 of the root length in 86.87% of the cases, considered lesser than the

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recommended length.[11] Similar results were observed in a study that radiographically evaluated the clinical situation of intraradicular metallic posts in 447 single rooted teeth, in which 93.29% of the posts were lesser than the 2/3 recommended.[13]

Another relevant factor is the post diameter. The diameter must be compatible to the maintenance of the root dentin, reduction of fracture and perforation risk, recommended as 1/3 of the root diameter.[11] This study shows that the larger the post, and thus lesser the root dentin thickness, the higher probability of root fractures.[14] In this study, in 31.31%, 20.20% and 11.11% of the teeth in the coronal, middle and apical thirds, respectively, this principle was not followed. This fact brings to unnecessary destruction of root dentin and, thus, tooth weakening.

The removal of root canal filling and the root canal shaping for the post is a treatment phase that requires maximum concentration, due to the risk of deviation and perforation of the root canal, what might compromise the treatment success.[11,15] The use of high-rotation rotatory instruments should be avoided, as enhances the risk of deviations and perforations of the root canal.[15] There was deviation in the root canal shaping for the post in 8 cases.

It is primordial that the intraradicular post and the sealer used fill the space created after the removal of the root canal filling. An empty space favors communication with the periodontium and could allow the development of periapical pathologies.[11] Empty spaces were found in 51.51% of the cases, what might lead to failure of the endodontic treatment.

To ensure the apical seal, primordial condition for endodontic treatment success, most authors agrees that the removal of root canal filling for the post should keep a reminiscent of at least 3 to 5 mm.[11,16] In this study, the maintenance of these recommended limits was seen in most teeth, mean higher than 6 mm.

Conclusion

Most rehabilitations of endodontically treated teeth by an intraradicular post did not follow the recommended principles for these treatments.

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Assessment of different clinical methods to identify mesiobuccal root canals of maxillary first molars

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ABSTRACT

Objective: The purpose of this study was to compare if the quantity of MB2 root canals found in the maxillary first molars increased when visualized at unaided eye and, posteriorly, with an Operating Microscope (OM). The influence of the operator's experience to localize the additional root canals was also evaluated. **Methods:** One hundred extracted maxillary first molars were evaluated by specialists in Endodontics and students of Endodontics specialization. Cone-beam computed tomography (CBCT) was used to confirm the quantity of root canals present in the mesiobuccal root and this evaluation was taken as gold standard for this research. The agreement level between examiners and CBCT images was evaluated by Cohen's Kappa Coefficient. **Results:** There were statically significant differences between specialists (k = 0.234) and students (k = 0.009) when using OM. The best agreement levels were achieved in the student group with Clinical Exam (CE) (k = 0.261) and the specialists with OM (k = 0.234). When the comparison was performed between the dentists there was reasonable agreement between the root canals identification methods: CE (k = 0.275) and OM (k = 0.4245). It was observed in the comparison between the evaluated root canals identification methods that there was moderated significance between specialists (k = 0.558) and students (k = 0.454). **Conclusion:** The evaluator experience and the OM employment influenced MB2 root canals identification.

Keywords: Operating microscope. First molar. Cone-beam computed tomography.

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Introduction

The aim of a successful endodontic treatment is the complete sealing of the root canal space and of the apical foramen with an inert sealing material.[1]

The knowledge of the internal tooth anatomy is essential for achieving this purpose, once it does not reproduce the simplicity of the external tooth anatomy. Smadi and Khraisat[2] reported that the maxillary first molar has the highest rate of endodontic treatment failure due to the presence of a second canal in some of the mesiobuccal roots (MB2) when the professional is unable to identify, prepare and fill this canal.

The MB2 occurrence has been related in a relatively high rate (95%).[3,4] Wolcott et al[5] compared the incidence of MB2 in the initial phase of endodontic treatments and retreatments, and found that the incidence of MB2 in the initial phase of endodontic treatment was 59%, while 67% in retreatment. The authors reported that this significant difference to identify and treat the MB2 canals may reduce the success of the endodontic therapy in long term.

Cleghorn et al,[6] assessing 8399 teeth from 34 studies, found 2 canals in the mesiobuccal root in 56.8% and 1 canal in 43.1%. The incidence of 2 canals in the mesiobuccal root was higher in laboratorial studies, 60.5% compared to 54.7% of the clinical studies. The more common use of the Operating microscope (OM) in recent clinical studies has led to an increased prevalence in the clinical detection of the MB2. Mesiobuccal root canal system in *ex vivo* studies are more leaning to show 2 canals in the maxillary first molar than *in vivo* studies, but the incidence seems to be increasing with the use of OM. Two canals leading to one foremen in the mesiobuccal root of the maxillary first molar are quite twice more usual than two canals and two foramens.

The technological advances from the last decade allowed the development and improvement of different techniques that were introduced to favor the evaluation of the internal anatomic variations of dental roots. The dental radiograph, commonly used in the dental practice, provides essential information to the treatment planning, diagnosis and follow up. However, a general problem in Endodontics is the limitation of radiographic images for the two-dimensional aspect and the superposition of the subjacent anatomy with the cortical bone density.[7] Even with the improvements in radiographic films quality and the digital receptors advent, the two-dimensional projection still being one limiting factor in the detection of MB2 in maxillary first molars.[8] On the other hand, the OM has been used in the dental practice in a search for increased visibility and lighting, which would favor the identification and handling of the MB2 in maxillary molars.[9,10]

Another resource is the Cone Beam Computed Tomography (CBCT), which was introduced to assist in three-dimension images visualization and has been used to evaluate the root canal anatomy. This three-dimensional system has a great potential in Endodontics, becoming a valuable resource in the diagnosis and treatment of endodontic issues, mainly, for the details observation, sometimes impossible to see using conventional radiographs.[11,12,7]

The aim of the present study was to compare the detection of additional canals in the mesiobuccal root (MB2) of maxillary first molars using different assessment methods: Clinical exam (CE), Operating microscope (OM) and Cone-beam Computed Tomography (CBCT). The clinical experience influencing the identification of MB2 was also evaluated by a comparison of different evaluators: Specialists in Endodontics and students of specialization in Endodontics.

Material and Methods

One hundred human maxillary first molars that had the mesiobuccal root were selected from the teeth stock.

After the teeth selection, the pulp chamber access was performed using carbide burr #2 (KG Sorensen, São Paulo, Brazil) and Endo-Z burr (Dentsply Maillefer, Ballaigues, Switzerland) at high rotation and under refrigeration.

The teeth were inserted in two acrylic uncolored slabs (210 x 110 x 5.0), previously perforated, letting the apical portion of the mesiobuccal roots positioned at the same level to guarantee the positioning during CBCT.

The teeth were inserted in two rectangle colorless acrylic slabs (210 x 110 x 5.0 mm), previously perforated, so that the mesiobuccal root apices were positioned at the same level to guarantee the poisoning for CBCT. The teeth were organized in five rows of 5 teeth each slab, taking care to dispose the buccal-lingual and mesiodistal axes at the same direction. The lines were identified with letters (A – J) and the rows with numbers (1 – 10) to identify the teeth (Fig 1).

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The examination of the MB2 presence or absence was performed by 6 dentists (3 specialists in Endodontics and 3 Endodontics specialization students) where the acquired data was duly registered in a pre-established table.

Clinical exam (CE) evaluation

The presence or absence of MB2 was verified with the aid of Flexo-file #10 (Dentsply Maillefer, Ballaigues, Switzerland) and the data were recorded.

Operating microscope (OM) evaluation

Subsequently, the teeth were examined with the aid of an operating microscope (MC – M1222, D. F. Vasconcellos, São Paulo, Brazil) (Fig 2) at 25X magnification and with Flexo-File #10 (Dentsply Maillefer), with the acquired data also recorded.

Cone-beam computed tomography (CBCT) evaluation

The acrylic slabs with the teeth were fitted to an acrylic base for the correct positioning in the tomograph Cone Beam l-Cat (Imaging Sciences International, Hatfield, PA, USA), using 120,000 kV and 46.72mA. The used scanning parameters were: 40 seconds acquisition time, small field of view (FOV = 6.0 cm), 800 x 800 pixels matrix. The scanning raw data were processed by Xoran-Cat software (Imaging Sciences International,

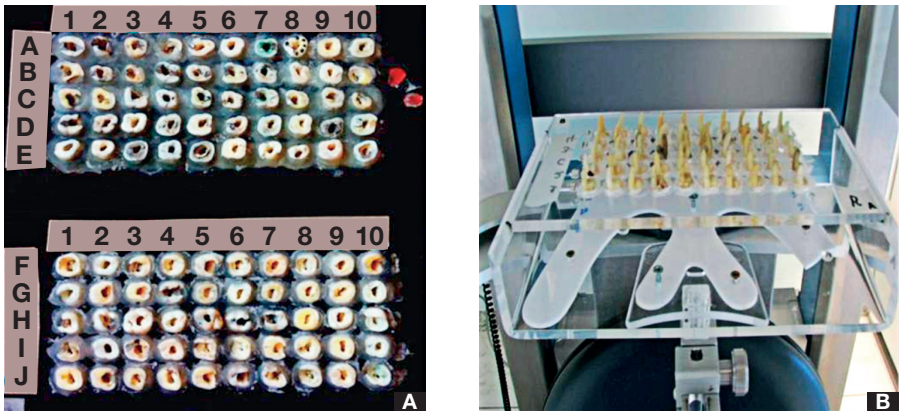


Figure 1. A) Acrylic slabs with teeth identified by lines and rows. **B**) Slab fitted to the acrylic base, in position for tomographic examination.

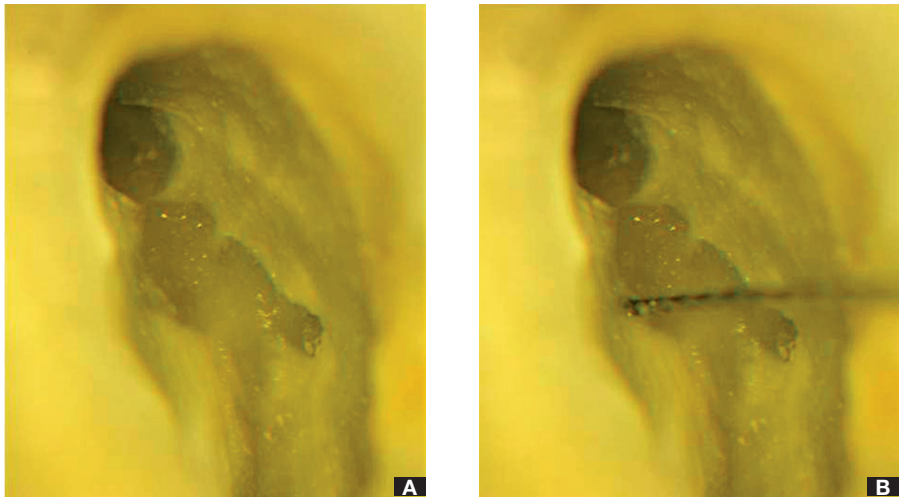


Figure 2. Teeth being examined by means of operating microscope and Flexo-File #10.

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Hatfield, PA, USA), with 394 axial slices obtained, which generated DICOM files with 8.97 MB.

The tomographic images obtained were analyzed with Cyclops MedStation (http://www.telemedicina.ufsc.br/cms/index.php?lang=en), in a lightless environment to favor the visualization of the same distortions and the data were recorded. The CBCT images were used to confirm the number of canals present in the maxillary first molars mesiobuccal root. This evaluation was used as gold standard (Fig 3).

Statistical analysis

The specialists and students evaluations were gathered to the analysis. When there was discordance between them, the value with higher frequency was used. The analysis was constituted of 500 evaluations, 400 performed by the dentists, and 100 by CBCT.

The level of concordance between examiners and the comparison with the CBCTs were evaluated by Cohen's Kappa Coefficient. The classifications of the Lapp values followed the information preconized by Landis and Koch.[13] The analysis was performed with software Microsoft Excel 2011 (Microsoft Corporate, Redmond, WA, USA) and SPSS 17 (SPSS Inc., Chicago, IL, USA).

Results

The results showed that specialists found a higher quantity of teeth with 2 canals in the mesiobuccal root than the students and the control group, regardless the identification method used (Table 1). In the CE exam, the specialists found 86 teeth with the MB2 canal, while the students found 62 teeth. Using OM, the specialists found 88 teeth with the presence of MB2 and the students found 60 teeth. The CBCT showed the presence of MB2 in 78 teeth.

There were statistically significant differences between the specialists (k = 0.234) and the students (k = 0.009) when using OM. The best agreement levels were found in the students group using CE (k = 0.261) and specialists using OM (k = 0.234). The lowest agreement level with the control group was seen in the students group using OM (k = 0.009).

The comparison between dentists showed reasonable agreement between CE and OM methods (k = 0.275 and 0.245, respectively). Comparing the identification methods it is observed that there was moderated agreement between the specialists (k = 0.558) and students (k = 0.454). The results of this study are described in Table 2.



Figure 3. Axial slice of a tomographic image showing the presence of MB2 in a specimen.

Table 1. Frequencies distribution of 100 specimens evaluated by CBCT, CE and OM.

Canals	CBCT	CE		OM	
		Specialists	Students	Specialists	Students
1	22	14	38	12	40
2	78	86	62	88	60

Table 2. Results description of Cohen's Kappa coefficient for pared comparison.

		CE		OM	
		Specialists	Students	Specialists	Students
CBCT		0.129	0.261	0.234	0.009
CE	Specialists		0.275	0.558	0.206
	Students			0.364	0.454
OM	Specialists				0.245

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Discussion

Since the 1990's, the OM and the CBCT started to be used in Dentistry, aiding to locate and, consequently, to treat root canals. Since then an exceptional level of care is taken in cases previously considered untreatable or of doubtful prognosis. Hess and Zurcher,[14] in a reference study from 1925, related the existence of MB2 in maxillary molars. In 1969, Weine et al[3] observed during the evaluation of endodontic prognosis that the failure in maxillary molars treatment occurred with high frequency in the mesiobuccal root region. Based in this observation, the authors studied and discovered that teeth with a fourth canal occurred more often than the ones with three canals (51.5% versus 48.5%, respectively).

Especially in the maxillary first molar, it is observed that the endodontic treatment failure happens commonly associated to the incapability of MB2 canal location,[15,3] although actually locating this canal is easier once new technologies were developed and inserted, as OM and CBCT.[15,16,17]

The OM use effectiveness for MB2 canal detection, in *ex vivo* and *in vivo* studies, compared to unaided eye, have been evaluated in several studies with diversified methodologies. Sempira and Hartnell, in an *in vivo* study using 200 maxillary molars, aided by OM observed the identification of MB2 increased by 30%. These values agree with the obtained by Coutinho Filho et al,[10] who found increased rate of canals localization from 53.7 to 87.96% when submitted to OM magnification. Carvalho and Zuolo[18] used 204 first and second molars extracted and found 641 canals without OM and 50 additional canals when magnification was applied, an increase of 7.8%. These results agree with the obtained in this research, where specialists using OM localized 4% more MB2 canals, similar values also found by Alaçam et al,[19] who found 5 additional MB2 canals from 100 teeth when using OM. Buhrley et al[20] performed a clinical study with specialists in Endodontics, reporting an increase of 71.1% for MB2 localization. In the present study there was an increasing in the localization of MB2 canals when submitted to OM examination, evaluated by specialists when compared to students. Baldassari-Cruz et al[21] found that 51% of the MB2 from 39 extracted maxillary molars were located with only explorer and dental mirror, and 82%, 12 additional canals found, when using OM. These studies confirm the results obtained in this research by the specialists group, which had an increase in the number of canals found when aided by the OM.

Disagreeing with these results, Görduysus et al[22] worked without visual magnification in 45 extracted maxillary teeth and localized the MB2 canal in 42 teeth (93%), and the use of OM increased the detection in just one tooth (96%). Similar results were found in the present study by the students group, where the OM was not critical for locating additional canals, on the contrary decreasing 12% when using the microscope. However, the students found 68% of the canals from a sample of 100 teeth. It can be concluded that the clinical experience is an important factor to the localization of additional canals.

Buhrley et al[20] stated that several factors may influence the low incidence of MB2 canals found when compared to *ex vivo* studies, including clinical environment, limited visibility and access, perforation risk, tooth position in the arch, general tooth condition (caries, restorations and prosthesis), patient's age and his/her tendency to stand a longer clinical session. Besides, the dentist experience might determine the quantity of root canals found. It is possible that, in some cases, the most important factor for canals identification is the professional persistence and not the image magnification. This might be a possible explanation to the low rate of MB2 canals found by students using OM in the present study. Due to the lower clinical experience and, consequently, lower contact with these canals, maybe the students had not already acquire the specialist persistence in the ceaseless search for additional canals.

In many cases, the MB2 identification might be facilitated by the presence of a sulcus, being the removal of a little quantity of dentin necessary in these cases. The use of OM in this phase aids in the identification and clinical detection of MB2,[23,22] once the OM light is parallel to the vision line and two to three times higher than the reflector, improving significantly the clinical vision of the pulpal chamber.[9]

Baldassari-Cruz et al[21] reported that different ways to access might increase the frequency of MB2 identification. The OM is very useful to execute this task, combined with the root canal system morphological knowledge and amplified vision of the area, allow the

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professional to achieve maximum results. This is confirmed by the increased number of MB2 canals found by specialists using the OM in the present study.

Coutinho Filho et al[10] state that the ability to identify MB2 canal rely on the professional skill, the anatomical complexity and the use of good lighting and magnification, similar to the conditions offered by OM. This statement is confirmed by this study, where the specialists using OM identified a higher number of additional canals.

New radiographic modalities are demonstrating viable applications to Endodontic, aiding the treatment of root canals. One is CBCT, which proves very useful to visualize the root canal morphology.[24,16] Blattner et al[15] evaluated the root canal identification ability with CBCT and concluded that this technology aided the identification of the presence or absence of MB2 with precision in 78.95% of the sample.

Kottor et al[25] described a case report where the authors found 7 canals in a maxillary first molar. The clinical detection was made by the OM and the confirmation with CBCT, since the initial diagnosis was done with a periapical radiography, which had not showed any anatomical variation. Based in these researches where the authors confirm the reliability in the identification of additional canals, we used CBCT as gold standard for the present study.

Conclusion

It is clear that for the identification of MB2 canal, the evaluator's experience and the clinical methods used influence the search for canals, although no method alone is 100% reliable. Several methods may be used to locate the additional canals, increasing successful maxillary first molars endodontic treatments.

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Endodontic treatment of three types of *dens invaginatus*: Report of four cases

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ABSTRACT

Dens invaginatus, also known as dens in dens, is a developmental abnormality that presents alterations in the form and volume of teeth, which may affect the crown and root. Its complex anatomy makes endodontic treatment much more difficult to be performed. Four cases of endodontic treatment in teeth with this type of anomaly are presented; one case type I, one case type II and two cases type III, according to Oehlers' classification. Endodontic treatment only was performed in three of these cases, and endodontic re-treatment and surgical complementation in one case. Long-term proservation of cases 2 and 4 demonstrated periapical repair with apical closure. Case 1 demonstrated total removal of invagination and the formation of an apical mineralized barrier. Proservation for case 3 was not possible because the patient moved away and contact was lost. Although the treatment of teeth with dens invaginatus is complex, it may be successfully performed when supported by correct diagnostic and planning. If necessary it can be complemented with surgical intervention.

Keywords: Endodontics. Calcium hydroxide. Advanced treatment.

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Introduction

During jaw formation some abnormalities may occur resulting in dental malformations such as *dens invaginatus* (DI).[1] Some authors describe DI as an alteration caused by the invagination of the enamel organ internal epithelium before its mineralization. In a specific moment during development, a relatively developed amelo-dentinal structure is formed inside the pulp.[2,3,4]

Teeth affected by this anomaly presents, radiographically, an invagination of the enamel and dentin that can extend deep into the pulpal cavity, and into the canal, sometimes reaching the root apex.[1] The cavity that is formed, generally from the tooth's palatal aspect, advance towards the pulpal cavity, involving it, but keeping communication with the outside through a small opening on the surface of the crown, which may lead to the retention of food residues.[5] This malformation may also be found in the literature as: Dens in dens, invaginated odontoma, dilated composed odontoma, telescopic tooth, dilated gestant odontoma, tooth inclusion. This wide range of names is the result of different theories on DI etiology.[1,2,3] The dental elements mostly affected by this abnormality are the permanent upper lateral incisors, followed by the central incisors, canines, upper premolars, as well as the lower incisors, canines and premolars. The occurrence of DI in the posterior teeth is rare but, when it occurs, is more frequently seen in the cervical area.[2,3,6,7]

Depending on the level of the tooth's involvement by the invagination, Oehlers[8] classified DI under three different forms: Type I, small invagination lined with enamel restricted to the dental crown, not extending beyond the cementoenamel junction; type II, moderate invagination lined by enamel, extending apically beyond the cementoenamel junction, which may or not be in communication with the dental pulp, but confined inside the root as in a blind sac; Type III, invagination lined by enamel, extending beyond the cementoenamel junction, penetrating along all the root's extension, normally without communication with the dental pulp and revealing a second lateral or apical foramen on the root's surface.

DI diagnostic may be performed clinically: The morphology of the affected crown may vary from normal to abnormal, depending on the size of the invagination, with a relatively deep pit on the tooth's palatal region, and a cingulate accentuation.[2] However, the main tool for identifying this anomaly is the radiograph which can show an image similar to a tooth inside another. The radiolucent image in the interior of DI should not be interpreted as lack of pulpal tissue, but just as the invagination intimacy.[9] Sometimes, DI may be recognized radiographically even before the tooth has erupted into the oral cavity.[2]

Several factors may influence in the choice of DI treatment. For instance, the patient's age, the patient's physical, psychological and financial situation, the type of invagination, the possibility of access, the form and the localization of the invagination opening in the crown, the root canal system configuration and the tooth's esthetical function.[2,3] Sometimes, the affected tooth may present incomplete rizogenesis, in which case apexification is recommended.[1] Therefore, several treatment modalities have been proposed for DI management, including preventive restorative procedures, conventional endodontic therapy, paraendodontic surgery, intentional dental re-implant. The treatment is complex and no method may be absolutely proposed due to the variety of existing malformations. Thus, the treatment is based on the anomaly's signs and symptoms, and the prognosis is frequently questionable.[2,7,10,11] Nevertheless, the literature has shown that, even in cases where endodontic therapy seems to be technically unsatisfactory, successful outcomes may be obtained.[12]

Therefore, due to the difficulties normally found in the endodontic management of DI, the objective of this paper is to report on the treatment of four cases of teeth presenting this developmental anomaly. All teeth presented periapical lesions and incomplete rizogenesis, contributing to the complexity in obtaining a satisfactory endodontic treatment. In addition to that, the paper presents the positive results obtained through the long-term clinical and radiographic proservation in two of these cases.

Case Reports

Case 1

A male patient, aged 31 years with a fistula in his left upper lateral incisor was referred to endodontic treatment in September 2010. There was no history of systemic diseases, and his last dental treatment had

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taken place 4 years previously. A gutta-percha cone was used to trace the fistula. A periapical radiograph showed a radiopaque projection inside tooth 22, lined by enamel (Oehlers's type I DI), apical radiolucency and an open apex (Fig 1A). Graphic resources from Microsoft Office PowerPoint 2010 were used to better visualize the image's volume (Fig 1B). Moreover, Corel Draw X5 was also used to better define the figure's outline on the radiographic image (Fig 1C). The tooth did not respond to the pulpal sensitivity, percussion and palpation tests, suggesting the probable clinical diagnostic of chronic periapical abscess.

After the administration of local anesthesia, a rubber dam was placed and stabilized with cyanoacrylate and dental floss. The access cavity was performed on the tooth's incisal edge with a high speed diamond bur until the invagination had been totally removed. Biomechanical preparation was achieved with manual endodontic files and 2.5% sodium hypochlorite solution, and the working length was confirmed (Fig 1D). The canal was dried with sterilized paper cones, and flooded with 17% EDTA trisodium solution for 3 minutes, after which the canal was dried once again. Calcium hydroxide paste with propylene glycol was placed inside the canal, which was replaced monthly to induce apexification, or the formation of a mineralized barrier on the apical foramen. After six months, the formation of an apical barrier was observed radiographically, which was detected with a gutta-percha cone as a probe. It was also possible to observe that the periapical healing was in the process of completion (Fig 1E). Final obturation was performed in March 2011 with a calcium hydroxide-based cement and gutta-percha by lateral condensation, followed by additional vertical condensation (Fig 1F).

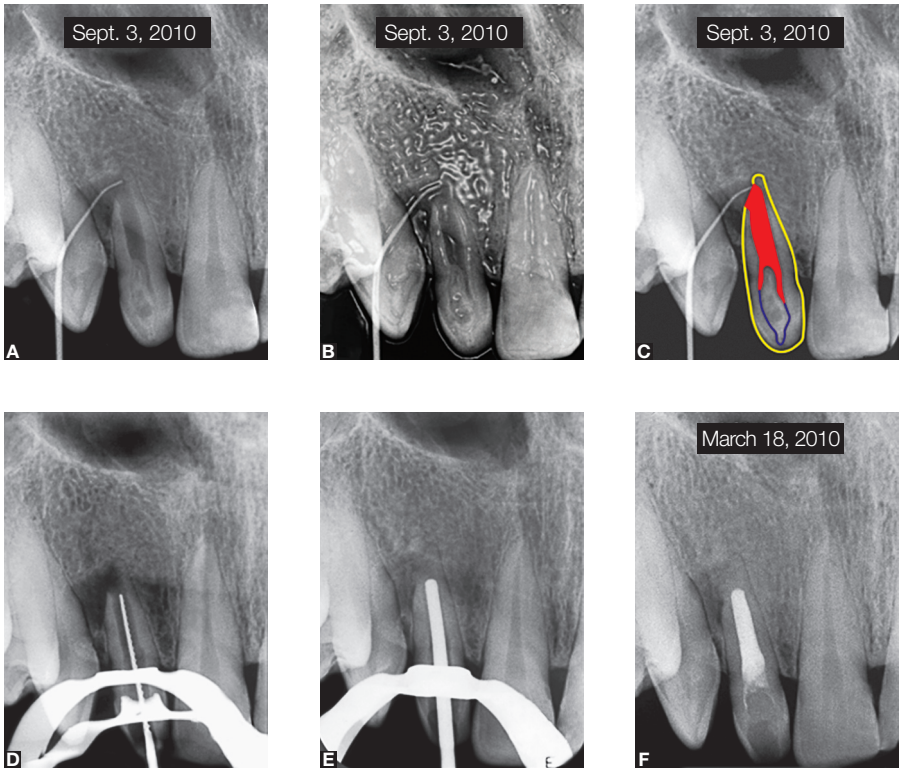


Figure 1. A) Initial radiograph showing fistula tracing. **B**) graphic resources, showing the tooth's volume. **C**) graphic resource defining the tooth's anomaly contour. **D**) Confirmation of working length. **E**) Cone test showing apical barrier. **F**) Final radiograph.

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Case 2

A 14-year-old female patient was referred to our private clinic in April 2001 with a periapical radiograph of tooth 12 showing an Oehlers's type II DI image associated to incomplete root formation, an open apex and a periapical radiolucent image, with the probable diagnostic of chronic periapical abscess (Fig 2A). In this case also, graphic resources were used as described previously for the same objectives (Fig 2B). The intraoral clinical examination showed the absence of edema, or pain on percussion and palpation, but there was a fistula which was traced with a gutta-percha cone (Fig 2C). There was no systemic diseases, but the patient reported to be a mouth breather and did not permit the placement of a rubber dam in any of the appointments. Hence, tooth's relative isolation was used only.

After local anesthesia and the tooth's isolation, the coronal access was performed through the incisal edge, because the crown was flared, and also to facilitate access to the canal. As it was an Oehlers's type II DI case, it was not possible to remove all the invagination, not even during the biomechanical preparation with manual endodontic files and 1% calcium hypochlorite solution. The working length was determined (Fig 2D). The canal was dried with sterile paper points and flooded with 17% EDTA trisodium solution for 3 minutes, after which the canal was dried once again and filled with calcium hydroxide paste, propylene glycol and iodoform (Fig 2E). The dressing was changed whenever radiographs showed that the intracanal medication had been partially reabsorbed. Five months later, the canal was obturated as described for Case 1. At this moment, radiographs showed that the cervical and apical to distal grooves had been filled by the obturating cement, and there was the partial repair of the periapical lesion and apical sealing (Fig 2F).

Three months after the case had been concluded, the patient was called for her first follow-up radiograph, which showed the evolution of the repair and apical closure (Fig 2G). The second follow-up visit was one year later when repair was almost concluded and a radiolucent area appeared inside the canal due to the obturating cement reabsorption (Fig 2H). Five years and nine months later, the patient returned for her third follow-up examination. The radiograph showed that the periapical lesion had been completely repaired, the obturating cement in the canal's apical grove had been totally reabsorbed, and the crown had been definitely restored (Fig 2I).

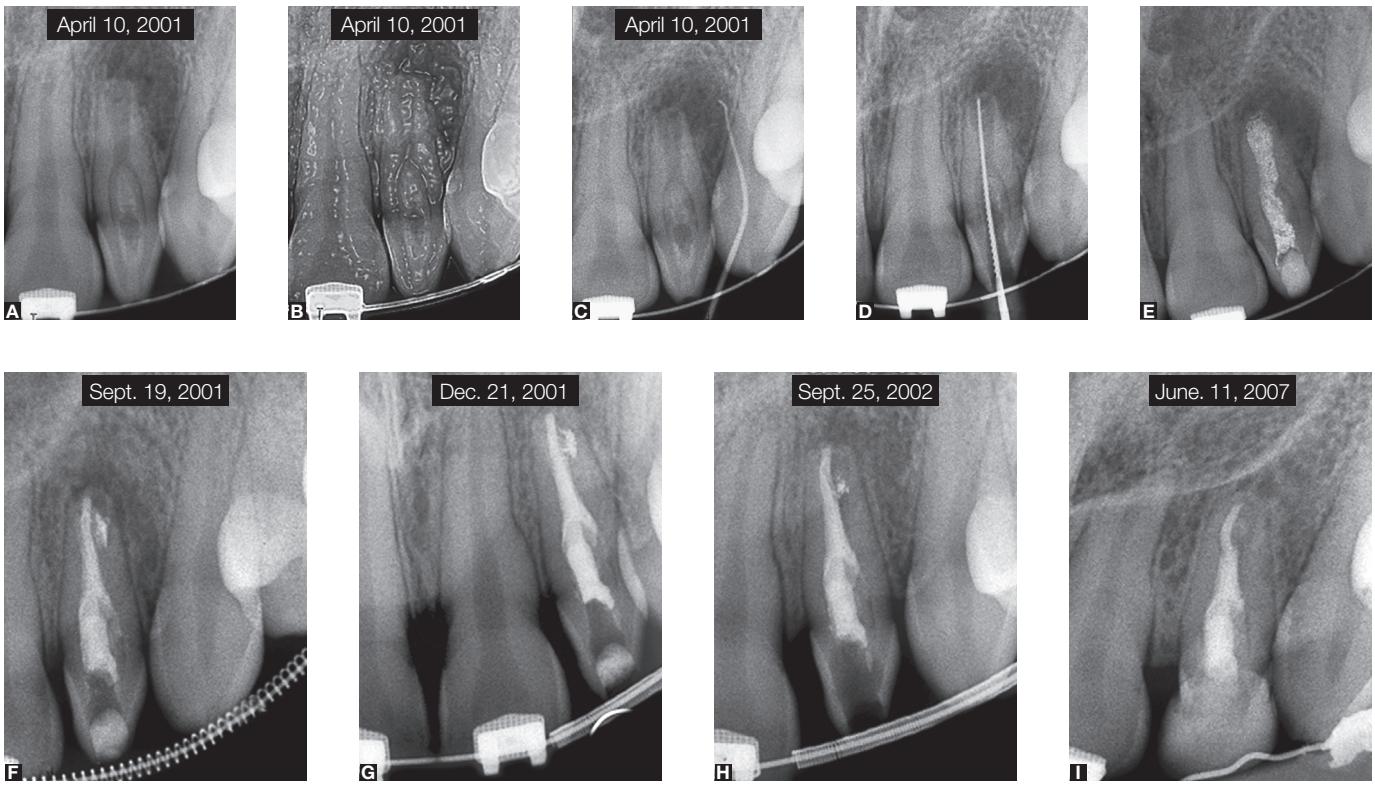


Figure 2. A) Initial radiograph; **B**) Graphic resource; **C**) Fistula tracing; **D**) Confirmation of working length; **E**) Intracanal dressing; **F**) Final radiograph; **G**) follow-up - 3 months; **H**) 1 year; **I**) 5 years and 9 months follow-up.

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Case 3

A male patient, aged 32 years was referred to treatment in April 1998 and reported that about 30 days previously had felt intense pain in the right lower canine region. On that occasion, coronal access and the placement of an intracanal dressing only had been performed. A periapical radiograph of tooth 43 showed Oehlers's type III DI, with open apex, and radiolucent lesion, suggesting chronic periapical abscess. During emergency, the clinician had accessed just one canal (Fig 3A and 3B). Clinically the tooth did not respond to the pulpal sensitivity, percussion and palpation tests, and a large volume was present in the dental crown.

After local anesthesia, a rubber dam was placed and the canals were accessed (Fig 3C). Both the biomechanical preparation and the application of 17% EDTA trisodium solution followed the same technique described for case 2. To assist in the sanitizing of the root canal, calcium hydroxide paste with propylene glycol was used as an intracanal dressing. As the patient was going to move away to another city, it was not possible to change the dressing in an attempt to induce mineralized tissue formation apically before the definitive obturation of the root canal. Hence, the gutta-percha cones test (Fig 3D and 3E) was carried out to verify their locking inside the canal and confirm the working length. After that, the canals were obturated as described in the previous cases. The obturation radiograph (Fig 3F) showed that a cone had exceeded the obturation limit in one of the canals, and there was a radiolucent area laterally to the cone in the other canal. It was proposed to the patient to endodontically re-treat the tooth, but he refused due to his urgency to have the treatment finished. Six months later, a contact was tried to invite the patient perform a follow-up radiograph. Unfortunately, it was not possible and thus, proservation was not performed.

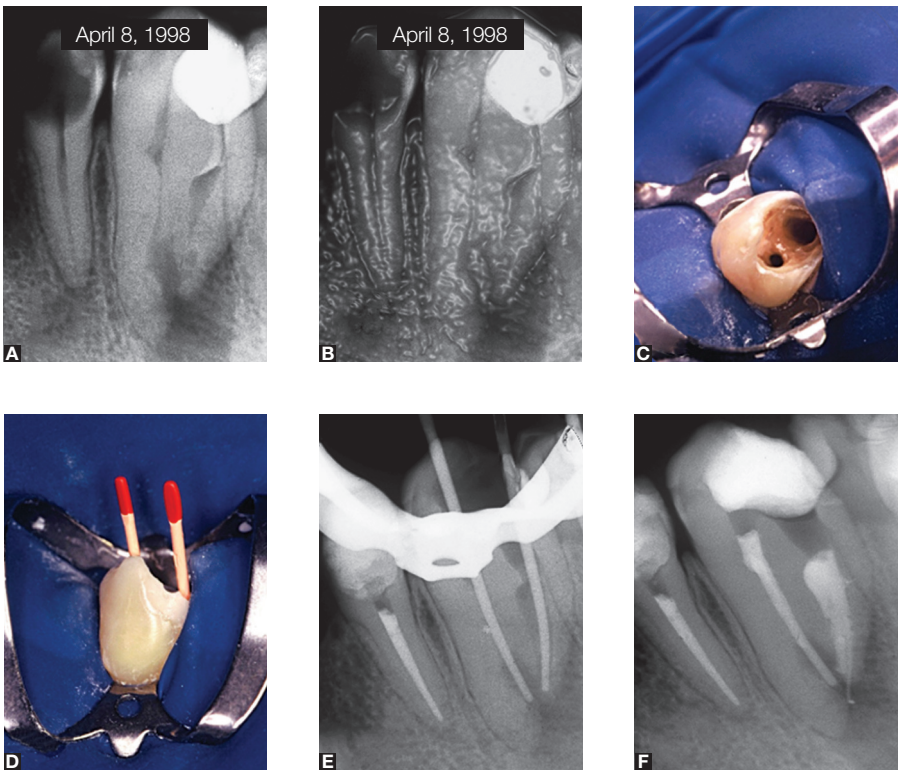


Figure 3. A) Initial radiograph showing the presence of dens invaginatus type III; **B**) With graphic resource showing volume; **C**) Coronal access to two canals; **D**) Photograph showing the main cone test; **E**) Radiograph showing the main cone test; **F**) Final radiograph.

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Case 4

A male patient was referred to endodontic treatment of tooth 22 in August 2001, due to the presence of a fistula. There was no systemic problems and, during the intraoral examination, the fistula was traced with a gutta-percha cone. A lateral radiograph (Fig 4A) confirmed the presence of an Oehlers's type III DI. The fistula was traced back to its origin, a possible root defect due to malformation. The radiographic images were manipulated with the same tools and objectives as in case 1 (Fig 4B and 4C). In order to correct the root defect, endodontic re-treatment complemented by a periapical surgery was scheduled.

After local anesthesia, a rubber dam was placed and the root canals were accessed. The obturating material was removed with Gattes Glidden burs, and the canals were shaped manually with endodontic files irrigated with 1% sodium hypochlorite solution. Then, the working length was confirmed (Fig 4D), and 17% EDTA trisodium solution was applied to provide better cleaning to the canals. The canals were dried with sterile paper points and filled with calcium hydroxide paste and propylene glycol. The dressing was changed just once, since this tooth would be referred to paraendodontic surgery. The canals were obturated in October 2001 with gutta-percha cones and a calcium hydroxide-based cement with the intentional extravasation of the obturating material, so that it could serve as a guide during surgery, as well as to promote a better sealing (Fig 4E).

The paraendodontic surgery was carried out by lifting a total thickness flap through two vertical relaxing incisions, keeping the interdental papillae in place, and exposing the root portion and the extravasated obturating material (Fig 4F). This extravasated material was then removed, the root defect was smoothed (Fig 4G), and the root lodging was obturated with MTA, until it was completely filled (Fig 4H). Finally, the gingival tissue was repositioned and sutured with Ethicon 6-0 thread (Fig 4I). The final periapical radiograph showed that the extravasated sealing material had been removed and the radiolucent area had been filled with obturating material (Fig 4J). The sutures were removed one week after the intervention.

Six years and one month later, the first follow-up radiograph showed clinical repair, characterized by the presence of a continuous lamina dura surrounding the whole periapical region, as well as the absence of a fistula and symptoms (Fig 4K). Nine years and six months after treatment, the patient returned for a second follow-up radiograph that showed the same characteristics as before, and the final prosthetic treatment given to the crown (Fig 4L).

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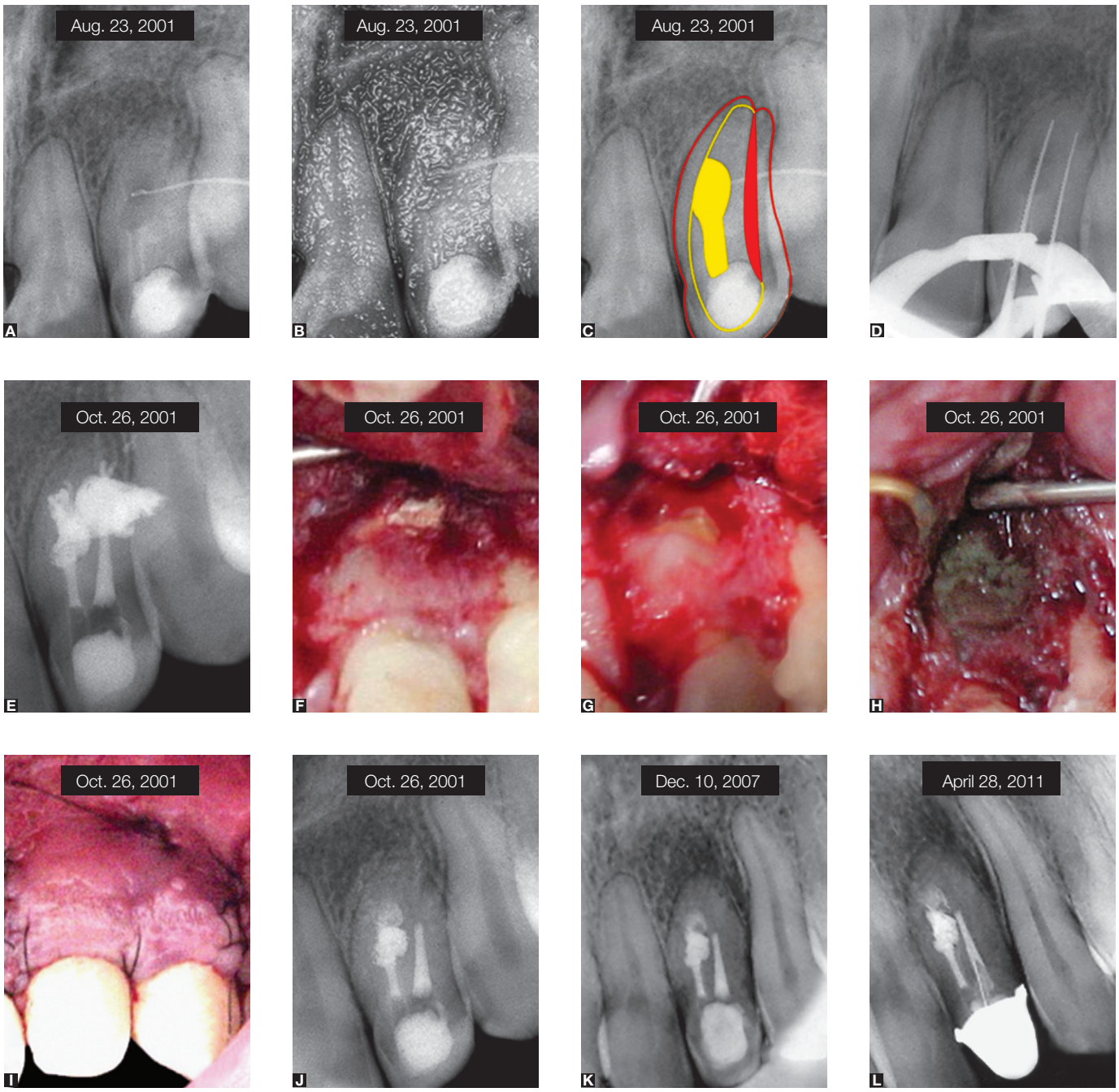


Figure 4. A) Initial radiograph showing fistula tracing; **B**) Graphic resource showing volume; **C**) Graphic resource defining the tooth's anomaly outline; **D**) Confirmation of working length; **E**) Extravasated obturating material; **F**) Transoperatory photograph showing the root portion with extravasated material; **G**) After extravasated material removal; **H**) MTA inserted in the root defect; **I**) Tissue repositioning; **J**) Final radiograph; **K**) Follow-up radiograph; **L**) Follow-up radiograph.

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Discussion

Previous studies report on a high incidence of DI in permanent upper lateral incisors,[2,3,6,7,10] which is in agreement with this report (three out of four cases). The radiographic images of this anomaly also showed rarefaction in the periapical area as a result of the evolution of the infectious process, and apexes with incomplete rizogenisis, as reported in the literature.[1,2,3,9] Therefore, after careful analysis, we opted for the initial endodontic intervention to stimulate the regression of signs and symptoms, since pulpal pathology was verified and endodontic therapy was indicated.[11]

The cases 1, 2, and 3 correspond to Oehlers's type I, II, III DI, respectively, and all presented chronic periapical lesions and incompletely formed apexes. In these conditions, endodontic therapy is faced with a complex root anatomy that hampers treatment. That is the reason why initial sanitizing, with the removal of necrotic tissue and the combat against bacterial infection with abundant irrigation with a sodium hypochlorite solution, was carried out.[13,14] Dressing with calcium hydroxide paste was used between sessions to complete disinfection and stimulate the deposition of mineralized tissue.[1,4,10,15,16] These procedures led to the clinical success in three cases without the need for periapical surgery.[17] These data corroborate the reports of other authors that demonstrated the influence of calcium hydroxide as an intracanal dressing, and the obturation with a calcium hydroxide-based cement for the repair of extensive periapical lesions in teeth with complete apexes.[16,18,19,20]

The frequency of pulpal necrosis in DI cases is explained by its coronal invagination, which makes teeth more susceptible to dental caries and pulpal infection, due to the structural defect existing at the bottom of the pit. Besides, the hypomineralization makes the internal enamel layer more fragile, or even absent, which facilitates the exposition of the dental pulp by fracture during chewing, or even by the natural microexposition.[2,3,11]

The case 4 is an Oehlers's type III DI. This tooth had been referred to endodontic re-treatment due to failure in the previous treatment. As in this type of anomaly the invagination may extend through the root in the shape of a cleft in the apical or lateral region without communication with the pulp, as shown by tracing the fistula with a gutta-percha cone (Fig 4A), endodontic as well as periodontal treatment was recommended.[21,22]

Therefore, paraendodontic surgery was planned for this case as a complement to the endodontic treatment. The literature states that this complementary approach is aimed at overcoming DI root canal irregularities, because the apical abnormality does not provide a good environment for the correct root canal debridement and obturation. In addition to that, the majority of cases treated between 1977 and 1994 used as a complement to endodontic treatment, the periapical surgery to seal the space existing between the root canal and the periapical tissues.[23-29]

Figures 4K and 4L illustrate a radiolucent image in the tooth's periapical region which is possible to observe the continuity of the lamina dura around the tooth, clinically indicating repair. This image suggests that it is a scar tissue since, according to Melcher[28] and Bosshardt and Sculean,[29] if the bone defect is not separated from the connective and/or epithelial tissue by a physical barrier, it will be filled by epithelial or connective cells that proliferates faster than bone and periodontal ligament cells, preventing the defect to be completely repaired, and generating this kind of radiographic image.

This work is in agreement with the work of Girsch and McClammy,[9] who admit that the prognosis of DI endodontic treatment is doubtful, specifically because of the complex morphology and communication among root canals, which makes the adequate cleaning and shaping of root canal systems more difficult. However, in cases 2 and 4, where clinical and radiographic proservation was possible, the treated teeth remained in the dental arch, keeping their esthetical and functional characteristics.

The four cases reported here demonstrate that DI is a challenge for the endodontic treatment, since this abnormality points to a complex root canal system. Moreover, the endodontic treatment of teeth with DI, although difficult to execute, can be successfully accomplished when supported correct diagnostic and planning and, if necessary, it may be complemented by surgical intervention.

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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

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

Yours sincerely,

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