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Área de Concentração: Odontologia Integrada

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RESUMO

Na endodontia, existem relatos de casos usando o Emdogain® (EMD) durante procedimentos cirúrgicos em casos de reparo de perfurações. Até o momento estudos laboratoriais e clínicos têm mostrado o potencial de eficácia do EMD utilizado em procedimentos endodônticos, como pulpotomia, capeamento pulpar direto, regeneração pulpar em ratos e medicação intracanal. Em dentes traumatizados ou fragilizados, como nos casos de dentes com ápices abertos e necrose pulpar seria interessante usar biomateriais durante procedimentos endodônticos regenerativos a fim de reestabelecer ou melhorar propriedades físico-mecânicas da dentina, tal como a microdureza dentinária radicular. Porém, até o momento não há estudos que relacionem o efeito do EMD na estrutura e composição da dentina radicular humana. Neste contexto, o presente estudo foi dividido em dois capítulos que abordaram o efeito do Emdogain® na microdureza e composição química de dentina radicular humana (capítulo 1 – estudo laboratorial) e seu desempenho clínico em procedimentos endodônticos regenerativos (capítulo 2 – série casos clínicos). No capítulo 1 dez terceiros molares humanos foram usados para produzir 30 espécimes de dentina (em forma de disco). As amostras permaneceram em contato com o EMD (gel Emdogain®) por 90 dias em estufa a 37ºC sob umidade. As medições de linha de base e 90 dias após o tratamento com EMD foram realizadas. A composição química foi avaliada com Espectroscopia de Infravermelho com Transformada de Fourier (ATR/FTIR); morfologia de superfície e análise de elementos foram avaliados com Microscopia Eletrônica de Varredura/Espectroscopia de Energia Dispersiva (SEM/EDS); e a microdureza da dentina (KMH) foi medida com um indentador Knoop com carga de 10g pelo tempo de 15 segundos. A análise estatística usada para dados de FTIR e KMH foram os

testes de Shapiro-Wilk ($p>0.05$) e igualdade de variâncias (Levene's test, $p>0.05$) e os achados de SEM/EDS foram relatados descritivamente. Após 90 dias de tratamento com EMD, os espécimes de dentina apresentaram diminuição dos valores de carbonato ($p=0,001$) e amida III ($p=0,002$), e as relações C:M (carbonato:mineral) também diminuíram ($p<0,001$), enquanto a amida I/amida III aumentaram. As imagens SEM mostraram que o tratamento com EMD não alterou a morfologia da superfície dentinária. A análise EDS mostrou uma ligeira diminuição na intensidade dos picos de cálcio (Ca) e fósforo (P) da dentina radicular. Não foram observadas diferenças significativas na microdureza superficial das amostras antes e após o tratamento ($p= 0,35$). Desta forma, concluiu-se que o uso de EMD, por 90 dias em contato com discos de dentina, não alterou a microdureza e a morfologia da dentina humana, embora a estrutura elementar/química da dentina tenha sido alterada, a redução do teor de carbonato está associada ao aumento da resistência à desmineralização. No capítulo 2, três casos de dentes permanentes com formação radicular incompleta, diagnosticados com necrose pulpar, foram tratados com procedimentos endodônticos regenerativos (PERs) usando EMD após a etapa de indução de sangramento. Os exames de acompanhamento mostraram que os dentes permaneceram funcionais sem quaisquer sinais ou sintomas. O exame radiográfico final aos 12 e 24 meses, respectivamente, revelou resolução completa das lesões e fechamento apical evidente nos três casos. Em 1 dos casos, havia imagens radiográficas sugestivas de zonas calcificadas dentro da luz do canal radicular que podem estar relacionadas ao uso de EMD. Além das vantagens já conhecidas dos PERs, conclui-se que também são um tratamento promissor para o espessamento dentinário e fechamento apical e que o EMD poderia potencializar esse processo, justificando ensaios controlados randomizados. Esta série de casos mostra como os PERs podem ter o potencial de formação contínua do comprimento radicular e fechamento apical, com considerável espessamento das paredes dentinárias radiculares. Os dois estudos permitem aferir que o uso de EMD possui viabilidade e potencial de utilização nos PERs.

Palavras-chave: Emdogain, derivado da matriz do esmalte, endodontia regenerativa.

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ABSTRACT

In endodontics, there are case reports using Emdogain® (EMD) during surgical procedures in cases of perforation repair. To date, laboratory and clinical studies have shown the potential effectiveness of EMD used in endodontic procedures such as pulpotomy, direct pulp capping, pulp regeneration in rats and intracanal medication. In traumatized or weakened teeth, as in the case of teeth with open apices and pulp necrosis, it would be interesting to use biomaterials during regenerative endodontic procedures in order to reestablish or improve the physical-mechanical properties of dentin, such as root dentin microhardness. However, to date, there are no studies that relate the effect of EMD on the structure and composition of human root dentin. In this context, the present study was divided into two chapters that addressed the effect of Emdogain® on the microhardness and chemical composition of human root dentin (chapter 1 - laboratory study) and its clinical performance in regenerative endodontic procedures (chapter 2 - six case series clinicians). In chapter 1 ten human third molars were used to produce 30 dentin (disk-shaped) specimens. The samples remained in contact with EMD (Emdogain® gel) for 90 days in an incubator at 37oC under humidity. Baseline measurements and 90 days after EMD treatment were performed. Chemical composition was evaluated with Fourier Transform Infrared Spectroscopy (ATR/FTIR); surface morphology and element analysis were evaluated with Scanning Electron Microscopy/Energy Dispersive Spectroscopy (SEM/EDS); and the dentin microhardness (KMH) was measured with a Knoop indenter with a load of 10g for a time of 15 seconds. The statistical analysis used for FTIR and KMH data were the Shapiro-Wilk tests ($p>0.05$) and equality of variances (Levene's test, $p>0.05$) and the SEM/EDS findings were reported descriptively. After 90 days of

EMD treatment, dentin specimens showed decreased carbonate ($p=0.001$) and amide III ($p=0.002$) values, and C:M (carbonate:mineral) ratios also decreased ($p<0.001$), while amide I/amide III increased. SEM images showed that EMD treatment did not alter the morphology of the dentin surface. EDS analysis showed a slight decrease in the intensity of calcium (Ca) and phosphorus (P) peaks in root dentin. No significant differences were observed in the surface microhardness of the samples before and after treatment ($p=0.35$). Thus, it was concluded that the use of EMD, for 90 days in contact with dentin discs, did not change the microhardness and morphology of human dentin. Although the elemental/chemical structure of dentin has been altered, the reduction in carbonate content is associated with increased resistance to demineralization. In chapter 2, six cases (6 teeth) of permanent teeth with incomplete root formation, diagnosed with pulp necrosis, were treated with regenerative endodontic procedures (PERs) using DME after the bleeding induction step. Follow-up examinations showed that the teeth remained functional without any signs or symptoms. Final radiographic examination at 12 and 24 months, respectively, revealed complete resolution of the lesions and evident apical closure in 3 of the six cases. In 1 of the cases, there were radiographic images suggestive of calcified zones within the root canal lumen that may be related to the use of DME. In addition to the already known advantages of PERs, it is concluded that they are also a promising treatment for apical root closure and that DME could potentiate this process, justifying randomized controlled trials. This case series shows how PERs can have the potential for continuous root length formation and apical closure, with considerable thickening of the root dentin walls. The two studies allow us to verify that the use of EMD has feasibility and potential for use in PERs.

Keywords: Emdogain, enamel matrix derivative, regenerative endodontics.

SUMÁRIO

LISTA DE ABREVIATURAS E SIGLAS

EDS – Energy dispersive spectroscopy

EDTA – Ethylenediaminetetraacetic acid

EMD – Enamel matrix derivative (Emdogain®)

EPT – Electric pulp test

FTIR – Fourier Transform Infrared Spectroscopy

HES – Hertwig epitelial sheath

KMH – Knoop Microhardness

MTA – Agregado de trióxido mineral

NaClO – Hipoclorito de sódio

PGA – Propylene Glycol Alginate

REP – Regenerative endodontics procedure

SEM – Scanning Electron Microscopy

TAP – Triple antibiotic paste

VAS – Visual analogue scale

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1 INTRODUÇÃO

A necrose pulpar em dentes permanentes imaturos representa uma situação clínica desafiadora, podendo levar a consequências devastadoras para o paciente, pois há um grande risco de fratura radicular, podendo a permanência destes dentes na cavidade oral variar de 39% a 89% (1,2).

Em dentes com necrose pulpar e ápices abertos a alternativa de tratamento convencional é a apicificação com o uso de Agregado de Trióxido Mineral (MTA) ou hidróxido de cálcio. No caso do hidróxido de cálcio, trocas sucessivas da medicação induzem a formação de uma barreira mineralizada apical. Esse tipo de tratamento demanda várias sessões, tornando o tratamento longo. Além disso, as propriedades proteolíticas e higroscópicas do hidróxido de cálcio podem induzir à degradação da porção orgânica da dentina, tornando esta frágil, podendo aumentar o risco de fratura (3). O uso do MTA permite a criação de uma barreira apical artificial e pode ser realizado em única sessão, o que torna uma vantagem relevante para a técnica. Entretanto, essas técnicas de tratamento têm a desvantagem de não permitirem a continuidade do desenvolvimento radicular, o que poderia manter a fragilidade radicular e aumentar a possibilidade de fratura (4).

Um novo protocolo clínico de tratamento (endodontia regenerativa/revascularização) vem sendo sugerido desde 2001, cujo primeiro passo consiste em uma correta descontaminação do canal radicular e após isso, a indução da formação de um coágulo sanguíneo que preencha o canal radicular (5,6). Esse coágulo carrega da região apical células tronco provenientes da papila apical e da bainha epitelial de Hertwig que estão presentes apenas até a completa formação da raiz (7), servindo como uma matriz que auxiliará a formação de um novo tecido vital neste espaço. O novo tecido formado ajudará no término do desenvolvimento radicular, com espessamento das paredes dentinárias e consequente fortalecimento radicular (8,9), podendo ainda reestabelecer a vitalidade de dentes não vitais (10,11).

O derivado de matriz de esmalte é o extrato de uma proteína usado para o tratamento de defeitos periodontais e recessão de tecidos moles. Seu uso em

endodontia tem sido objeto de exploração, principalmente em procedimentos regenerativos (12).

Está bem documentado que a secreção de proteínas derivadas da matriz de esmalte pela bainha epitelial de Hertwig desencadeia uma cascata de reações que estimulam a odontogênese (13,14). Este derivado de matriz de esmalte está disponível comercialmente como Emdogain® (EMD) e é bem reconhecido em Periodontia pelo seu potencial regenerativo (14). No tratamento conservador da polpa dentária, o EMD induz a formação de dentina reparadora, protegendo o tecido pulpar da inflamação e degeneração (15-17). No entanto, seu potencial em endodontia regenerativa ainda não está totalmente compreendido, embora tenha mostrado um papel importante na odontogênese, com potencialização de reparo e regeneração tecidual da polpa (12). Outras propriedades da EMD incluem dentinogênese, cementogênese e angiogênese (18-22). Além disso, o alginato de propilenoglicol, veículo em que é fornecido o EMD apresenta propriedades antibacterianas (23-25).

Na endodontia, existem relatos de casos usando o EMD durante procedimentos cirúrgicos em casos de reparo de perfurações (26). Até o momento estudos laboratoriais e clínicos têm mostrado o potencial de eficácia do EMD utilizado durante procedimentos endodônticos, tais como pulpotomia (27-29), capeamento pulpar direto (30), regeneração pulpar em ratos (31) e medicação intracanal (32).

Em dentes traumatizados ou fragilizados, como nos casos de dentes com ápices abertos e necrose pulpar, seria interessante usar biomateriais durante procedimentos endodônticos regenerativos que possam reestabelecer ou melhorar propriedades físico-mecânicas da dentina, tal como a microdureza dentinária radicular. Porém, até o momento não há estudos que relacionem o efeito do EMD na estrutura e composição da dentina radicular humana. Desta forma, o objetivo do estudo laboratorial, descrito no Capítulo 1, foi avaliar o efeito deste material na microdureza superficial e composição química da dentina radicular humana.

Até o momento, nenhum estudo clínico randomizado e controlado traz evidências da eficácia do EMD utilizado durante procedimentos endodônticos regenerativos (PERs) com desfecho centrado na indução da maturação da raiz (espessamento das paredes radiculares, continuidade na formação do comprimento radicular e fechamento do ápice radicular). Assim, o objetivo da série de casos clínicos, descrita no Capítulo 2, foi avaliar o efeito do EMD na indução da maturação radicular em dentes permanentes imaturos com necrose pulpar através da análise clínica e radiográfica.

2 CAPÍTULO 1

EFFECT OF AN ENAMEL MATRIX DERIVATIVE (EMDOGAIN®) ON THE MICROHARDNESS AND CHEMICAL COMPOSITION OF HUMAN ROOT DENTIN*

ABSTRACT

Introduction: The purpose of this study was to evaluate the effect of an enamel matrix derivative (Emdogain®) on the surface microhardness and chemical composition of human root dentin. **Methods:** Ten human third molars were used to produce 30 dentin specimens (disk-shaped). Specimens remained in contact to EMD (Emdogain® gel) for 90 days into an incubator at 37ºC under humidity. Baseline and 90-days after EMD treatment measurements were performed. Chemical composition was assessed with Fourier Transform Infrared Spectroscopy (FTIR); surface morphology and element analysis were assessed with Scanning Electron Microscopy/Energy Dispersive Spectroscopy (SEM/EDS); and dentin microhardness (KMH) was measured with a Knoop indenter with a load of 10g for a time of 15 seconds. Statistical analysis was used for FTIR and KMH data, and SEM/EDS findings were descriptively reported. **Results:** After 90-days of EMD (Emdogain®) treatment, dentin specimens had decreased values of carbonate ($p=0.001$) and amide III ($p=0.002$), and the C:M (carbonate:mineral) ratios also decreased ($p<0.001$), while the amide I/amide III increased. SEM images showed that the EMD (Emdogain®) treatment did not change dentin surface morphology. EDS analysis showed a slight decrease in the intensity of the calcium (Ca) and phosphorus (P) peaks of the root dentin. No significant differences were observed in the surface microhardness of the samples before and after treatment ($p = 0.35$). **Conclusion:** The use of EMD (Emdogain®) for 90 days in contact with dentin disks did not alter the microhardness and morphology of human dentin. Although the elemental/chemical structure of dentin was altered – the alteration was correlated with increasing resistance to demineralization.

Keywords: Emdogain, enamel matrix derivative, regenerative endodontics, dentin microhardness, dentin chemical composition.

* Article submitted for publication in the journal Scientif Reports.

INTRODUCTION

During the development of the human dental-pulp complex, the secretion of endogenous enamel matrix derivative (EMD) by the Hertwig's epithelial root sheath triggers a cascade of reactions that stimulate odontogenesis (1,2). In addition to that, the Hertwig's sheath deposit enamel matrix proteins on the root surface prior to cementum formation, and these proteins are the initiating factor for cementogenesis (3).

Emdogain® is the commercial name for a synthetic gel containing EMD. The substance is a mixture of natural proteins aiming to induce biological processes that usually take place during the development of the periodontium and to stimulate cells involved in the healing process of soft and hard tissues. Clinically, the Emdogain® has been traditionally used for the treatment of periodontal defects and soft tissue recession.

In endodontics, the EMD (Emdogain®) has been mostly studied as an adjunct to improve regenerative approaches (4). Although the potential of EMD in regenerative endodontics is not yet fully understood, it is proved that it has an important role in odontogenesis, improving pulp tissue healing and regeneration (5). When the EMD is used as an adjunct to a conservative pulp treatment, it induces the formation of reparative dentin, protecting the pulp tissue and consequently preventing pulp degeneration (6-9). The endodontic literature has also stated potential advantages of using Emdogain® in surgical sealing of root perforations (10), direct pulp capping (11, 12) pulpotomy (13, 14), pulp regeneration in rats (15), and as intracanal medication (16). Although EMD Emdogain® has been investigated as a potential substance for use in endodontics, producing good results in relation to its clinical indications, the information on its influence in root dentinal properties has not been studied yet.

When necrosis and apical periodontitis occur in an immature tooth, the weakened of tooth structure is expected because the incomplete apposition of dentin in the root canal walls and incomplete root development. Regenerative procedures aiming pulp revascularization is one of the options to treat these type of cases – and the use of EMD as an intracanal substance could be a manner to strength the tooth structure, improving the physical and chemical properties of dentin. Therefore, this in vitro investigation aimed to test if the EMD-gel (Emdogain®) when used intracanal for 90 days had influence on mechanical or the surface chemical composition of dentin. The null hypotheses tested were: (1) EMD (Emdogain®) would not alter the root dentin Knoop microhardness or morphology; and (2) EMD (Emdogain®) would not affect the calcium and phosphorus content of root dentin or the ratios of mineral/matrix, carbonate/mineral, amide I/amide III and amide I/CH2.

MATERIAL AND METHODS

Enamel matrix derivative EMD (Emdogain®) was obtained from the manufacturer (Institut Straumann® AG, Basel Switzerland). For this experiment approximately 6 gel-syringes of the product were needed. Ten human third molars, extracted for therapeutic reasons, were used to produce 30 dentinal specimens (three slices were acquired from each tooth). This project was approved by the local Research Ethics Committee (Approval number: 3.540.098).

Inclusion criteria for teeth comprised closed apex, no radiographic signals of calcification/mineralization (nor diffuse neither localized), no evidence of internal resorption, and absence of previous endodontic initiated therapy or root canal obturation.

Specimens preparation

Teeth were cleaned and maintained in distilled water for a maximum of 6 months after extraction. Firstly, the crown was separated from the root using a diamond disc attached to a cutting machine (Isomet 1000 Precision Saw Buehler).

Secondly, new slices (from the cervical third of the root) were made to produce dentin disks (n=30). Specimens measured 3 mm diameter x 3 mm height (Figure 1).

For SEM/EDS analysis and microhardness test, the specimens were built-in synthetic plastic polymer (polyvinyl chloride tubes) using acrylic resin (TDV, Pomerode, SC, Brasil) for fixation of the disk base. The disk dentin surfaces were polished using silicon carbide sandpaper in decreasing grains (#400, #600 and #1200). The specimens were polished with felt cloths soaked in diamond paste attached to a slow-speed handpiece (Diamond, FGM, Joinville, SC, Brazil). Then, specimens were washed in an ultrasonic vat with distilled water for 30 min. After taking the baseline measures, the specimens were stored in an oven at 37°C, under humidity (dentin disks remained in well plates covered with 2x2 gauze moistened with distilled water).

Enamel matrix derivative (EMD) treatment

EMD (Emdogain®) was injected directly from the manufacturer syringes into petri dishes. The dentin specimens were then set down over the EMD, having one of the disk surfaces immersed into the substance. Specimens remained for 90 days into an incubator at 37ºC and 100% humidity. After EMD treatment, the specimens were again washed in an ultrasonic vat with distilled water for 30 min, and new measurements for the tests (FTIR, SEM/EDS and MH) were obtained.

Chemical Composition with Fourier Transform Infrared Spectroscopy (FTIR)

Chemical composition of the samples (n=10) was determined using attenuated total reflectance/Fourier transform infrared spectroscopy (FTIR; Vertex 70, Bruker, Ettlingen, Germany). Evaluations were made before and 90-days after EMD (Emdogain®) treatment.

The dentin surfaces (without being included in acrylic blocks) were positioned against the diamond crystal of the FTIR unit. Spectra were recorded in the range from 400 to 4.000 cm^{-1} at 4 cm^{-1} of resolution. Each specimen was scanned 32 times in each FTIR measurement, and the final spectrum acquired was

the average of all these scans. Spectra were recorded and analyzed by OPUS 6.5 software (Bruker, Ettlingen, Germany). After baseline correction and normalization, the area under each band was integrated by using the appropriate tools from the software. Each spectrum was normalized according to the phosphate band (1.190 - 702 cm^{-1}). FTIR spectra were analyzed by calculating the following parameters: (1) mineral/matrix ratio M:M (the ratio of the integrated areas of phosphate v1, v3 stretching mode at 1.035 cm^{-1} to the collagen amide I at 1.655 cm^{-1}); (2) carbonate/mineral ratio C:M (the ratio of the integrated areas of carbonate v2 at 872 cm^{-1} to the phosphate v1, v3 at 1,035 $cm^{-1)}$; (3) amide I/amide III ratio (the ratio of the integrated areas of amide I at 1.655 cm^{-1} to the amide III at 1.235 cm^{-1}); (4) amide I/CH2 ratio (the band ratio of the integrated areas of amide I at 1.655 cm^{-1} to the CH2 scissoring at 1.450 cm^{-1}) (17, 18).

Surface Morphology and Element Analysis with Scanning Electron Microscopy/Energy Dispersive Spectroscopy (SEM/EDS)

Images and spectra of the dentinal surface were obtained before and 90-days after the treatment with EMD (Emdogain®) on a Scanning Electron Microscope (SEM) (TM3030, Hitachi, Tokyo, Japan). Specimens were fixed in a metallic stub using a double-sided carbon tape and evaluated at 1000x and 2500x magnifications, in electron backscatter mode, having as reference point the central region of the sample. Subsequently, the EDS spectra were collected from the same dentinal surfaces to identify calcium and phosphorus elements, similarly to SEM images.

Knoop Microhardness (KMH)

Baseline microhardness readings were obtained from the specimen surface before the treatment with EMD (Emdogain®). Dentin microhardness was measured with a Knoop indenter at 40x magnification (Shimadzu HMV-2000; Shimadzu Corporation, Kyoto, Japan), with a load of 10 g for 15 seconds. The average length of the two diagonals produced by the indenter was used to calculate the KMH value. Four indentations were made in each specimen at 20 µm far away

from the root canal lumen. The representative microhardness value for each sample was the average result of the four indentations. Ninety days after EMD (Emdogain®) treatment, the specimens were washed in an ultrasonic vat with distilled water for 30 min, and new microhardness measurements were obtained identically as described above.

Statistical analysis

Data were tested for normal distribution (Shapiro-Wilk's test, $p>0.05$) and equality of variances (Levene's test, $p>0.05$). FTIR and KMH data were analyzed by a paired t-test, comparing before and after EMD (Emdogain®) treatment. Sigma Plot statistical package (version 12.0, Systat Software, Inc., San Jose, CA, USA) was used for analysis and a p-value of lower than 0.05 was considered statistically significant. SEM/EDS findings were descriptively reported.

RESULTS

Chemical Composition (FTIR)

The mean and standard deviation values for chemical parameters and ratios obtained by FTIR are shown in Tables 1 and 2. After EMD (Emdogain®) treatment, the samples showed a significant decrease in the values of carbonate ($p=0.001$) and amide III ($p=0.002$). The C:M ratios decreased in the samples after EMD (Emdogain®) treatment ($p<0.001$), while the amide I/amide III increased.

Figure 2 shows the FTIR spectra of EMD (Emdogain®) and cervical root dentin before and after treatment. The EMD (Emdogain®) spectrum presented three more evident bands observed at ~ 575, 1,637 and 3,340 cm-1 wavenumbers. The band at 3,340 cm^{-1} is attributed to O-H vibrations in adsorbed water or as hydroxyl group, while the band at 1.637 cm^{-1} is dominated by the C=O stretch vibrations of the peptide linkages present in amide I (19, 20). The band at 575 cm^{-1} is associated with phosphate v4 ($PO4^{3-}$) (18, 21).

Surface Morphology and Element Analysis (SEM/EDS)

Representative SEM/EDS images of specimens before (baseline) and 90-days after treatment with EMD (Emdogain®) are illustrated in Figure 3. The SEM images showed that the EMD (Emdogain®) treatment did not change the surface morphology. After 90 days, the EDS analysis showed a slight decrease in the intensity of the calcium (Ca) and phosphorus (P) peaks of the root dentin.

Dentin Microhardness (KMH)

The results of the Knoop microhardness analysis are shown in Figure 4. No statistically significant differences were observed in the surface microhardness of the samples before and after treatment with EMD (Emdogain®) ($p = 0.35$).

DISCUSSION

The enamel matrix derivative (EMD) (Emdogain®) has been indicated for promoting regeneration of dental tissues. Emdogain® is a composed mainly of amelogenin and amelin, proteins that play an important role in dentinogenesis and promote an increase in the level of mineralization markers in odontoblasts (22). This class of proteins is known to induce the growth and proliferation of cells of the periodontal ligament, which has propylene glycol alginate (PGA) as a vehicle with an important antibacterial action (23-26).

The literature has shown that EMD induced formation of mineralized tissue on root canal walls, contribute to the root development and, even, the helped in periapical healing. In 2012, the efficacy of EMD was compared with a triple antibiotic paste (TAP) as an intracanal drug for regeneration of immature teeth in rats with pulp necrosis. EMD and TAP were able to reduce periapical lesion size loss and increase root length and thickness. EMD promoted narrower canals compared to TAP, a positive finding that could strengthen the tooth. Another experiment proved

that EMD, when used in pulpotomy therapy, induced the formation of substantial amount of dentin-like tissue. The beginning of hard tissue formation was radiographically observed 2 weeks post-operative and it was located only in the affected pulp region. In comparison, the authors showed that dentin-like tissue was also formed in teeth treated with Dycal®, but in limited amount, and at the expense of the whole width of the pulp chamber floor, narrowing of the root canals entrance. The total amount of dentin formed in the teeth treated with EMD was significantly higher than in the samples treated with Dycal® (15).

The findings of this present study added information to the body of knowledge on the benefits of using EMD (Emdogain®) for 90 days in contact with root canal dentin. We showed that EMD (Emdogain® gel) did not alter either microhardness of human dentin or its morphology, accepting the 1st null hypothesis.

The FTIR analysis is a reliable way to generate evidence about the presence of functional groups present in the structure of a sample, which can be used to identify a compound or to investigate its chemical composition (20). Since EMD (Emdogain®) is a mixture of hydrophobic enamel matrix proteins [derived from 6-month-old porcine tooth buds] containing amelogenin, enamelin, tuftelin, amelin, and ameloblastin, in a propylene glycol alginate (PGA) (27), its proteins guide tissue regeneration and induce remineralization of enamel and dentin (28, 29).

This current study observed a reduction in dentin carbonate values after EMD (Emdogain®) treatment, which also impacted the C:M ratio. This ratio indicates the extent of carbonate incorporation in the hydroxyapatite lattice (30). As carbonate is responsible for the acidic solubility of dental hard tissues, the reduction in carbonate content is related to the increased resistance to demineralization (31), which occurred after EMD (Emdogain®) treatment. These findings rejected the 2nd null hypothesis.

Amelogenins are responsible for regulating the mineralization process and for organizing the apatite crystals into juxtaposed prisms (Moradian-Oldak, 2001). The amelogenin protein molecule is divided into three amino acid domains,

which are: central domain, C-terminus (COOH) and N-terminus (NH2) (32). Both terminus types play key roles in proteolytic processes (33) and can interact with chemical components of the dental tissue, altering them quantitatively or changing their molecular conformation. Filamentary structure in amelogenin may induce ionic interactions, through acidic residues present in the C-terminal domain, for example (33). This can result in modifications to the amide bands of the FTIR spectrum. In our study, these alterations found in the organic portion of dentin are represented by changes in amide III values. It is one of the amides present in the collagen structure; however, it is a very unstable and complex band depending on the details of the force field, the nature of the side chains and hydrogen bonding (18, 34). The reduction in amide III values may mean a disorganization in the secondary structure of the collagen fiber-forming protein unit (35, 36). Amide I/amide III ratio has also been altered and this represents a change in organization of collagen within the samples after Emdogain treatment (37, 38). Since collagen is the most abundant protein in dentin, its proteolysis has a significant impact on the structural integrity of this tissue, which can become mechanically and functionally compromised (17,18). However, the chemical modifications did not repercuss in significative changes in dentin microhardness and surface morphology.

This is a pioneering study on the action of emdogain on human root dentin in the endodontic literature. Nevertheless, we showed that EMD (Emdogain®) is a potential substance to use intracanal – not interfering in dentin microhardness and contributing to increase resistance to demineralization (consequence of the reduction of carbonate levels). One of the strengthens of this study is the initial understanding of what occurs in the macro and microstructure of EMD treated dentin. The findings of this current study may prompt further studies such as: tooth esthetic/color analysis when EMD is used into the root canal, analysis of other physicochemical-biological dentin properties, etc.

CONCLUSION

The use of EMD (Emdogain®) for 90 days in contact with dentin disks did not alter the microhardness and morphology of human root dentin. The elemental structure of dentin was altered, because there was a reduction in carbonate content, which is related to the increased resistance to demineralization. The findings of this study suggest that EMD (Emdogain®) may be an interesting option to be used as intracanal dressing in regenerative therapies.

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Tables

Table 1 - Means and the standard deviations of the integrated area of each chemical component analyzed by FTIR before and 90-days after EMD (Emdogain®) treatment.

Chemical components	*Assessment time*		
	Before (control)	After	*p-value*
Phosphate	13.15 (1.30)	11.95 (1.79)	=0.103
Carbonate	0.23 (0.03)	0.16 (0.03)	=0.001*
Amide I	2.06 (0.36)	2.22 (0.73)	=0.685
Amide III	0.22 (0.05)	0.16 (0.02)	=0.002*
CH_2	0.12 (0.04)	0.14 (0.05)	=0.395

** Indicates differences in root dentin chemical components (in rows) obtained by paired t-test ($p<0.05$).*

Table 2 - Means and the standard deviations for M:M; C:M, amide I/amide III and amide I/CH_2 ratios analyzed by FTIR before and after EMD (Emdogain®) treatment.

Ratios	*Assessment time*		
	Before (control)	After	*p-value*
M:M	6.58 (1.40)	5.72 (1.46)	=0.293
C:M	0.017 (0.001)	0.013 (0.001)	<0.001*
Amide I/amide III	9.70 (1.38)	12.71 (1.71)	<0.001*
Amide I/CH_2	17.94 (4.43)	17.50 (6.42)	=0.857

** Indicates differences in root dentin ratios (in rows) obtained by paired t-test (p<0.05).*

Figure Legends

Figure 1 – Schematic illustration of the experimental design.

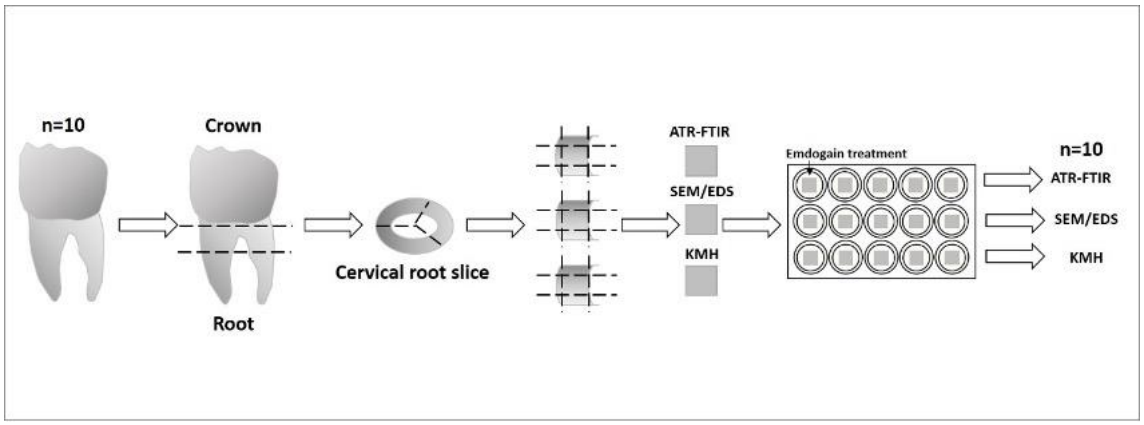


Figure 2 - Representative imagens of FTIR spectra. A -EMD (Emdogain®) material. B- Cervical root dentine spectra before (black line) and after EMD (Emdogain®) treatment (red line).

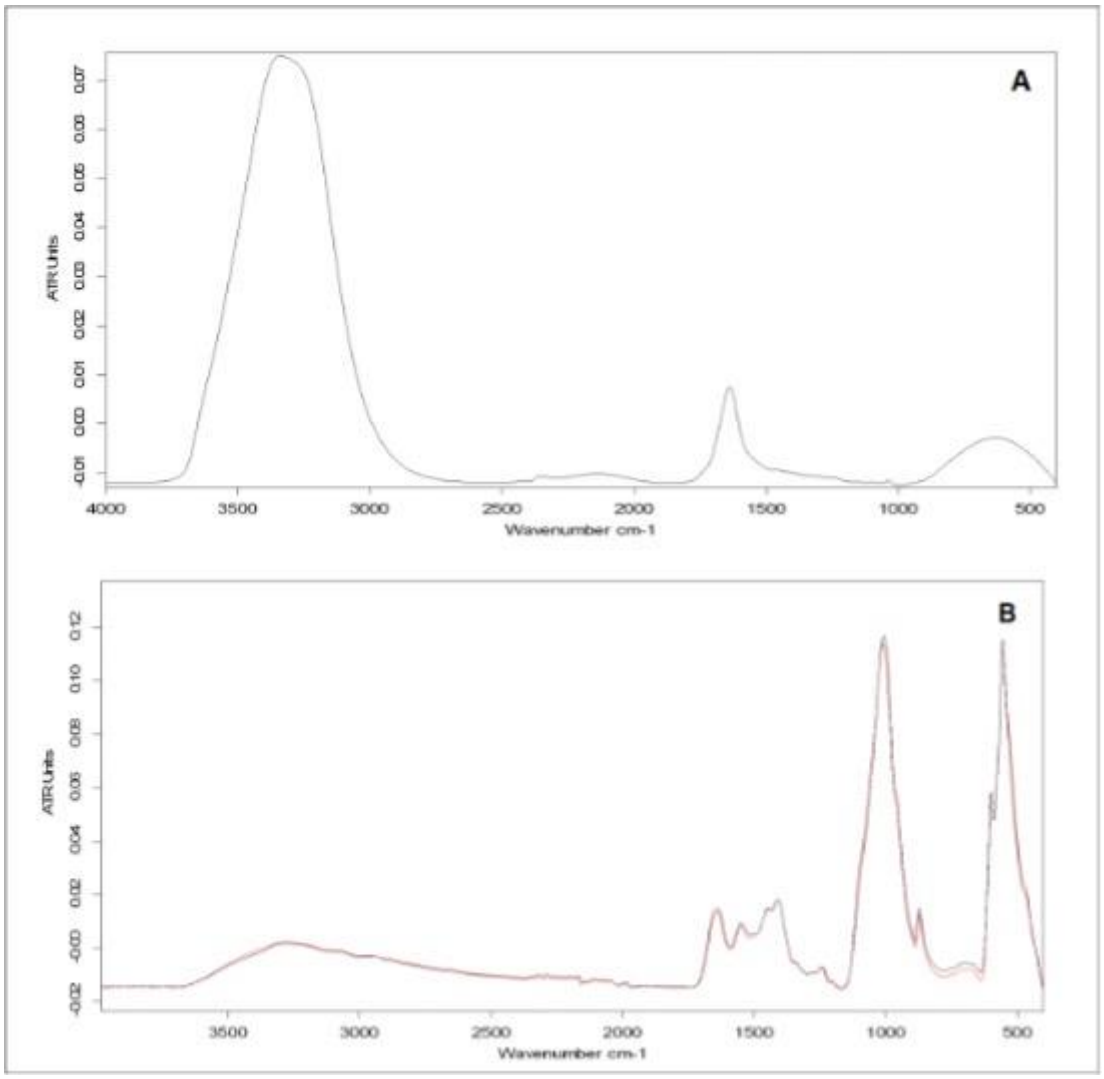


Figure 3 – Representative images/spectra of specimens` surface (dentinal surface) analysed by SEM/EDS: (A) is showing the SEM image and the spectra (right side) before treatment with EMD (Emdogain®) (baseline); and (B) is showing the SEM image and the spectra (right side) 90-days after treatment with EMD (Emdogain®).

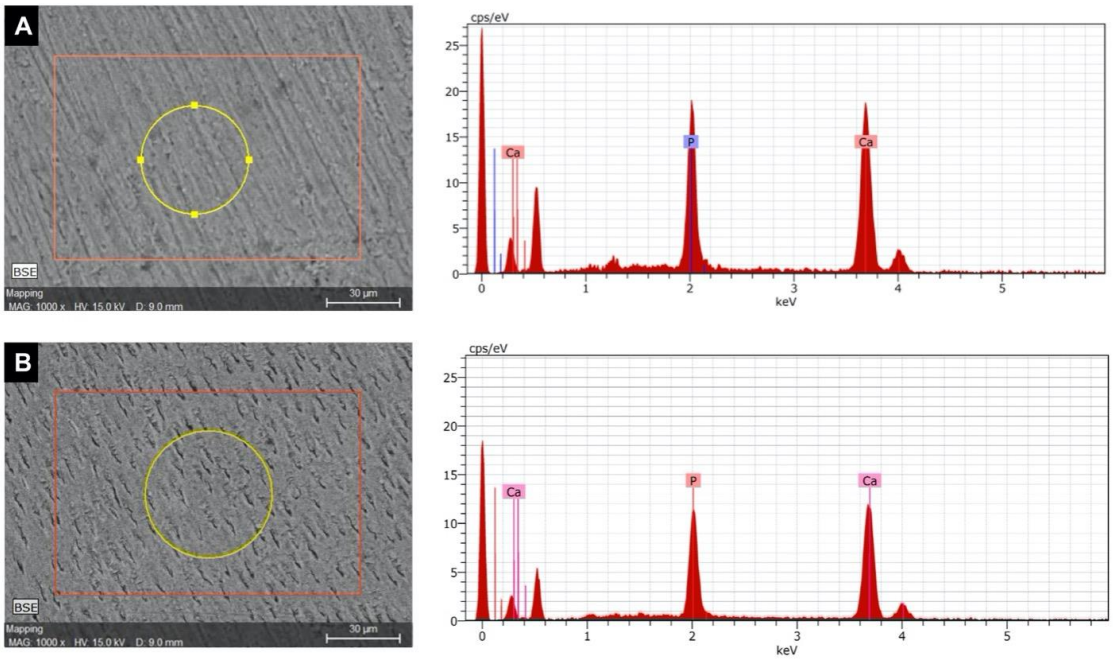
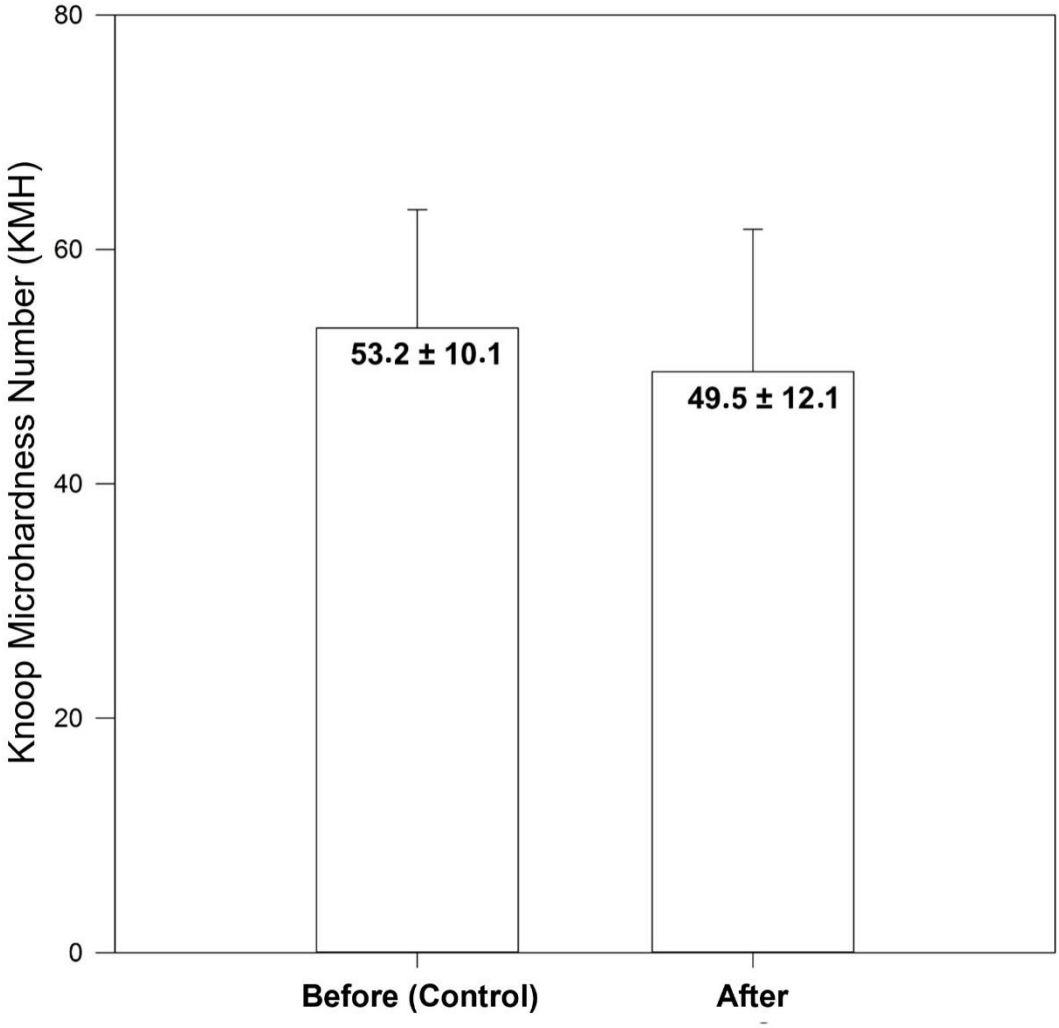


Figure 4 – Mean microhardness values (KMH) of root dentin before (baseline) and after 90-days treatment with EMD (Emdogain®).



Highlights

- Emdogain® is the commercial name for a synthetic gel containing enamel matrix derivative (EMD), and it induces biological processes in the dental periodontium and stimulates cells involved in the healing process of soft and hard tissues
- The use of EMD (Emdogain®) for 90 days in contact with dentin disks did not alter the microhardness and morphology of human root dentin
- The use of EMD (Emdogain®) for 90 days in contact with dentin disks altered the elemental/chemical structure of dentin – but the alteration was correlated with increasing resistance to demineralization.

Significance

EMD (Emdogain®) is a potential substance to use intracanal – not interfering in dentin microhardness and contributing to increase resistance to demineralization. This could reinforce dental structures during regenerative procedures.

3 CAPÍTULO 2

REGENERATIVE ENDODONTIC PROCEDURE USING EMDOGAIN®: CASE SERIES

ABSTRACT

Introduction: Emdogain® (EMD) is an enamel matrix derivative, whose potential in regenerative endodontics procedures (REPs) is not yet fully understood, although it has shown an important role in odontogenesis with potentiation of pulp tissue repair and regeneration. The aim of this study was to evaluate the effect of EMD on the induction of root formation in immature permanent teeth with pulp necrosis through clinical and radiographic analysis**. Methods:** Immature permanent teeth with necrotic pulp (n=3) from 8- and 12-year-old patients were treated with REP using EMD. Protocol was divided into two sessions with two-weeks interval: in both, intracanal irrigation and drying were performed, and in the second session, bleeding was induced using EMD, with subsequent sealing with mineral trioxide aggregate cement (MTA), placement of restorative glass ionomer cement and restoration with composite resin. **Results:** Follow-examinations showed that the teeth remained functional without any signs or symptoms. Final radiographic examination at 12 and 24 months, respectively, revealed complete resolution of the lesions (when presente) and evident apical closure. An increase in root length and thickening of the root dentin walls could be observed in all cases. In one of the cases, there were radiographic images suggestive of scattered calcified zones in the root canal lumen that may be related to the use of EMD. **Conclusions:** This case series shows how REPs may have the potential for continuing root length formation and apical closure, with considerable thickening of the root dentin walls and that EMD could potentiate this process, justifying randomized controlled trials.

Keywords: Regenerative endodontics, dental pulp necrosis, enamel matrix derivative, odontogenesis.

INTRODUCTION

The regenerative endodontic procedure was suggested as an option for the treatment of necrotic teeth with incomplete rhizogenesis in 2004 by Banchs and Trope, whose first step consists of correct decontamination of the root canal and after that, the induction of the formation of a blood clot that fills the root canal (1,2). This clot carries from the apical region cells from the apical papilla and Hertwig epithelial sheath (HES) that are present only until the complete formation of the root (3), serving as a matrix that will help the formation of new vital tissue in this space. The presence of cells will help in ending root development with thickening of the dentinal walls and consequently root strengthening (4,5), can also restore the vitality of non-vital teeth (6,7). Protocols for regenerative endodontic procedures vary in terms of the use of irrigants (8,9,10), intracanal medication (2,11,12), scaffolds (13,14) and materials for cervical sealing (10,11,15,16). Any manipulation inside the root canal, such as the use of irrigants or intracanal medication, should be considered under the premise of creating the best possible environment for the cells that were carried into the canal during the induction of bleeding to exert their regenerative potential. This implies controversy, as the correct disinfection of the root canal system is as important as preventing damage to the target cells (17).

Resolution of apical periodontitis associated with immature permanent teeth with necrotic pulps after regererative endodontic therapy is predictable if clinicians use an effective disinfection protocol to eradicate infection (18). It could depend on the severity, source and duration of apical periodontitis or the severity of damage caused by the dental trauma to HES and the apical papilla (19). Clinically it is impossible to know their conditions; therefore, the outcomes of regererative endodontic therapy in immature permanent teeth with pulp necrosis/ apical periodontitis following dental trauma are unpredictable.

A blood clot has been the main scaffold in the published case studies (8,10,16, 20-23, 25), but it would be advantageous if regenerative strategies using advanced scaffolds and growth factors could favour pulp and dentine regeneration. Many types of biomaterials have been investigated and have been

proposed as scaffold and some tested for regenerative endodontic therapy in animal models, as alginate, hyaluronic acid, chitosan PLLA NF-MS with BMP-2, PLGA-PEG nanoparticles and Vitrogel 3D with SDF- 1a and BMP-2 (26).

It is well documented that the secretion of enamel matrix-derived proteins by Hertwig's epithelial sheath triggers a cascade of reactions that stimulate odontogenesis (27,28). The enamel matrix derivative is commercially available as Emdogain® (EMD) and is well recognized in Periodontics for its regenerative potential (28). In the conservative treatment of the pulp EMD induces the formation of reparative dentin, protecting the pulp tissue from inflammation and degeneration (29-31). However, its potential in regenerative endodontics is not yet fully understood, although it has shown an important role in odontogenesis, with potentiation of pulp tissue repair and regeneration (32). The aim of this study was to evaluate the effect of EMD on the induction of root formation in immature permanente teeth with pulp necrosis through clinical and radiographic analysis.

MATERIAL AND METHODS

Clinical and radiografic procedures

Three patients aged 8 and 12 years old who had teeth (n=1 per patient) with incomplete root formation and a diagnosis of pulp necrosis were treated by a single REP protocol with prior approval from the Research Ethics Committee of Ceuma University (approval number: 2.997.609).

Medical and dental history was obtained for all patients, followed by a thorough dental examination. Clinical and radiographic examinations in combination with a thorough periodontal evaluation and clinical testing (pulp and periapical tests) were used to confirm preliminary diagnosis. Mobility tests and thermal sensitivity to cold, electrical test for diagnosis of pulp condition and tests of horizontal and vertical percussion and apical palpation were performed. Quantitative assessments of crown shades of treated teeth were determined using the VITA Easyshade® Advance 4.0 spectrophotometer (VITA Zahnfabrik,

Bad Sackingen, Germany) before and after REP, as well as during follow-up visits. This measurement was performed in the most cervical area of the vestibular surface of the teeth.

The same treatment approach was undertaken by all six patients and is described step by step in Chart 1. At the first treatment appointment, local anesthesia with 4% articaine containing 1:100,000 epinephrine (Nova DFL, Rio de Janeiro, Brasil) was initially administered. The tooth was then isolated with a rubber dam, and the access cavity was prepared. Root canals were detected and then pulp chamber and root canal orifices were irrigated with 10mL 2.5% sodium hypochlorite (NaClO - Fórmula e Ação, São Paulo, Brazil). Working length was determined radiographically and a light mechanical preparation of the cervical and middle third of the canals was performed. Each canal was then irrigated with another 10 mL of 2.5% NaClO using a 27-G irrigating needle tip (Ultradent Products Inc. South Jordan, Utah, USA). After, 10mL of ethylenediaminetetraacetic acid (EDTA-Fórmula e Ação, São Paulo, Brazil) was applied. Each canal was then irrigated with another 10mL d of 2.5% NaClO, 10mL of saline solution and dried with sterile paper points and medicated with calcium hydroxide (Ultracal - Ultradent, EUA). The access cavity was sealed with sterile cotton pellet temporary filling material (Coltosol®, Coltene, Rio de Janeiro, Rio de Janeiro) as well as restorative glass ionomer cement Riva Light Cure (SDI, Victoria, Austrália).

All teeth were asymptomatic and two weeks later, the second treatment session was scheduled. The teeth were isolated with rubber dam after local anesthetic infusion without a vasoconstrictor (2% lidocaine, Nova DFL, Rio de Janeiro, Brazil). The root canals were accessed once more and irrigated with 10 mL of EDTA to remove calcium hydroxide, followed by a final rinse with 10 mL of saline solution and drying with sterile paper points. The root canals were again accessed and irrigated with 10 mL of EDTA for the initial removal of calcium hydroxide followed by a final rinse with 10mL of saline solution and the canals were then dried with sterile paper points. Bleeding was generated by disrupting periapical tissues 2 mm beyond the root apex with a size #40 K file.

Following the initial input of blood into the root canal, EMD (Emdogain®,Institut Straumann AG, Basel, Switzerland) was injected straight into the canal in the full amount of the syringe (0.15mL). After a suitable blood clot/EMD had been generated, a little amount of white MTA (MTA Angelus Odontológica, Londrina, Paraná, Brazil) was gently deposited and condensed in the coronal third of each channel. Restorative glass ionomer cement was placed over the MTA, followed by a composite resin restoration (3M ESPE, St. Paul, MN).

Chart 1 - Regenerative Endodontics Procedure Protocol.

Step	First Session	Second Session
1	Local anesthesia with 4% articaine containing 1:100,000 epinephrine	Local anesthesia with 2% lidocaine without vasoconstrictor
2	Absolute isolation and access to root canals	Absolute isolation and access to root canals
3	Irrigation with 10mL of 2.5% NaClO	Irrigation with 10mL of EDTA to remove Ultracal
4	Determination of working length through periapical radiography and light mechanical preparation in the cervical and middle thirds	Irrigation with 10 mL of saline
5	Re-irrigation with 10 mL of 2.5% sodium hypochlorite with a 27-G irrigating needle tip	Drying with absorbent paper points
6	Irrigation with 10 mL of EDTA	Bleeding induction with K-file size #40, going to 2 mm from the root apex
7	Irrigation with 10mL of 2.5% NaClO	Determination of bleeding time in seconds
8	Irrigation with 10 mL of saline	Application of the total syringe (0.15 mL) of EMD, mixing with the blood
9	Drying with absorbent paper points	Placement and condensation of white MTA in the cervical third of root canals
10	Insertion of calcium hydroxide (Ultracal)	Restorative glass ionomer cement insert
11	Access sealing with sterile cotton	Restoration with composite resin
12	Temporary filling with Coltosol + Riva Light Cure restorative glass ionomer cement	X-Ray to confirm MTA adaptation

The final radiograph was taken to confirm the MTA placement in all cases. The patients were recalled at different times after 3, 6, 12, 18 and 24 months. Subjective pain was measured using the Visual Analogue Scale (VAS) with degrees numbered from 0 (no pain) to 10 (worst possible pain). The assessment times were: during REP (shortly after induction of bleeding) and seven days following the procedure. Table 1 describes the patient age, gender, tooth, diagnosis, bleeding time, trans operative pain, radiolucency resolution, tooth discoloration, results for electric pulp test and cold sensitivity test, root thickening, root lengthening, apical closure, quality of coronal restoration and follow-up.

RESULTS

Case report 1 (M.S.M.)

The 8-year-old girl was referred to the integrated children's clinic at Ceuma University, with facial swelling and fever. The person in charge reported painful symptom. The medical history was unremarkable. Clinical examination revealed edema in the apical region and tests resulted in negative for cold and electrical test and positive for palpation. When performing the intraoral examination, it was noted that tooth #19 (mandibulary left first permanent molar) had been previously accessed and had a provisional restoration, however, carious tissue was still present. Probing depths were 3 mm. Percussion testing revealed tenderness. Radiographic examination showed the opened apex as well as the periradicular lesion to the distal canal (Fig. 1A). The diagnosis of necrotic pulp with acute apical abscess was made, and REP was elected as the treatment of choice. Clinical procedures for REP were performed as described previously. Intraoperative and postoperative pain was evaluated using a form containing a visual analogue scale (VAS). The patient claimed the worst possible pain during the induction of bleeding (duration of bleeding time during induction in the 2nd session was 60 seconds), however she had no discomfort for the entire week of postoperative pain assessment. The tooth was asymptomatic and negative in all

clinical tests at the 3-month follow-up session, and radiographs revealed that the periradicular region had healed (Fig. 1B).

Changes in root width and length were noted at the 6-month and 12-month follow-up examination (Fig. 1C, 1D), tooth continued to be asymptomatic and revealed a positive response to thermal and electrical testing with percussion and palpation responses within normal limits. A normal periodontal ligament aspects of the root.

All clinical signs remained stable at the 24-month follow-up, including a positive response to cold and electrical sensitivity tests and a color shade of A3. The apex of the mesial canals was closed and the periodontal ligament was within normal limits on radiographic evaluation (Fig. 1E).

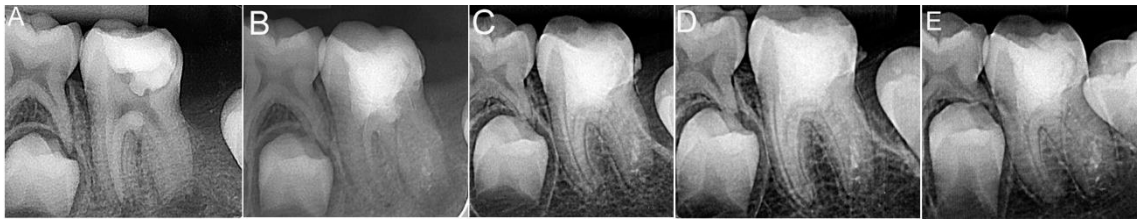


Figure 1 – case 1: tooth #19. (A) Preoperative periapical radiograph. (B) Periapical radiograph after 3 months of REP with EMD. (C) Periapical radiograph after 6 months postoperatively showing the continuity of root length formation. (D) Periapical radiograph taken 12 months after REP, showing the complete absence of a periapical lesion. (E) Periapical radiograph 24 months after the REP, with closure of the root apex.

Case report 2 (Y.G.S.F.)

The 8-year-old boy was referred to Ceuma University's integrated children's clinic. The person in charge reported trauma. Enamel-dentin fracture was observed in tooth #8 (maxillary right permanent central incisor). Medical history was normal. Percussion and palpation examinations, as well as thermal and electrical testing, yielded negative results during clinical evaluation. Tooth color was initially determined in B2. Radiographic examination showed open apex and absence of periapical lesion (Fig. 2A). The diagnosis of necrotic pulp with normal apical tissues was made, and REP was elected as the treatment of choice. Clinical procedures for REP were performed as described previously. The duration of bleeding time during induction in the 2nd session was 50 seconds and

the patient reported grade 8 intraoperative pain (severe pain). In the seven days following the procedure, no pain was reported (grade zero) and on the seventh day, the crown was restored with composite resin. At the first follow-up visit (3 months after REP), the tooth was asymptomatic and tested negative for both the thermal and electrical tests. There was a change in tooth color to C3. Radiographic examination showed visible continuity of root length formation and a radiopaque image in the region in the first root third, suggestive of tissue formation (Fig. 2B). At the second follow-up visit (6 months after REP), the tooth remained asymptomatic and once again tested negative for both the clinical tests. There was a change in tooth color to D4.

The radiographic examination showed evidence of continuity in the formation of the root length and radiopaque images circumscribed in the region in the first root third, suggestive of tissue formation (Fig. 2C). At the third visit (12 months after REP) there was a positive response to thermal and electrical tests. Upon color determination with Vita Easyshade® V, D4 color maintenance was observed. Digital radiographic examination showed continuity in root formation, with onset of apical closure and further intraradicular radiopaque images (Fig. 2D). At the last visit (24 months after REP), all clinical signs remained unchanged, including the positive response to the clinical tests, with determination of C4 color. The root apex was closed and periradicular regions as well as the periodontal ligament, were all within the normal range on radiographic inspection (Fig. 2E).

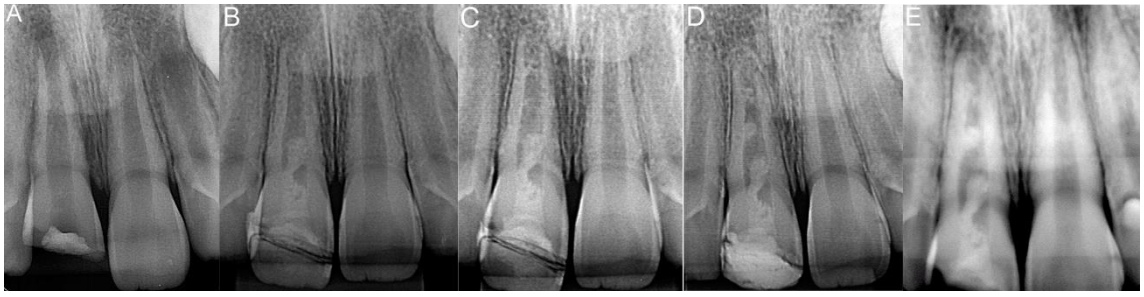


Figure 2 – case 2: tooth #8. (A) Preoperative periapical radiograph. (B) Periapical radiograph after 3 months of REP with EMD. (C) Periapical radiograph after 6 months postoperatively showing continuity of root length formation and appearance of radiopaque images (suggestive images of

calcified material) within the root canal. (D and E) Periapical radiograph taken 12 and 24 months after REP, showing radiopaque images and dentinal thickening and apical closure.

Case report 3 (E.S.A.)

The 12-year-old boy was referred to Ceuma University's integrated children's clinic. The person in charge reported trauma more than a year ago. Provisional restoration was observed in tooth #9 (maxillary left permanent central incisor). Medical history was normal. On clinical examination, that tooth did not respond to percussion, palpation, thermal, or electrical tests. Tooth color shade was initially determined on A3. The radiograph revealed an open apex with a periapical lesion (Fig. 3A). The diagnosis of necrotic pulp with asymptomatic apical periodontitis was made, and REP was elected as the treatment of choice (Fig. 3B). Clinical procedures for REP were performed as described previously. The duration of bleeding time during induction in the 2nd session was 27 seconds and the patient reported grade 8 intraoperative pain (severe pain). In the seven days following the procedure, no pain was reported (grade zero).

At the first follow-up visit (3 months after REP), the tooth was asymptomatic and tested negative for both the thermal and electrical tests. There was a change in tooth color to C3. At the second follow-up visit (6 months after PER), the tooth remained asymptomatic and once again tested negative for both clinical tests. When utilizing Vita Easyshade® V to determine color, the C3 color remained unaltered. Radiographic examination showed continuity of root length formation, with a tendency to close the root apex (Fig. 3C).

In the third appointment (18 months after REP) a negative response to the thermal and electrical tests remained. Upon color determination, a color change to B3 was observed. Digital radiographic examination showed continuity in root formation, with slight apical closure and periradicular regions and periodontal ligament within the normal range with regression of the lesion (Fig. 3D).

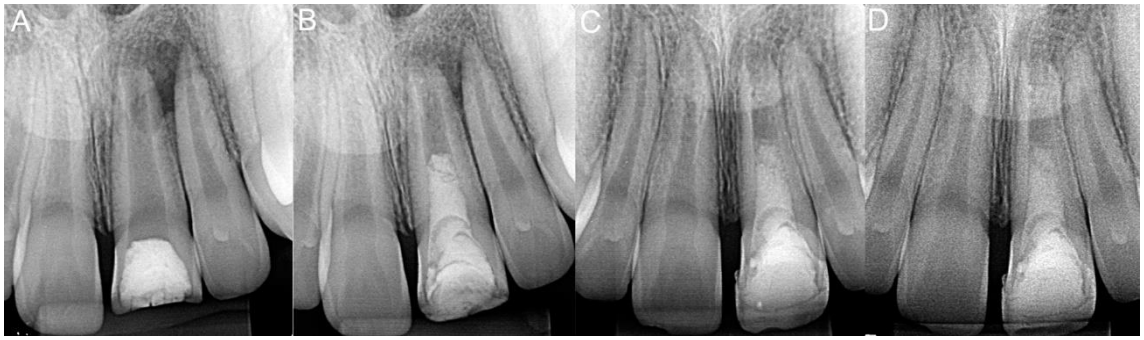


Figure 3 – case 3: tooth #9. (A) Preoperative periapical radiograph. (B) Periapical radiograph after REP with EMD (C) Periapical radiograph after 6 months postoperatively showing mild root thickening. (D) Periapical radiograph taken 18 months after REP, showing mild root thickening, tendency to close the root apex and regression of the periapical lesion.

Table 1 - Patient data and regenerative endodontic procedure.

Pacient age	Gender	Tooth #	Diagnosis	Bleeding time (seconds)	TOP VAS	Radiolucency resolution	Tooth discoloration	EPT 3/6/12/24	CST 3/6/12/24	Root thickening 3/6/12/24	Root lengthening 3/6/12/24	Apical Closure 3/6/12/24	QCR (S or I)	Follow-up (months)
08	F	19	PN/AAA	60	10	Yes	A2 – A3	-/+/+/+	-/+/+/+	-/+/+/+	-/-/+/+	-/-/-/+	S	24
08	M	8	PN/NAT	50	8	-	B2 – C4	-/-/+/-	-/-/+/-	-/-/+/+	-/-/-/+	-/-/+/+	I	24
12	M	9	PN/AAP	27	9	Yes	A3 – B3	-/-/-/-	-/-/-/-	-/-/-/+	-/-/+/+	-/-/-/+	S	24

Legend: F, female; M, male; PN, pulp necrosis; AAA, acute apical abscess; AAP, asymptomatic apical periodontitis; NAT, Normal apical tissues; TOP, trans operatory pain; VAS, visual analogic scale; EPT, Electric pulp test; CST, Cold sensibilty test; QCR, Quality of Coronal Restoration; S, Satisfactory; I, Insatisfactory.

DISCUSSION

Current focus on Regenerative Endodontic Procedures (REP) with has been on immature teeth with pulp necrosis/apical periodontitis. There are many variables that may affect the clinical outcomes which have been defined in the AAE Clinical considerations for a Regenerative Procedure (AAE 2018) and defined as primary (resolution of signs and symptoms of infection), secondary (further root maturation is desirable) and tertiary goals (return of neurogenesis). It has been suggested that a 20% increase in root canal wall is required for there to be a significant clinical effect of the REP. In this protocol there is little or no instrumentation, using only irrigating agents with antimicrobial (sodium hypochlorite), chelating (EDTA) and alkalizing (saline saline) purposes (33).

In 2012, Scarparo et al. (34) compared the effectiveness of EMD against a triple antibiotic paste (TAP) as an intracanal medication for regeneration of immature rat teeth with pulp necrosis. The results showed that both EMD and TAP were able to reduce periapical lesion size, in addition to increasing root length and thickness, although EMD promoted narrower root canals compared with TAP. Results showed that EMD treatment induced mainly the formation of a cementum like tissue on the apical region of the root's external surfaces as well as ingrowth of cementum like tissues into the root canal space. This ingrowth of hard tissue may potentially increase the resistance to fractures and concluded that EMD may be an alternative for enhancing root development in immature necrotic teeth.

Another study (32) evaluated the effects of EMD on the proliferation and differentiation of human dental pulp cells (hDPCs) in vitro. They concluded that EMD could enhance the mineralization of hDPCs and increased the expression of markers for odontoblast/osteoblast-like cells.

In the present case series, all cases showed dentinal thickening and a tendency to apical closure. Also, calcified deposits were observed in the root canal. Although obliteration of the root canal lumen does not mean failure of the regenerative endodontic procedure, its occurrence may indicate that the tooth needs conventional endodontic treatment. Two cases showed positive responses

to pulp sensitivity tests at different moments (case 1 from 6-months to 24-months follow-up and case 2 in 12-months follow-up). Most cases had a history of dental trauma (cases 2 and 3), some with associated injuries. All teeth showed resolution of signs and symptoms, in addition to functional restoration.

Ideal root development pattern in immature teeth includes increase in root length, increase in root wall thickness, and formation of the root apex. In some studies, the outcome of REP was lower than ideal, including absence of increase in root length (8,15), absence of increase in root wall thickness (8,22), or lack of formation of tooth apex. Formation of a hard-tissue barrier inside the canal between the coronal MTA plug and the root apex (22) is another reported unfavorable outcome. It has been further speculated that there may be a relationship between the duration of pulp necrosis and the outcome of treatment. Case 2 showed radiopaque images inside the root canal, suggestive of calcified tissue. There might be a correlation between dental history and quality of root development, the longer the duration of pulp necrosis, the lower the quality of root development after regenerative endodontic treatments (15).

Lack of continued root development after REPs is likely due to severe damage to the HES because it regulates root development. If the apical papilla is severely damaged, there will be no newly differentiated odontoblasts to produce root dentine. The survival of the apical papilla, Hertwig's epitelial root sheath and dental follicle in immature permanent teeth with pulpal infection or dental trauma is not under the full control of the clinician. Some immature permanent teeth with necrotic pulps/apical periodontitis after REPs respond to thermal or electric pulp test (EPT) (18). All vital tissues are vascularized, and most are innervated and therefore can respond to thermal and EPT tests. The fact that a tooth after REPs does respond to sensibility tests does not necessarily indicate that a more organized vital pulp tissue has been regenerated in line with what is described in the tertiary goal of 'AAE Clinical consideration for a regenerative procedure' (35) because histological examination has shown that repair with periodontal- and bone-like tissues is achieved rather than regeneration of a pulp–dentine complex (36-42).

Trans and postoperative pain was evaluated in this case series and can impact quality of life, especially in childhood and pre-adolescence, and make it difficult to continue treatment. Other impairments may result from this pain, such as speech, chewing and psychological aspects, such as difficulty relaxing and irritability (43,44). In the 3 cases described here, pain was manifested during the operation, especially at the time of induction of bleeding for clot formation, ranging from 1 (mild pain) to 10 (worst possible pain). To facilitate bleeding after root canal disinfection, using local anesthetics without vasoconstrictors is recommended (45), however, alternatives to pain mitigation should be sought at this stage, as patient collaboration is essential for a good performance of the clinical procedure. Postoperatively, there was no manifestation of pain. It is necessary for the professional to be aware of the impacts felt by the patient in the trans and postoperative phases, so that he can take precautions in future treatments.

Regarding tooth discoloration after REP, it was observed that it was a unanimous occurrence. Only in one of the cases there was a color change from a more yellowish hue to a lighter hue. However, this case has a follow-up of only 3 months, which makes it possible for this color change to follow the pattern of the other cases. Studies reported a percentage ranging from 44% - 83% of coronal discoloration (46,47) The mechanism of discoloration is most evident when tetracycline-based antibiotics (minocycline or doxycycline) are used as intracanal medication. In the present study, only calcium hydroxide (Ultracal) was used as medication, however the rate of tooth discoloration was high.

MTA is another material that can induce discoloration (48,49). Discoloration after treatment of teeth that were treated with calcium hydroxide might be related to the presence of MTA in cervical portion of the root channel space in 2 of 20 cases (22). A report on pulp capping in anterior teeth revealed that the presence of white MTA in the crown can cause considerable discoloration (50). One difficulty observed in this study was the insertion of MTA to seal the entrances of the root canals, especially in cases of single-rooted teeth, whose greater volume of the canal lumen seems to favor the extravasation of material beyond the cervical third of the root. Case three exemplifies this difficulty very

well, as it was observed radiographically, an image suggestive of this extravasation.

Future studies are warranted to discover alternative repair materials to overcome this drawback.

Further studies are required, and adoption of standardized approaches is necessary to evaluate outcomes. Biomaterials have been used in REP and this study is the first case series using EMD in these clinical procedures. Jung et al. (24) state that, although the case series do not provide definitive evidence to support the treatment protocol, they have the advantage of being performed in real patients and may provide future guidelines for randomized clinical trials.

CONCLUSION

This case series raised the possibility of beneficial clinical effects of Emdogain® in regenerative endodontic procedures. Outcomes such as continuity of root formation, thickening of the root dentin walls and closure of the root apex were observed.

Randomized controlled clinical studies, with clinical and radiographic follow-ups associated with the assessment of the pulp and periodontal condition, can provide scientific support for establishing a consistent and more predictable clinical protocol.

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4 CONSIDERAÇÕES FINAIS

O uso de EMD (Emdogain®) por 90 dias em contato com discos de dentina não alterou a microdureza e a morfologia da dentina radicular humana. A estrutura elementar da dentina foi alterada, pois houve redução no teor de carbonato, o que está relacionado ao aumento da resistência à desmineralização. Os achados deste estudo sugerem que o EMD (Emdogain®) pode ser uma opção interessante para ser utilizado como medicação intracanal ou em terapias regenerativas.

O Emdogain mostrou potencial para uso intracanal – não interferindo na microdureza da dentina e contribuindo para aumentar a resistência à desmineralização. Os achados deste estudo atual podem levar a novos estudos, como: análise estética/cor do dente quando o EMD é usado no canal radicular, análise de outras propriedades físico-químicas-biológicas da dentina.

Referente à série de casos clínicos, há especial interesse quanto ao potencial uso de derivado de matriz de esmalte (Emdogain®) em procedimentos de endodontia regenerativa. Estudos clínicos randomizados controlados, com acompanhamentos clínico e radiográfico associados à avaliação da condição pulpar, podem fornecer subsídios científicos para que se estabeleça um protocolo clínico consistente e mais previsível.

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ANEXO 1 – PARECERES DE APROVAÇÃO DO CEP

CENTRO UNIVERSITÁRIO DO MARANHÃO - UNICEUMA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: AVALIAÇÃO CLÍNICA E RADIOGRÁFICA DA EFICÁCIA DA APLICAÇÃO DE UM DERIVADO DE MATRIZ DE ESMALTE (EMDOGAIN) NA ENDODONTIA REGENERATIVA: ESTUDO CLÍNICO RANDOMIZADO

Pesquisador: Ceci Nunes Carvalho

Área Temática:

Versão: 2

CAAE: 96193518.0.0000.5084

Instituição Proponente: Centro Universitário do Maranhão - UniCEUMA

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.997.609

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1137375.pdf	04/09/2018 09:51:31		Aceito
Recurso Anexado pelo Pesquisador	CartadeRespostaCEPdefinitivo.docx	04/09/2018 09:50:43	Karime Tavares Lima da Silva	Aceito
Projeto Detalhado / Brochura Investigador	ProjetoDoutoradoDefinitivo.docx	04/09/2018 09:49:11	Karime Tavares Lima da Silva	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TALECEPFINAL.docx	04/09/2018 09:48:40	Karime Tavares Lima da Silva	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLECEPFINAL.docx	04/09/2018 09:48:18	Karime Tavares Lima da Silva	Aceito
Declaração de Instituição e Infraestrutura	CartaAnuenciaAssinada.pdf	03/08/2018 11:22:45	Karime Tavares Lima da Silva	Aceito
Folha de Rosto	FolhaRostoAssinada.pdf	03/08/2018 11:22:15	Karime Tavares Lima da Silva	Aceito
Cronograma	CronogramaCEP.docx	03/08/2018 10:31:22	Karime Tavares Lima da Silva	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

SAO LUIS, 02 de Novembro de 2018

Assinado por:
RUDYS RODOLFO DE JESUS TAVAREZ
(Coordenador(a))

CENTRO UNIVERSITÁRIO DO MARANHÃO - UNICEUMA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITO DA APLICAÇÃO DE UM DERIVADO DE MATRIZ DE ESMALTE (EMDOGAIN) NA COMPOSIÇAO QUÍMICA E ESTRUTURA SUPERFICIAL DE DENTINA RADICULAR HUMANA - ESTUDO IN VITRO

Pesquisador: Karime Tavares Lima da Silva

Área Temática:

Versão: 2

CAAE: 15975419.1.0000.5084

Instituição Proponente: CEUMA-ASSOCIACAO DE ENSINO SUPERIOR

Patrocinador Principal: MINISTERIO DA CIENCIA, TECNOLOGIA E INOVACAO

DADOS DO PARECER

Número do Parecer: 3.540.098

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1295620.pdf	10/07/2019 10:50:23		Aceito
Declaração de Instituição e Infraestrutura	AnuenciaProjLabUFU.pdf	10/07/2019 10:49:54	Karime Tavares Lima da Silva	Aceito
Projeto Detalhado / Brochura Investigador	ProjetoDoutoradoLabCEP.pdf	10/07/2019 10:43:43	Karime Tavares Lima da Silva	Aceito
Cronograma	CronogramaProjLabCEP.pdf	10/07/2019 10:43:17	Karime Tavares Lima da Silva	Aceito
Declaração de Instituição e Infraestrutura	AnuenciaProjLaboratorial.pdf	07/06/2019 09:47:34	Karime Tavares Lima da Silva	Aceito
Folha de Rosto	FolhaRostoProjLabAssin.pdf	02/04/2019 12:53:58	Karime Tavares Lima da Silva	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLEdentesdoados.pdf	02/04/2019 12:53:24	Karime Tavares Lima da Silva	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

SAO LUIS, 29 de Agosto de 2019

Assinado por:
RUDYS RODOLFO DE JESUS TAVAREZ
(Coordenador(a))

ANEXO 2 – REBEC

ReBEC
Registro Brasileiro de Ensaios Clínicos

Languages karimelima

Approveds

Last update:	10/02/2019
Title:	Clinical and radiographic evaluation of the efficacy of pulpal revitalization treatment: randomized clinical trial

To view Renew

RBR-336mnh Clinical and radiographic evaluation of the efficacy of pulpal revitalization treatment: randomized clinical trial

Date of registration: 10/02/2019 (mm/dd/yyyy)
Last approval date : 10/02/2019 (mm/dd/yyyy)

Study type:

Interventional

Scientific title:

en
Clinical and radiographic evaluation of the efficacy of the application of an enamel matrix derivative (Emdogain) in regenerative endodontics: a randomized clinical study

pt-br
Avaliação clínica e radiográfica da eficácia da aplicação de um derivado de matriz de esmalte (Emdogain) na endodontia regenerativa: estudo clínico randomizado