FACULDADE DE ODONTOLOGIA

EFEITO DO AGREGADO TRIÓXIDO MINERAL INTRACANAL EM UM MODELO DE REIMPLANTE TARDIO EM DENTES DE RATOS - ANÁLISE HISTOLÓGICA E IMUNOISTOQUÍMICA

BEATRIZ FARIAS VOGT

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PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL

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Tese apresentada como parte dos requisitos para a obtenção do título de Doutor em Odontologia, Área de Concentração em Cirurgia e Traumatologia Bucomaxilofacial, pelo Programa de Pós-Graduação da Faculdade de Odontologia da Pontifícia Universidade Católica do Rio Grande do Sul.

Profª. Drª. Maria Martha Campos
– Orientadora –

Profª. Drª. Adriana Etges
– Co-orientadora –

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Dedicatória

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Resumo

RESUMO

O reimplante é o tratamento de escolha da avulsão dentária. Deve ser realizado imediatamente, a fim de evitar que ocorra necrose pulpar e do ligamento periodontal e, consequentemente, o desenvolvimento de reabsorção radicular. Porém, quando a reabsorção estiver instalada, a terapia com hidróxido de cálcio deve ser iniciada tão logo quanto possível. Pelo fato deste material ainda apresentar alguns inconvenientes, como a necessidade de trocas frequentes e o aumento da fragilidade da estrutura dentária quando utilizado por longos períodos, outros materiais estão sendo utilizados como substitutos do hidróxido de cálcio. Sendo assim, o objetivo deste trabalho foi de, através de uma revisão da literatura, conhecer a aplicabilidade do agregado trióxido mineral no tratamento de dentes traumatizados. Além disso, também foi realizado um estudo experimental para avaliar o efeito do MTA Branco (WMTA) (Angelus, Londrina, Brasil) e do MTA Fillapex (Angelus, Londrina Brasil) como medicação intracanal na prevenção da reabsorção radicular, em dentes de ratos, reimplantados tardiamente. Neste estudo foram utilizados 48 ratos machos (Wistar) que tiveram o incisivo central superior direito extraído e mantido em meio seco por 30 minutos. Os animais foram divididos em quatro grupos: 1 – WMTA; 2 – MTA Fillapex; 3 – Hidróxido de cálcio; 4 – Controle negativo. Após a remoção química do ligamento periodontal, os canais radiculares foram secos, preenchidos com o material correspondente a cada grupo e reimplantados. Após 10 e 60 dias, as peças operatórias foram submetidas ao processamento para realização de cortes histológicos no sentido longitudinal. As lâminas foram coradas com HE para análise histológica qualitativa e quantitativa. Os resultados foram analisados estatisticamente por meio de análise de variância (ANOVA), seguida pelo Teste de Tukey, com nível de significância de 5%. A avaliação qualitativa demonstrou que os grupos do WMTA e MTA Fillapex apresentaram áreas de reabsorção inflamatória, reabsorção por substituição e anquilose alvéolo-dentária, similares às observadas no grupo do hidróxido de cálcio, nos dois períodos avaliados. Um processo inflamatório maior foi observado nos grupos WMTA e MTA Fillapex. Na análise quantitativa, a avaliação do processo inflamatório mostrou diferença significativa entre o grupo do hidróxido de cálcio e o grupo controle, aos 10 dias ($p<0.05$). Aos 60 dias, áreas de anquilose alvéolo-dentária foram significativamente maiores no grupo MTA Fillapex quando comparado ao grupo controle ($p<0.05$). Os resultados mostraram que nenhum dos materiais avaliados foi capaz de prevenir completamente o desenvolvimento da reabsorção radicular, apesar de ambos os materiais à base de MTA terem apresentado resultados comparáveis aos do hidróxido de cálcio. Sendo assim, os cimentos de MTA podem apresentar algumas vantagens; porém, as consequências dos seus efeitos pro-inflamatórios devem ser mais bem investigadas.

Palavras-chave: reimplante dentário, avulsão dentária, agregado trióxido mineral, hidróxido de cálcio[1].

[1] DeCS – Descritores em Ciências da Saúde, disponível em www.decs.bvs.br , acessado em 07 de junho de 2011.

Abstract

ABSTRACT

The replantation is the treatment of choice for tooth avulsion. It should be performed immediately, in order to avoid the occurrence of pulp and periodontal ligament necrosis, culminating in root resorption development. However, when resorption is present, calcium hydroxide therapy should be initiated as soon as possible. Other materials have been tested as substitutes for calcium hydroxide. Therefore, the aim of this study was to evaluate the effect of White MTA (WMTA) and MTA Fillapex as intracanal medications to prevent root resorption, in a rat model of delayed tooth raplantation. Maxillary right central incisors of 48 male rats were extracted, and left exposed to dry environment for 30 min. The animals were allocated into four groups: 1 – WMTA; 2 – MTA Fillapex; 3 – Calcium hydroxide; 4 - Negative control. Following periodontal ligament removal, root canals were dried and filled with the corresponding material for each group and replanted. After 10 and 60 days, all surgical specimens were subjected to histological processing. The slides were stained with HE for histological qualitative and quantitative analysis. The results were statistically analyzed by ANOVA followed by Tukey's post-hoc test, with a significance level of 5%. Qualitative evaluation revealed that both WMTA and MTA Fillapex groups showed inflammatory and replacement resorption and dento-alveolar ankylosis profiles, which were similar to that observed for calcium hydroxide, in both time-points of evaluation. A slight increase of the inflammatory process was observed in either MTA groups. Quantitatively, inflammation score analysis showed a significant difference between the calcium hydroxide and the control group at 10 days ($p<0.05$). On 60 days, dento-alveolar ankylosis was found significantly increased in the MTA Fillapex, in comparison to the control group ($p<0.05$). Our data showed that none of tested materials was able to fully prevent the root resorption, although either MTA-based intracanal medications presented an outcome comparable to that seen for calcium hydroxide. Therefore, MTA cements might present some advantages, but the consequences of the pro-inflammatory effects of MTA cements need to be further investigated.

Keywords: tooth replantation, tooth avulsion, mineral trioxide aggregate, calcium hydroxide [2].

[2] DeCS – Descritores em Ciências da Saúde, disponível em www.decs.bvs.br , acessado em 07 de junho de 2011.

Lista de Ilustrações

LISTA DE ILUSTRAÇÕES

Lista de Abreviaturas, Siglas e Símbolos

LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

OPG – osteoprotegerina

pH – potencial hidrogeniônico

RANK - ativador nuclear kappa-β

RANKL – ligante do ativador nuclear kappa-β

RS – Rio Grande do Sul

® - marca registrada

% - por cento

< - menor

> - maior

Sumário

SUMÁRIO

Introdução

1 INTRODUÇÃO

A avulsão dental representa o deslocamento total do dente para fora do seu alvéolo. O tratamento de primeira escolha é o reimplante dentário, que consiste no reposicionamento do dente no alvéolo e deve ser realizado para evitar problemas funcionais, estéticos e psicológicos aos pacientes. O armazenamento do dente avulsionado em meio adequado, até que o reimplante seja realizado, bem como, a conduta do cirurgião-dentista no primeiro atendimento, são fatores extremamente importantes para o sucesso do tratamento. Entretanto, na maioria dos casos, os dentes são reimplantados tardiamente ou em condições desfavoráveis. Isso compromete o prognóstico do tratamento, pois há necrose das células do ligamento periodontal devido ao tempo excessivo de permanência extra-alveolar em meio seco, o que favorece o desenvolvimento de reabsorções radiculares que, em pouco tempo, podem levar à perda do dente (ANDREASEN e ANDREASEN, 2007; FLORES et al., 2007).

O período extra-alveolar está diretamente relacionado ao prognóstico do tratamento, pois, quanto menor for o tempo de permanência do dente fora do alvéolo, melhores serão as condições para cicatrização. Estudos experimentais indicaram que o meio de armazenamento, mais do que a duração do período extra-alveolar, determina o prognóstico (ANDREASEN, 2000). Várias substâncias são estudadas e comparadas com bastante frequência em modelos experimentais, no intuito de investigar aquela que melhor contribui para a manutenção da vitalidade e viabilidade das células do ligamento periodontal (PILLEGGI et al., 2002; SCHWARTZ et al., 2002; SIGALAS et al., 2004; MARTIN e PILLEGI, 2004). Outros fatores como o grau de rizogênese, as condições dos tecidos periodontais adjacentes e da polpa, a manipulação do elemento dentário avulsionado, a contaminação local e os métodos de contenção, estão associados de forma a interferir no prognóstico do reimplante (ANDREASEN, 1981a; ANDREASEN, 1981b; OKAMOTO e OKAMOTO, 1995; OKAMOTO et al., 1998).

A reabsorção radicular é considerada uma das mais importantes sequelas decorrentes do reimplante dental. Na tentativa de conhecer e controlar, ou mesmo, de inibir estes efeitos, muitos estudos avaliaram a resposta do ligamento periodontal frente a diversas substâncias utilizadas, não só como meio de armazenamento, mas

também no tratamento tópico da superfície radicular, como, por exemplo, fluoreto de sódio, hidróxido de cálcio, antibióticos, formulações de própolis, dentre outras (MARTIN e PILLEGI, 2004; Al-SHAHER et al., 2004; ÖZAN et al., 2007; FLORES et al., 2007; VOGT, 2008; SILVA et al., 2009). Além disso, em casos de presença de reabsorção inflamatória, a utilização da pasta de hidróxido de cálcio é indicada como medicação intracanal (FELLIPPE et al., 2005; TROPE 2011). Independente do veículo, o uso do hidróxido de cálcio, tem por finalidade principal o retardo do processo de reabsorção ou, quando muito, a sua paralisação. Apresenta ação alcalinizante e antibacteriana, que parecem ser os principais atributos responsáveis pela sua ação benéfica, mesmo nos casos de reabsorção dentária por substituição ou anquilose alveolodental (CONSOLARO, 2005). A condição ideal para a ocorrência da reabsorção nos tecidos duros é a presença de um meio ácido. Sendo assim, as hidrolases ácidas envolvidas estão ativas, provocando a desmineralização dos tecidos. A razão para o tratamento da reabsorção inflamatória, é que o ácido lático, produto dos osteoclastos, pode ser neutralizado pela ação do hidróxido de cálcio, prevenindo a dissolução dos minerais do dente (TRONSTAD et al., 1981). A utilização da pasta de hidróxido de cálcio no preenchimento do canal radicular, previamente ao reimplante, objetiva aumentar o pH, através da difusão dos íons hidroxila pelos canalículos dentinários, o que determina a diminuição da atividade osteoclástica e o aumento da atividade da fosfatase alcalina, presentes nas áreas de reabsorção (TRONSTAD et al. 1981; ESBERARD et al., 1996; FELLIPPE et al., 2006). Na presença de pH alcalino, a atividade da fosfatase alcalina é estimulada, o que parece exercer um papel crucial na formação dos tecidos duros. Por esse motivo, a continuidade do processo de reabsorção torna-se improvável com o uso do hidróxido de cálcio, e o reparo pode acontecer (TRONSTAD et al.1981).

Negri et al. (2008) avaliaram a resposta tecidual ao reimplante tardio, em dentes de ratos que foram tratados endodonticamente e tiveram os canais radiculares obturados com pasta de hidróxido de cálcio, Sealapex® e Endofill®, sem a utilização de cones de guta-percha. Seus resultados demonstraram que o grupo tratado com pasta de hidróxido de cálcio e propilenoglicol apresentou áreas de reabsorção inflamatória em menor frequência. Os autores concluíram que a utilização desses materiais, quando comparados ao hidróxido de cálcio, sem a utilização de cones, não apresentou resultados superiores.

Um dos principais problemas enfrentados por pacientes com reabsorção radicular inflamatória está na necessidade frequente de trocas do hidróxido de cálcio, o que leva a um maior risco de re-contaminação e enfraquecimento da estrutura dentária (TUNA et al., 2011). Em razão disso, devem ser investigados outros materiais capazes de induzir o reparo dos tecidos periapicais, até mesmo com deposição de tecido mineralizado, como o cimento Agregado Trióxido Mineral (*Mineral Trioxide Aggregate* - MTA), possibilitando ainda, o tratamento em um número reduzido de sessões (JACOBOVITZ e PONTES LIMA, 2008).

O MTA foi lançado no mercado apresentando na sua composição silicato tricálcico, aluminato tricálcico, óxido tricálcico e óxido de silicato. Apresenta duas fases específicas constituídas por óxido de cálcio, na forma de pequenos cristais, o que explica, teoricamente, um mecanismo de ação similar ao do hidróxido de cálcio e, por fosfato de cálcio, como estrutura amorfa (LEE et al., 1993; TORABINEJAD et al., 1995).

O MTA tem sido empregado não apenas em cirurgias parendodônticas (LEE et al., 1993), mas também nos casos de pulpotomia, perfuração radicular (BARBOSA, 1999), apecificação, como tampão cervical em clareamentos dentários internos, no reparo de fraturas radiculares verticais e, ainda, como material obturador (TORABINEJAD e CHIVIAN, 1999; JACOBOVITZ et al., 2009).

Holland et al. (2002) ao compararem a resposta tecidual ao MTA com a que se obtém quando do emprego do hidróxido de cálcio, observaram similaridade entre os dois materiais. Ambos pareceram estimular a neoformação de tecido duro (cemento e dentina). O óxido de cálcio, componente do pó, ao ser misturado com a água é convertido em hidróxido de cálcio, que, em contato com os fluidos tissulares, se dissocia em íons cálcio e hidroxila. Ao reagir com o gás carbônico, os íons cálcio dão origem às granulações de calcita. A fibronectina se acumula junto a essas granulações permitindo a adesão e diferenciação celular, formando uma ponte de tecido duro (HOLLAND et al., 2002).

O MTA é apresentado na forma de um frasco contendo pó, um frasco contendo líquido (água destilada) e uma colher dosadora. Segundo o fabricante, a composição do MTA é: SiO_2 ,K_2O, Al_2O_3, Na_2O, Fe_2O_3, SO_3, CaO, Bi_2O_3 MgO e resíduos insolúveis (sílica cristalina, óxido de cálcio e sulfato de potássio e sódio). O cimento possui um pH alcalino (pH 12). Não é necessário aguardar a presa do

material, uma vez que este tem as propriedades melhoradas, quando em contato com meio úmido.

O MTA apresenta maior radiopacidade que outros materiais como guta-percha, IRM, sendo melhor visualizado radiograficamente. Além disso, sua ação antimicrobiana é atribuída ao fato de promover a elevação do pH, na presença de umidade, alcalinizando o meio onde está sendo empregado (TORABINEJAD et al., 1995).

Özdemir et al. (2008) sugerem que o MTA tem grande potencial para aplicação em casos de reabsorção radicular inflamatória, já que, em seu estudo, encontraram a ocorrência de liberação de íons Ca^{++}, através dos túbulos dentinários, após a aplicação de material no interior do canal radicular. Apesar do MTA não possuir propriedades físicas adequadas para ser empregado como material obturador do canal radicular, Holland et al. (2002) mencionaram a necessidade de correção oportuna dessa condição, pois sua boa capacidade seladora e de biocompatibilidade, estimulando a neoformação dos tecidos duros, poderiam fazer do mesmo um bom material obturador.

Considerando que o mecanismo de ação do MTA é muito semelhante ao do hidróxido de cálcio, ele vem sendo utilizado em casos descritos na literatura, reconhecendo seus efeitos bastante promissores. Apesar de serem poucos os relatos da utilização na clínica do MTA para o tratamento das reabsorções radiculares e os períodos de acompanhamento ainda não serem muito longos, os resultados obtidos têm sido bastante satisfatórios (JACOBOVITZ e PONTES LIMA, 2009; JACOBOVITZ e PONTES LIMA, 2008; PACE et al., 2008; JACOBOVITZ et al., 2009).

Com o intuito de conhecer melhor os mecanismos de reabsorção dos tecidos dentários, por meio de análise imunoistoquímica, outras pesquisas estão sendo realizadas, com base nas vias moleculares de diferenciação dos osteoclastos, através do ligante do ativador nuclear kappa-β (RANKL), do ativador nuclear kappa-β (RANK) e, da osteoprotegerina (OPG), a qual bloqueia o RANKL. Essas proteínas pertencem à família do fator de necrose tumoral e apresentam algumas características importantes (KHOSLA, 2001, LÖSSDERFER et al., 2002):

a) Osteoprotegerina (OPG): é uma proteína solúvel, sintetizada pelos osteoblastos, com ação de inibir a diferenciação dos osteoclastos a partir da ligação com RANKL

b) Ativador nuclear kappa-β (RANK): receptor presente na superfície das células progenitoras dos osteoclastos.
c) Ligante do ativador nuclear kappa-β (RANKL): sintetizado pelos osteoblastos; estimula a osteoclasia através do contato célula-célula. A reabsorção se dá através da ligação com RANK.

Basicamente, o mecanismo de ação do sistema OPG-RANK-RANKL se dá através da indução da diferenciação das células progenitoras dos osteoclastos pelo RANKL. A OPG inibe essa diferenciação pela sua ligação com RANKL, impedindo-o de se ligar ao RANK (LERNER, 2004).

Sendo assim, o presente estudo compreende dois trabalhos apresentados sob a forma de artigos científicos. Através de uma revisão da literatura, o primeiro artigo tem como objetivo fundamentar a utilização do MTA em casos relacionados ao trauma dentário. O segundo artigo, experimental, compara os efeitos do MTA e de um cimento endodôntico à base de MTA aos efeitos do hidróxido de cálcio, através de análise histomorfométrica. Finalmente, ainda são apresentadas algumas imagens preliminares de imunoistoquímica, com determinação da expressão das proteínas OPG, RANK e RANKL, relacionadas aos processos de reabsorção óssea, a fim de estender as evidências acerca dos possíveis mecanismos de ação dos cimentos MTA.

2 ARTIGO 1

O artigo a seguir intitula-se *“Mineral trioxide aggregate applications in management of dental trauma: a literature review”*, o mesmo foi formatado de acordo com as normas e submetido ao periódico *Dental Traumatology* (Anexo A).

Mineral trioxide aggregate applications in management of dental trauma: a literature review.

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Keywords: apexification, dental trauma, mineral trioxide aggregate, root resorption.

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Abstract: This article was aimed to review the potential of mineral trioxide aggregate (MTA) in dental trauma management. Pertinent dental traumatology and endodontic literature was revised, and either *in vivo* or *in vitro* studies testing MTA have been included. Case reports were added too, in order to show the clinical outcomes of this material. Apexification, prevention and/or treatment of root resorption and complete root canal filling were the most common treatments related to dental trauma. In the majority of cases, the MTA use presented good results, and this material has been indicated as a potential calcium hydroxide substitute. This can be supported by some MTA advantages, such as the biomineralization ability and the needless of repeated changes, reducing the total treatment period and the patient compliance.

2.1 GENERAL CONSIDERATIONS

Dental trauma might involve one or more teeth, without a predictable pattern of intensity and extent (1). The management of dental trauma requires an appropriated long-term follow-up chart (2,3). Teeth with partially resorbed roots can be preserved, considering both functional and aesthetic aspects. The long-term prognosis is questionable and shows great inter-individual variations (4). Correct post-traumatic management is the best way to avoid complications and to improve the long-lasting prognosis of avulsed teeth (4,5). Independent on the initial clinical situation, the occurrence of future problems, such as pulp necrosis, discoloration, root resorptions, or sinus tract pain is not necessarily immediate, but it can appear in several months (6-8).

Calcium hydroxide is the most used root filling material, and it is able to induce the repair of radicular and periapical tissues, even with the deposition of mineralized tissue. Other calcium-based materials, such as Mineral Trioxide Aggregate (MTA),

have also such properties, with the advantage of a fewer number of sessions to complete the treatment (9).

MTA was first described as a root-end filling material (10). Subsequently, various studies pointed out further attractive properties for this material. Nowadays, MTA is suggested to display many advantages over previously used materials, and some researchers consider it an uprising material in contemporary endodontics and dental traumatology (11). In fact, MTA has several clinical applications including the repair of root perforations, pulp capping, pulpotomy, apexogenesis, apical barrier formation in teeth with open apexes, and root canal filling (12).

MTA composition is based on Portland cement, which includes tricalcium silicate, tricalcium aluminate, tricalcium oxide and silicate oxide, and some other mineral oxides that are responsible for its chemical and physical properties. However, the biocompatibility of MTA is likely superior to that described for the Portland cement (13). Nowadays, there are two MTA forms: the gray (GMTA) and the white (WMTA). There are some differences between them - the GMTA has FeO, which could cause teeth discoloration, whereas WMTA was developed to solve this problem. Moreover, WMTA present thinner and homogeneous particles in comparison to GMTA, what makes easier the handling of the material (12).

MTA displays inductive and conductive effects on hard tissues for both tooth apical regions and areas of previous external resorption (2). Furthermore, some case reports using MTA for treatment of internal and external resorptions, as result of avulsed, luxated or intruded teeth or in horizontal root fractures, have demonstrated high successful rates (8,14-16). It has been suggested that this material is able to allow adhesion, to support cellular proliferation, and to induce migration of bone marrow-derived mesenchymal stem cells (17). When used in traumatized teeth, the treatment might be directed to induce apexification in cases of incomplete

apexogenesis, to stop or to prevent root resorption areas, and as a root filling material. Additionally, in cases of traumatic crown fractures with pulpal exposure, MTA might be used in place of calcium hydroxide for pulp capping. In this case, MTA does not appear to deteriorate and disintegrate with time, avoiding microleakage formation, as it is commonly described for calcium hydroxide (18). This conservative treatment results in pulp preservation, allowing the normal root development and preservation of bone (19-21). Despite these attractive results, more clinical data are needed to prove the long-term efficacy of MTA for the abovementioned purposes (14,22).

2.1.1 Apexification

Dental trauma in children is commonly associated to incomplete root development. In such cases, the canal remains large, with thin and fragile walls, and the apex architecture remains divergent. These features difficult the instrumentation of the canal and hinder the formation of an adequate apical stop. Then, to allow the condensation of the root filling material and to promote an apical seal, it is imperative to create an artificial apical barrier or induce the closure of the apical foramen with calcified tissue, which is known as apexification (23).

Once pulp necrosis, gangrene or infection have been established, the use of dressing materials is mandatory for neutralizing bacteria and their by-products. Endodontic dressings are also required in the cases of teeth with incomplete apex, to induce apexification (15,24). For many years, calcium hydroxide has been largely used to induce apexification when root development has been arrested, due to its ability of inducing hard tissue formation and anti-bacterial activity (25,26). However, induction of apexification with calcium hydroxide is associated with certain difficulties, such as the very long time of treatment required, the possibility of tooth fracture, the

incomplete calcification of the bridge, and the dependence on patient compliance (27-29). This last factor is very important to be considered, as apexification does not occur when calcium hydroxide is left in the canal for long periods, without renovation (24). Nevertheless, the frequent renewal of calcium hydroxide paste results in detrimental effects on the repair and apexification process, although the overall intensity of inflammatory process is found reduced. Then, the treatment of teeth with open apices depends on the formation of calcified tissue, suggesting that renewal of calcium hydroxide paste should not be performed for a period of 5 months (30). In traumatized teeth, this might represent a problem, as infectious and/or inflammatory process is present, and this long period could be less effective to control root resorption when it is present. Thus, a temporary calcium hydroxide dressing should precede the application of MTA mixture to limit bacterial infection, and this procedure has been adopted in most case reports in the literature (9,14).

Erdem et al. (6) presented a series of five cases of dental trauma involving immature teeth. The signs observed included radiolucent areas in the apical region and mobility fistulae. The treatment consisted of calcium hydroxide dressing between 1 and 6 weeks, followed by placement of an MTA apical plug. It is known that lipopolysaccharide (LPS) has direct stimulatory effects on the later stages of receptor activator of NF-κβ ligand (RANKL)-mediated osteoclast formation. Calcium hydroxide dressing appears to create a favorable environment, because it significantly inhibits osteoclast formation by the detoxification of LPS (31).

In a study conducted in monkeys, MTA and calcium hydroxide were tested in cases of immediate replantation, and the results showed that they were similar, revealing the biological sealing of some apical lateral canals, with newly formed cementum and absence of root resorption (32).

For immature tooth treatment, MTA can be considered a very effective material and a valid option for apexification in infected teeth, with the advantage of a shorter time for therapy completion, although a long-term follow-up is needed (6,23,33). The use of an apical plug of MTA for open apices has gained popularity in recent years, as a calcium hydroxide alternative, with optimal results (16,34-37).

In teeth with open apical foramen, the risk of material extrusion is high. However, the presence of MTA beyond the root end does not seem to affect the healing of periapical tissues, probably duo to its biologic properties (29,38). Even so, excessive pressure during root filling must be avoided, since this might dislodge the apical dome. In addition, the amount of heat used should be limited, as the root walls are thin and the heat may be transmitted to the periodontal ligament, with potential for adverse effects (24).

2.1.2 Root resorption

Root resorption is a very common and important complication of traumatic dental injuries, including intrusion, avulsion and luxation (39). Basically, there are two most common forms of root resorption: the inflammatory and the replacement resorption. In replacement resorption cases, there is a progressive replacement of dental tissues by bone tissues, when an injury is severe and causes necrosis of periodontal ligament cells (40). Inflammatory root resorption commonly occurs in cases of necrotic pulp with infection, and might involve the internal or external radicular surface (5,25,41). The presence of bacteria, either in infected pulp space or sulcus might lead to tooth loss, if no treatment is implemented (42,43). The extensive nature of the external root resorption compromises the structural integrity of the root (2). To prevent this, the treatment must aim the complete removal of the resorptive

tissue from the root canal system, in an attempt to prevent further defeats of hard tissue (44).

When pulp necrosis is present due to trauma, the healing process can be interrupted, and the integrity of periradicular tissues can be compromised (8). The alkaline environment provided by calcium hydroxide and MTA is important for preventing resorption in traumatized teeth (9). This occurs because MTA can also release calcium ions through dentinal tubules in resorption defect areas, favoring the repair in surrounding tissues (45). Calcium hydroxide is widely used as intracanal medication in inflammatory resorption treatment (30). It is employed in order to retard the resorption process, or at best, to promote its arrest. In fact, calcium hydroxide paste is used to fill the root canal prior to tooth replantation, to induce pH elevation, by diffusion of hydroxyl ions through dentinal tubules, which likely reduces the osteoclast action and increases alkaline phosphatase activity present in resorption area (27,46,47). It is believed that calcium hydroxide prevents the dissolution of tooth mineral components, by neutralizing osteoclast-derived lactic acid (46). In young patients, because of the wider dentinal tubules, the alkaline pH of MTA could block the inflammatory process and retard the odontoclastic activity (5). An *in vitro* study with simulated root resorption defects revealed that the pH was significantly higher in teeth filled with MTA, in comparison to calcium hydroxide, but the authors suggested the needing of additional studies to determine whether this difference is clinically significant (48). Furthermore, calcium hydroxide displays high antibacterial effects, which seem to be the main responsible feature for its beneficial actions, even in cases of replacement resorption or ankylosis (49).

The long-term exposure to calcium hydroxide appears to alter the physical properties of dentine (50). It is assumed that presence of calcium hydroxide for long periods weakens the root structure, favoring cervical fractures. The disruption could

take place due to neutralization, dissolution, or denaturing of the acid proteins and proteoglycans, which serve as bonding agents between the collagen network and the hydroxylapatite crystals in dentin (51).

A recent article showed that MTA root canal filling improved the healing outcomes in cases of severe inflammatory root resorption in young permanent teeth, as observed by clinical and radiographic findings (8). Concerning resorption involving only the tooth cervical region, Pace et al. (7) reported a case in which cervical invasive root resorption was detected two years after the occurrence of an uncomplicated coronal fracture. In this situation, MTA was effective in promoting the repair of resorption, without any clinical symptoms or periodontal pockets. Following trauma, it is controversial if the resorptive process is purely inflammatory and activated by sulcular microorganisms, or if it represents a type of benign proliferative fibrovascular disorder in which microorganisms have no pathogenic role, but may become secondary invaders. Then, different from inflammatory root resorption, in which pulp is necrotic and infected before its occurrence, in cervical invasive resorption, the pulp remains intact, due to the protecting layer of predentin and dentin (52).

2.1.3 Root canal obturation

MTA can be used as a root canal filling material, although clinicians should be aware of some of its limitations, such as the difficulty in controlling the length of the filling, the chance of producing void spaces and the absence of a known solvent for MTA removal (53). Bogen and Kuttler (54) have suggested that for obturation purposes, MTA displays a greater resistance to leakage, as an impediment against oral pathogens. This might overcome other supposed disadvantages of MTA, such as the complex manipulation, placement and removal. Moreover, it is important to

remember that when used in anterior teeth, white MTA should be employed, as gray MTA have greater FeO concentrations, and this could be primarily responsible for color variations and could provoke discoloration of teeth and gingival surfaces (55). These details cannot be disregarded, as most of the avulsed teeth involve aesthetic aspects and patient's life quality (15). However, this aesthetic problem is not completely solved with white MTA, because it still displays a slight gray discoloration at cervical region, when used as a root canal filling (44,56). Of note, coronal discoloration was observed in 22.7% of teeth following white MTA placement as apical plug (57).

Osseous reaction investigations have shown that the bone response to MTA is relatively mild and with minor inflammation (53). The study conducted by Bogen and Kuttler (54) suggested that teeth obtured with MTA not only presented increased resistance to fracture, but also displayed significant effects on severe infection. Extending this notion, another *in vitro* study showed that long-term use of calcium hydroxide dressing induced weakness of the root structure, which was superior to that observed for BioAggregate® or MTA (58). Nevertheless, an *in vitro* study showed that MTA-based material obturation presented a higher ratio of bacterial leakage, when compared to AH Plus and Sealapex (59).

Despite MTA formulations are not recommended to be used as sealers, many of their properties support the use alone as a root canal filling/sealing material, although the placement in canals may be challenging (60,61). Additionally, the use of MTA as an obturation material might provide long-term benefits that enhance the prognosis and retention of the natural dentition, not only in conventional, but also in complex therapies (54,62).

The present review article shows a series of advantages for MTA-based cements, as demonstrated by numerous literature case reports of dental trauma.

Moreover, this material seems to represent an excellent substitute to calcium hydroxide, even showing superior results. However, it is still necessary to evaluate the effectiveness of MTA cements, after several years of records, in order to confirm the success rates for the different clinical applications.

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3 ARTIGO 2

O artigo a seguir intitula-se *“Evaluation of two mineral trioxide aggregate formulations as intracanal medications in a rat model of delayed tooth replantation”*, o mesmo foi formatado de acordo com as normas e submetido ao periódico *International Endodontic Journal* (Anexo B).

Evaluation of two mineral trioxide aggregate formulations in a rat model of delayed tooth replantation

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Running Title: MTA use on tooth replantation

Keywords: ankylosis, calcium hydroxide, mineral trioxide aggregate (MTA), root canal therapy, root resorption, tooth avulsion.

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Abstract

Objective: This study aimed to evaluate the effects of White MTA (WMTA) and MTA Fillapex® to prevent root resorption, when used for root canal filling, in a rat model of delayed tooth raplantation.

Methodology: Maxillary right central incisors of 48 male rats were extracted, and left exposed to dry environment for 30 min. The animals were allocated into four groups: 1 – WMTA; 2 – MTA Fillapex; 3 – Calcium hydroxide; 4 - Negative control. Following periodontal ligament removal, root canals were filled with the corresponding material for each group and replanted. After 10 and 60 days, both qualitative and quantitative histological analyses were carried out. The results were statistically analyzed by ANOVA followed by Tukey's post-hoc test, with a significance level of 5%.

Results: Qualitative evaluation revealed that both WMTA and MTA Fillapex groups showed inflammatory and replacement resorption and dento-alveolar ankylosis profiles, which were similar to that observed for calcium hydroxide, in either 10 or 60 days. Notably, a slight increase of the inflammatory process was observed in both MTA groups. Quantitatively, inflammation score analysis showed a significant difference between the calcium hydroxide and the control group at 10 days ($p<0.05$). On 60 days, dento-alveolar ankylosis was found significantly increased in the MTA Fillapex, in comparison to the control group ($p<0.05$).

Significance: Our data showed that none of tested materials was able to fully prevent the root resorption, although both MTA-based cements presented an outcome comparable to that seen for calcium hydroxide. MTA cements might present some advantages when considering no need of frequent changes, but the consequences of the pro-inflammatory effects of MTA cements need to be further investigated.

Keywords: ankylosis, calcium hydroxide, mineral trioxide aggregate (MTA), root canal therapy, root resorption, tooth avulsion.

3.1 Introduction

Studies on delayed replantation of avulsed teeth have investigated several substances as storage media for preserving the viability of the periodontal ligament, or as root surface treatments, intended to prevent the occurrence of root resorption and dentoalveolar ankylosis [1,2,3]. Various studies have also evaluated a series of substances as intracanal medications, including calcium hydroxide, sodium alendronate [4], Endofill [5], and Sealapex [5], corticosteroids [6,7,8], bisphosphonates [9], and Ledermix paste® [10,11], although none of them was able to prevent root resorption.

Calcium hydroxide is the most widely used temporary intracanal medication in cases of delayed replantation, due to its ability to raise the pH and the pronounced antimicrobial action [12]. In spite of that, the needing for frequent changes of calcium hydroxide dressings is commonly associated to inflammatory root resorption, as well as to increased recontamination risk and tooth fragility [13,14].

During the last years, mineral trioxide aggregate (MTA) has emerged as a reliable bioactive material with extended applications in endodontics, which include the obturation of root canals. This material might have huge advantages because of its superior physiochemical and bioactive properties, such as the great sealing and elevated biocompatibility [15,16]. Furthermore, MTA is highly effective in inducing periapical tissue repair, allowing the treatment of root resorption in a reduced number of sections [14,17]. Of high interest, a study conducted by Holland *et al.* [18] (2002) demonstrated that MTA shares several beneficial properties with calcium hydroxide. MTA has also been successfully used as a permanent obturation material following tooth traumatic injuries, such as luxation, intrusion or avulsion [19]. Nevertheless, only few clinical studies have evaluated the long-term outcome of MTA-obtured

traumatized tooth [14,20,21]. Therefore, the present study evaluated the effects of two MTA formulations, WMTA and MTA Fillapex®, when employed for root canal filling, in a rat model of delayed tooth replantation.

3.2 Materials and Methods

This study is in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Animal Committee, at Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Brazil.

Forty-eight male Wistar rats (300 and 350 g) were used. The animals were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg), dosed by intraperitoneal route. Following intraoral and perioral antiseptic procedures, the maxillary right central incisor of each animal was extracted. The teeth were fixed by the crown to a sterile bone wax plate, and left exposed for 30 min. The animals were then distributed into four groups: 1 – WMTA (Angelus, Londrina, Brazil); 2 – MTA Fillapex® (Angelus, Londrina, Brazil); 3 – Calcium hydroxide paste (Calen®, S.S.White, Rio de Janeiro, Brazil); 4 - Negative control. Tooth replantation was done after the treatment proposed for each group.

Teeth of groups 1, 2 and 3 had the pulp removed apically with a #15 size endodontic file (Flex-R Roane Tip File, Moyco Union Broach, New York, USA). Canals were cleaned with 5 ml of 0.9 % NaCl solution (Texon®, Viamão, RS, Brazil).

Chemical ligament removal was performed by immersion of tooth in 1 % sodium hypochlorite solution (Cloro Rio, Rioquímica, São Caetano do Sul, SP, Brazil) for 30 min, and immediately after, teeth were immersed for 20 min in 5 ml of 2 % sodium fluoride solution (Vigodent, DFL, Rio de Janeiro, RJ, Brazil) at pH 5.5. Root external and internal surfaces were then irrigated with 20 ml of 0.9 % NaCl solution. Subsequently, the root canals were dried with absorbent sterile paper points and filled with the MTA formulations and calcium hydroxide paste, according to the experimental group. Sockets were carefully probed to check for unorganized

coagulation before replantation. Dental retention was not employed [22,23]. The negative control group received no treatment, and following 30 min of exposing, the teeth were replanted.

Animals received a single intramuscular administration of penicillin G benzathine (Benzetacil, Eurofarma Laboratórios Ltda., São Paulo, SP, Brazil) as systemic antibiotic therapy (20,000 units per kg). For the control of postoperative pain, the animals received paracetamol (50 mg/kg) (Merck, Rio de Janeiro, RJ, Brazil), by intraperitoneal route, until five days. For the first 30 days after replantation, the rats were fed with commercial powdered chow, returning to (Nuvital Nutrientes S/A, Colombo, PR, Brazil) pelleted chow after that period. Water was available *ad libitum* throughout all the experimental period.

After 10 and 60 days, 6 animals of each group were euthanized by isoflurane overdose. The hemi-maxillae containing the replanted teeth were removed and immersed in 10% formalin for 24 h. After, they were decalcified in 5% acid nitric solution during 3 weeks. For the histological analysis, slides were prepared from each tooth, with 5-μm-thick longitudinal sections, from the cervical to the apical region, and stained with hematoxylin and eosin (HE). A qualitative and quantitative analysis of the palatal surface of the middle third of the root was carried out, using a light microscope (Olympus BX50, São Paulo, Brazil). The sections were analyzed with respect to alveolar bone, PDL space, cementum and dentin characteristics, inflammatory resorption, replacement resorption, ankylosis and inflammatory process.

The middle third of each specimen was visualized at a microscope (Olympus BX51, São Paulo, Brazil) with image capture system. Histomorphometric analysis was carried out with *Image Tool 3.0* (Texas, USA), and the measures were calculated in percentage, on the basis of the of total root surface area. The

inflammatory process was analyzed at 100x magnification, according to the following scores: 0, absence of inflammatory cells; 1, until 10 % of inflammatory cells; 2, 11 to 50 % of inflammatory cells; 3, more than 51 % of inflammatory cells. The results were statistically analyzed by ANOVA followed by Tukey's post-hoc test, with a significance level of 5%.

3.3 Results

The analysis and description of the slides was focused on the following aspects: inflammatory process, PDL space, presence of replacement resorption, inflammatory resorption or ankylosis, and bone tissue characteristics. Descriptive and quantitative analysis are provided for both 10 and 60 days of evaluation.

Ten days

For the MTA Fillapex group, the bone tissue presented irregular surface in remodeling process, with clastic and blastic cells in five specimens. In most slides, PDL space was filled by an organized and dense connective tissue, with chronic inflammatory process, showing intense hyperemic areas (Fig. 1A). In some specimens, it was possible to observe material in adjacent tissues, with multinucleated giant cells surrounding the material. Two specimens presented small and localized areas of inflammatory resorption in dentine (Fig. 1a).

In this time period, alveolar bone tissue presented regular surface containing osteocytes in the WMTA group. In the majority of specimens, the PDL space was filled by a dense fibrous connective tissue, with fibers disposed organized and parallel to the root surface, with an intense acute inflammatory process and extensive hemorrhagic areas. There were few areas with newly formed bone tissue interwoven to connective tissue, and in contact with tooth surface featuring ankylosis. Two specimens presented few and small localized areas of inflammatory resorption (Fig. 1B).

In the calcium hydroxide group, the bone tissue presented a quite regular surface in resorption process, with clastic cells. Three specimens had localized areas of newly formed bone tissue filling partially the PDL space, interwoven by a dense

connective tissue. This fibers were disposed parallel to the root surface, with few lymphocytes and some neutrophils surrounding. Two specimens presented small areas of initial inflammatory root resorption, filled by clastic cells and a slight layer of fibrous tissue (Fig. 1C).

In the negative control group, which did not receive any treatment, bone tissue presented irregular surface in resorption process. Osteoclasts were observed periferically, and large spaces were occupied by osteocytes. The PDL space was filled partially by a layer of dense connective tissue with a lot of fibroblasts, intense vascularization, and acute inflammatory cells, predominantly neutrophils. In all specimens, hemorrhagic areas were observed. In dentin, two specimens showed localized lacunae of inflammatory resorption with clastic cells filled by fibrous connective tissue, surrounded by few lymphocytes (Fig. 1D).

The quantitative analysis revealed that there was no significant difference among the experimental groups regarding the inflammatory process scoring (Fig. 2A) and the inflammatory resorption (Fig. 2C), at 10 days following dental avulsion. Interestingly, the inflammation score analysis showed statistically significant difference between calcium hydroxide and control group ($p<0.05$).

Sixty days

In MTA Fillapex group, the bone tissue presented irregular surface, populated by osteocytes. Loose connective tissue areas were interlinked in bone tissue, with intense chronic inflammatory process. The PDL space was filled by newly formed bone areas. In all specimens, bone tissue was localized in direct contact with cementum, all along the root surface area (Fig. 1E). Some areas of replacement resorption were observed. In most slides, the apical third portion showed an intense acute inflammatory process, beyond multinucleated giant cells and clasts, causing

dentine and bone resorption (Fig. 1b). Some specimens showed the material in adjacent tissues surrounding by multinucleated giant cells, with an aggressive inflammatory reaction. Regarding the WMTA group, the bone tissue was found organized in most specimens. Few small and localized areas of loose connective tissue were interwoven in bone tissue, containing chronic inflammatory process. The PDL space was filled by dense connective tissue with fibers disposed parallel to the root surface in just one specimen. In the other five specimens, the PDL space was filled by newly formed and organized bone tissue. Most specimens showed large replacement resorption areas that were regular and quite invasive along the root middle third. Some areas of ankylosis were also observed. Newly formed bone trabeculae were observed at the bottom of the socket in most specimens (Fig. 1F).

The analysis of calcium hydroxide group revealed bone tissue with regular surface containing osteocytes, and a great layer of loose connective tissue with chronic inflammatory cells. In all specimens, ankylosed areas were observed, with bone tissue filling the PDL space, in direct contact with cementum. Moderately invasive, but extended replacement resorption in dentine was observed throughout the root surface. Three specimens also presented slight localized areas of inflammatory resorption (Fig. 1G).

In the negative control group, the bone tissue was found irregular, compact and highly cellularized, containing loose connective tissue areas with some neutrophils. PDL space was filled by a layer of connective tissue, with chronic inflammatory process in most cases. Dentine presented extended inflammatory resorption areas along the root surface, filled by connective tissue and clastic cells. It was also possible to observe some few areas of replacement resorption and ankylosis Fig. (1H).

In this time period, only ankylosis extension showed statistically significant difference between the MTA Fillapex and the control group ($p<0.05$) (Fig. 2E). The other evaluated parameters were not significantly different at 60 days of evaluation ($p>0.05$) (Fig.2 B,D,F).

3.4 Discussion

Several studies use the rat as an experimental model because of the ease of manipulation, as well as the existence of previous publications related to dento-alveolar trauma in this animals species [4,24-28].

MTA materials have been shown to have a biocompatible nature and have promising potential for endodontic use [29]. MTA was first described in 1993; subsequently, several formulations have been introduced in the dentistry market [30]. MTA composition is based on Portland cement, which includes tricalcium silicate, tricalcium aluminate, tricalcium oxide and silicate oxide, and some other mineral oxides that are responsible for its chemical and physical properties. Of note, new root canal sealer MTA-based cement denoted MTA Fillapex, has been recently developed in Brazil. It is composed by salicylate resin, diluting resin, natural resin, bismuth trioxide, nanoparticulated silica, MTA, and pigments. Among its properties, it is worth to remark the excellent viscosity for insertion, as well as the high radiopacity. MTA has two specific phases: calcium oxide which reacts with tissue fluids to form calcium hydroxide; and calcium phosphate as an amorphous structure [31]. It is described that calcium release from MTA yields a desirable healing effect [32], probably by stimulating hard tissue deposition at the apical level [18].

It was previously demonstrated that MTA is able to induce the over-expression of the anti-inflammatory cytokine IL-10, in dentin tubes implanted in the mouse dorsum [33]. The same authors have also found that MTA induced a pro-inflammatory and pro-resolution environment in their experimental paradigm, by modulating the expression of several inflammatory components. In our study, at the 10-days period of evaluation, MTA groups presented minor inflammatory alterations, especially when considering the qualitative analysis, in comparison to the negative

control group, what might be likely related to the anti-inflammatory effects of MTA-based cements. However, in comparison to the calcium hydroxide group, the inflammatory process was greater. Interestingly, in the MTA Fillapex group, at both 10- and 60-days period, the histological analysis revealed an inflammatory process with some neutrophils, macrophages and multinucleated giant cells, and with the presence of connective tissue surrounding the cement. Moreover, this could be associated with the presence of resins on their formulation, which could contribute to the aggressive inflammatory process. In addition, this fact suggests some degree of toxicity for this material, or even that biomineralization is necessarily preceded by an acute inflammatory reaction.

MTA Fillapex and WMTA groups showed fewer extended areas of inflammatory resorption along the root surface, but they were more invasive in dentin when compared to the other groups. An *in vitro* study performed by George *et al*. [34] (2009) demonstrated that Ca^{2+} is continuously released from MTA, at constant levels, pointing out this material as a potential alternative for inflammatory root resorption cases. Likewise, the diffusion of calcium ions through exposed dentinal tubules following MTA application, for root canal filling, constitutes a time-related process [32]. Furthermore, the antimicrobial actions of MTA are likely attributed to humidity presence that potentiates the alcalinization of surrounding tissues [31].

It is well known that osteoclasts and odontoclasts induce the resorption of mineralized tissues by lowering the pH in the resorption lacuna, followed by tissue degradation through the secretion of matrix metalloproteinases [35]. One might infer that MTA cements are able to modulate these events, although additional studies are required to prove this.

At the 60-days time-point, we observed that WMTA, MTA Fillapex and calcium hydroxide groups showed replacement resorption areas. In addition, some areas of

newly formed bone tissue occupying PDL space were observed at the 10-days period. This could be associated to the release of calcium, and its effects on the early expression of BMP-2 in human PDL cells [36]. The authors suggested that production of BMP-2 likely stimulates the osteoblastic/cementoblastic differentiation of PDL cells via BMP-2 receptors.

Several biological mechanisms in combination with the bioactivity of MTA may explain its ability to induce mineralized tissue deposition [37]. Hakki *et al*. [38] (2009) demonstrated that MTA induces mineralization by modulating the expression of bone sialoprotein and collagen type I, which plays a critical role in cemental repair and regeneration, in the process known as cementogenesis. This could explain data obtained in our study, showing that MTA Fillapex and WMTA groups displayed more ankylosis areas than other groups.

3.5 Conclusion

Our study shows that none of tested materials for root canal filling was able to fully prevent the root resorption, in the rat model of delayed tooth replantation presented in our study. Of note, WMTA presented a tissue response similar to that observed for calcium hydroxide. Nevertheless, MTA Fillapex displayed visible inflammatory effects, suggesting some degree of tissue toxicity. Although MTA fillapex showed promising results as a root filling material, additional investigations about its mechanisms of action and biologic responses are needed, especially concerning its application in cases of delayed tooth replantation.

Acknowledgments

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3.7 Figures

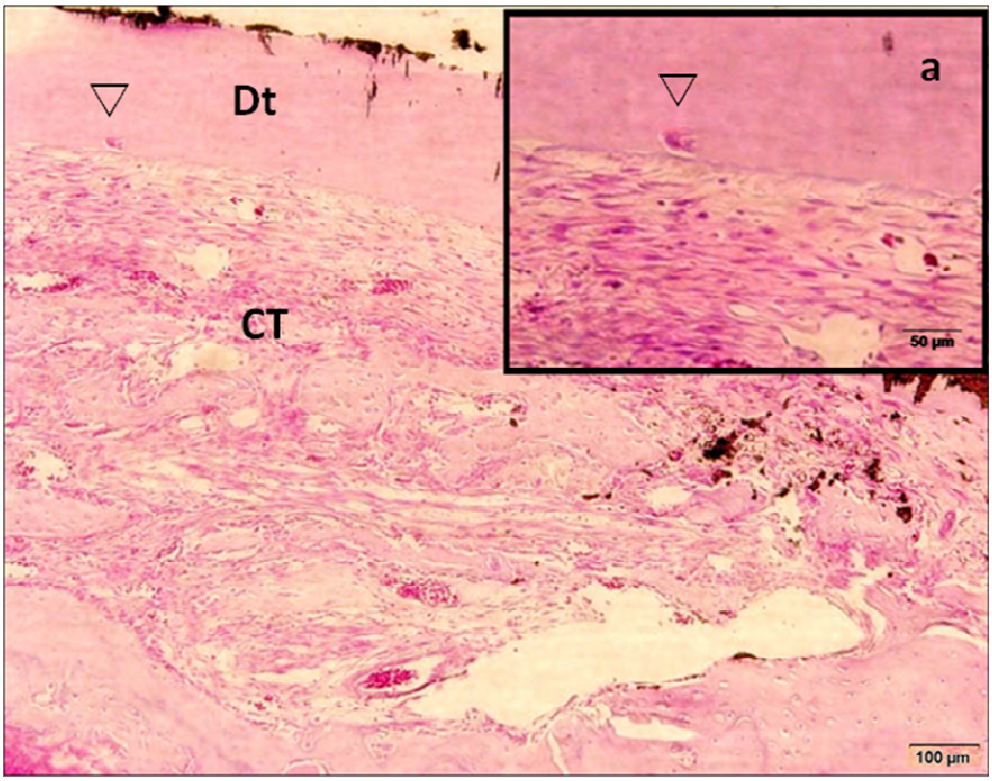

Figure 1 A - Photomicrograph of MTA Fillapex group showing inflammatory resorption area (▽) at 10-days period, dentine (Dt).

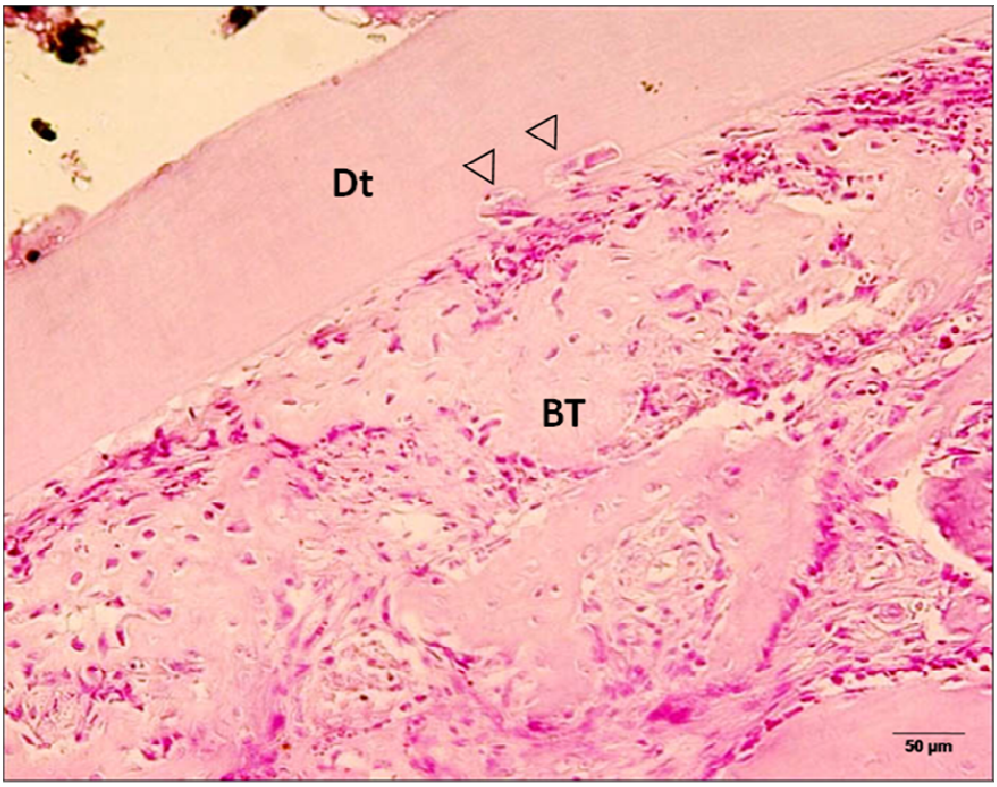

Figure 1 B - WMTA group showing areas with newly formed bone tissue interwoven to connective tissue (BT), and inflammatory resorption (▽) at 10 days, dentine (Dt).

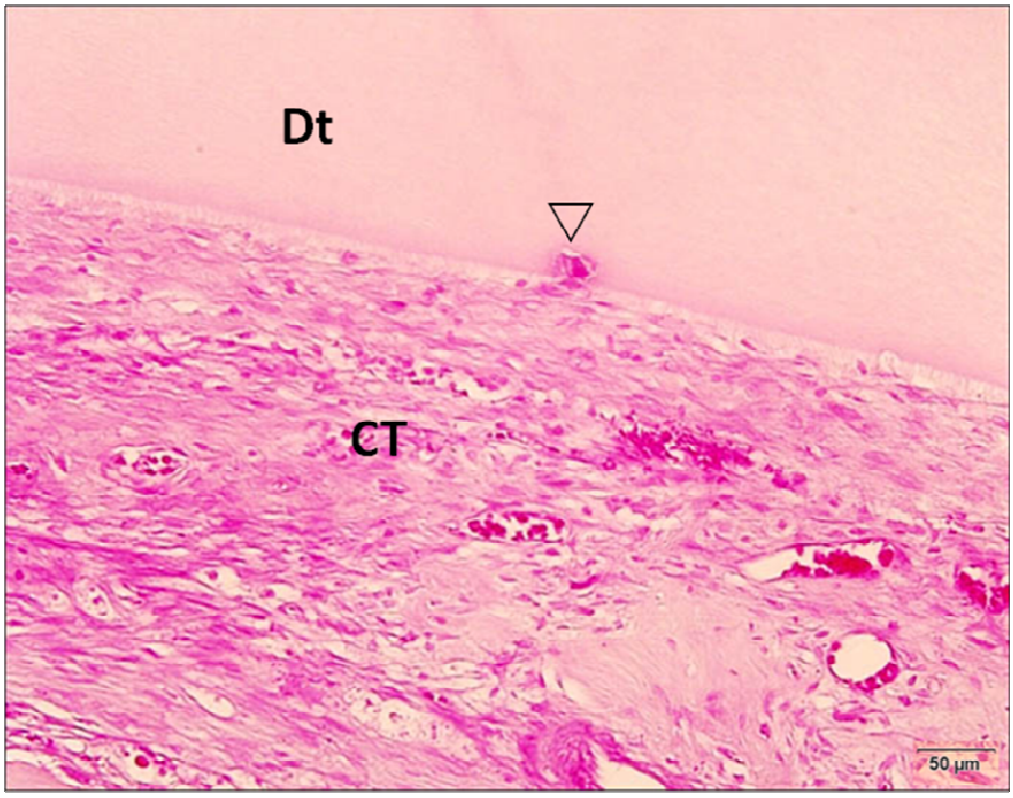

Figure 1 C - Calcium hydroxide group showing small inflammatory resorption area filled by clastic cells (▽), connective tissue (CT) at 10 days.

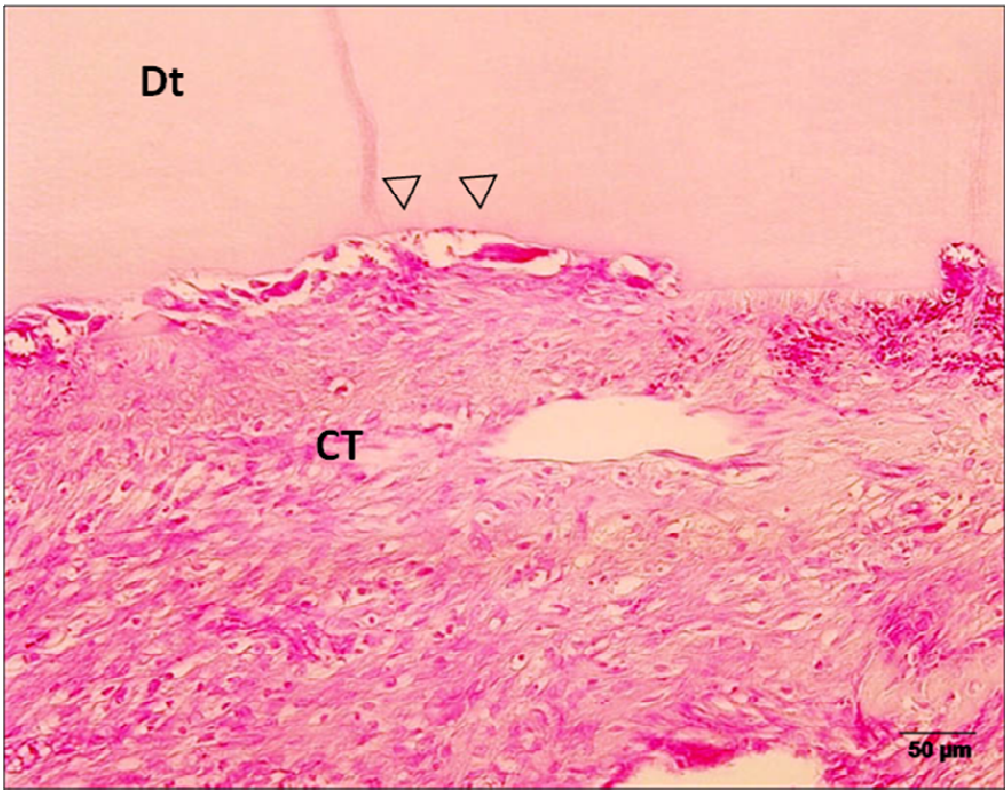

Figure 1 D - Negative control showing more extensive inflammatory resorption areas (▽) at 10 days.

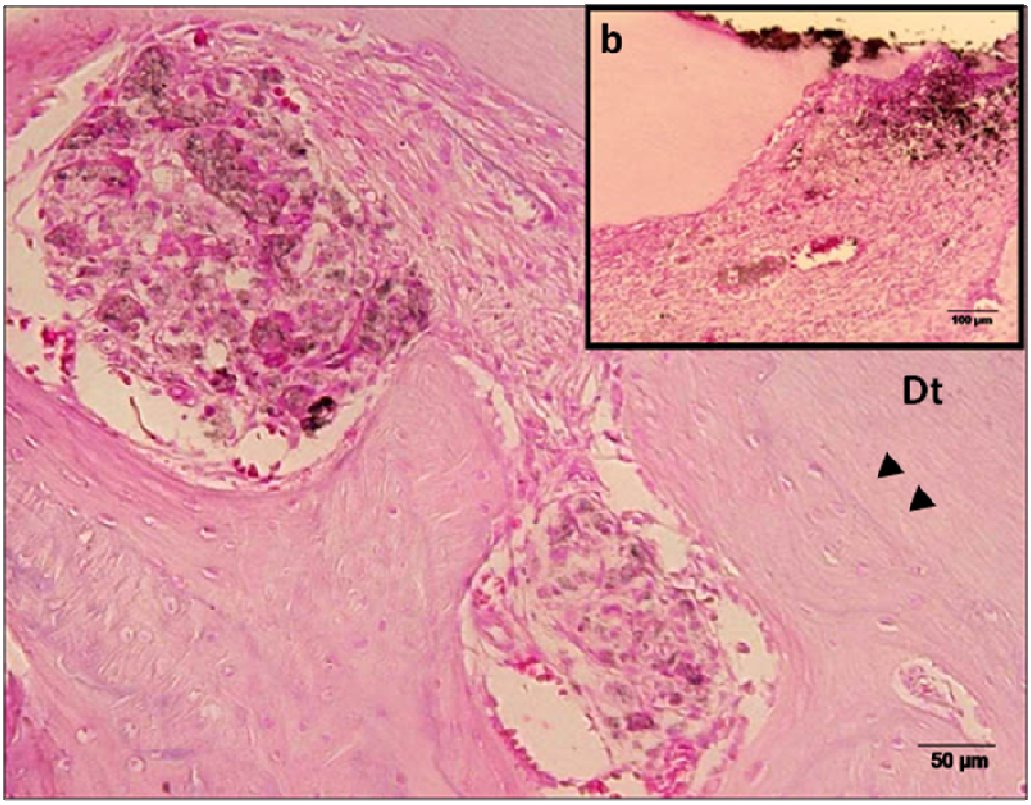

Figure 1 E - MTA Fillapex group with dento-alveolar ankylosis (▼) and severe invasive inflammatory resorption area (1b).

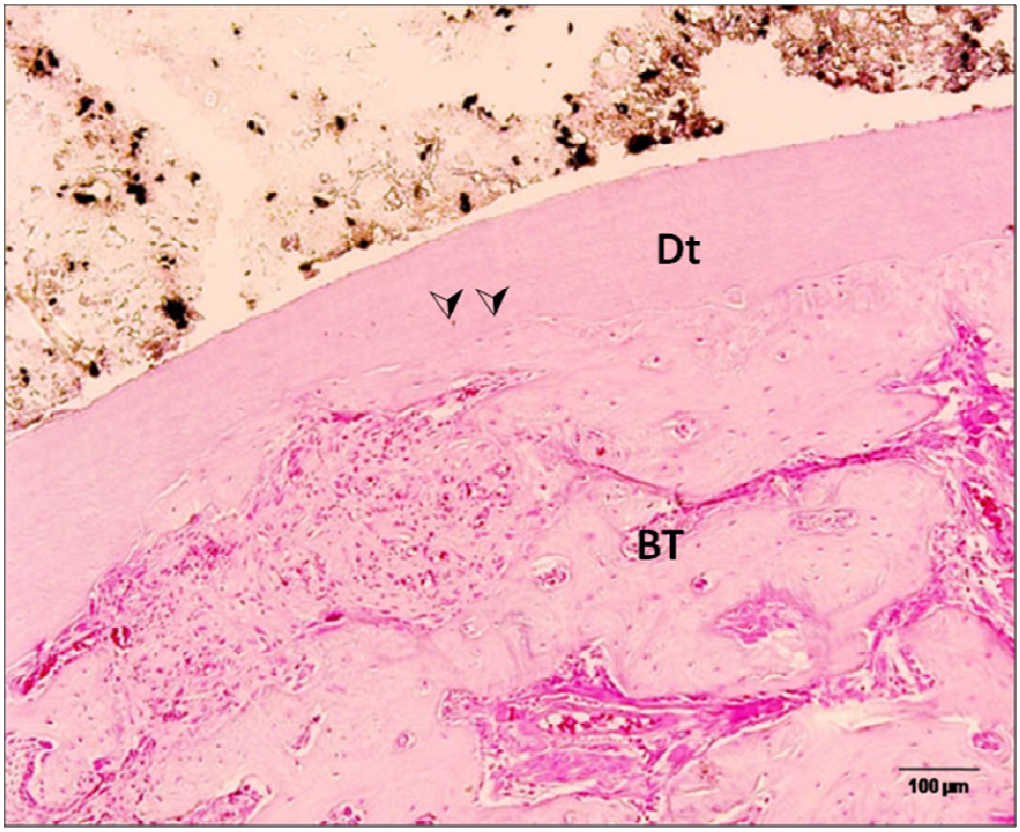

Figure 1 F - WMTA group with replacement resorption areas (⮟) at 60-days period, bone tissue (BT).

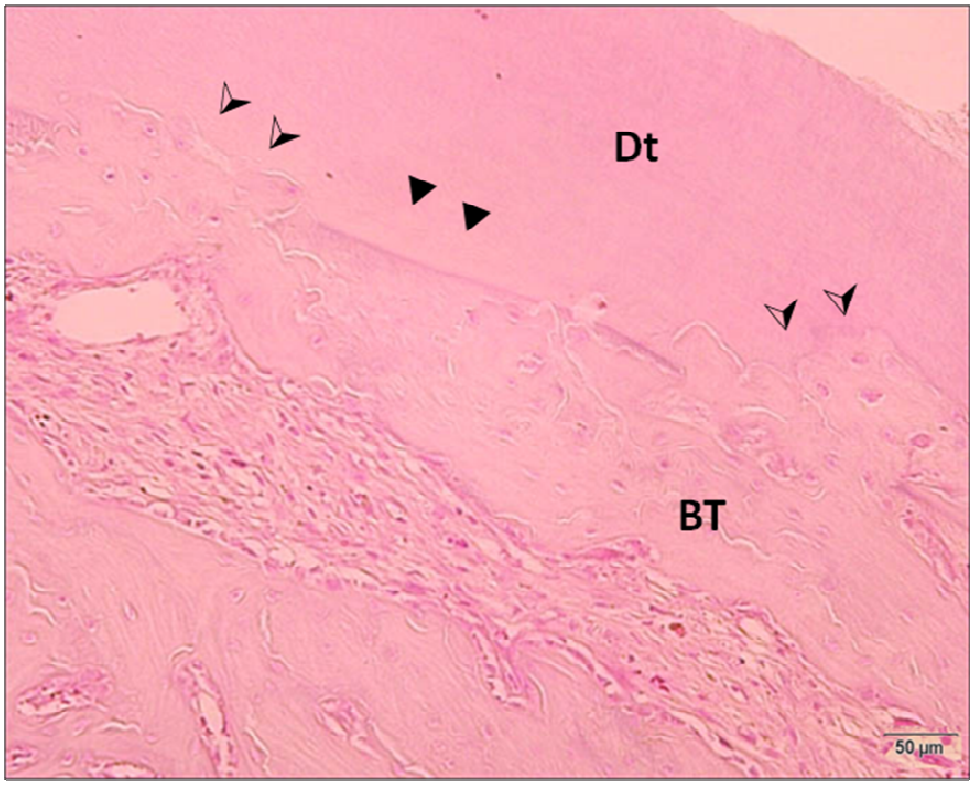

Figure 1 G - Calcium hydroxide group showing dento-alveolar ankylosis (▼) and replacement resorption (▽) with few inflammatory process at 60-days period, bone tissue (BT).

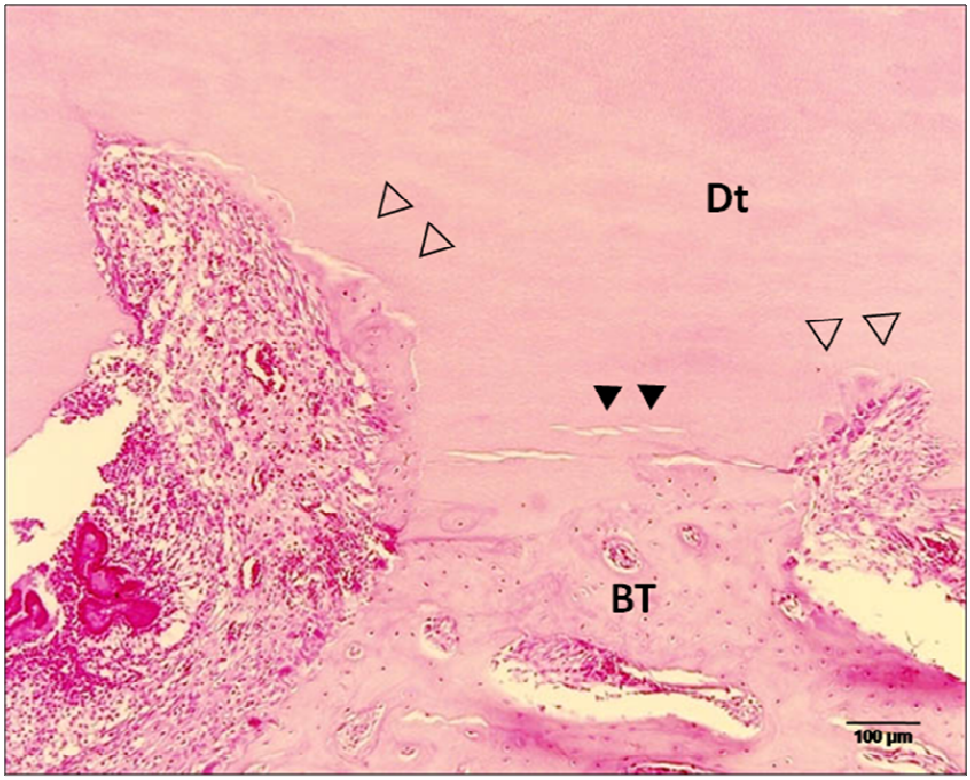

Figure 1 H - Negative control group showing more invasive inflammatory resorption areas (▽) and dento-alveolar ankylosis (▼) at 60 days, bone tissue (BT).

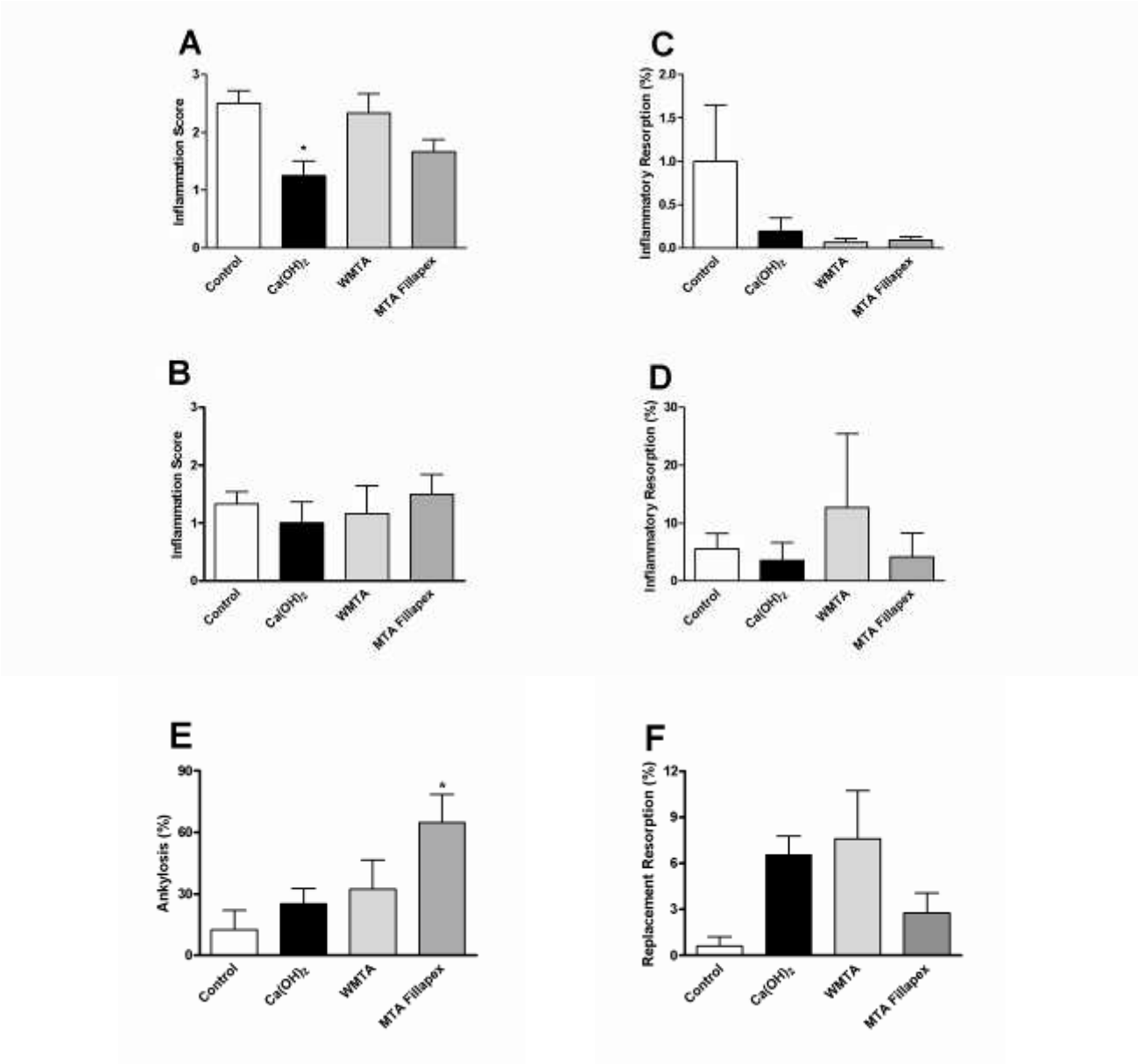

Figure 2. (A) Inflammation score showing a statistically significant difference between calcium hydroxide and control group (*p<0.05) at 10 days; (B) Inflammation score at 60-days period. Inflammatory resorption at (C) 10 and (D) 60 days. (E) Dentoalveolar ankylosis at 60-days period, showing a statistically significant difference between the MTA Fillapex and the control group (*p<0.05). (F) replacement resorption at 60 days. The columns represent the mean of 6 animals and the vertical lines indicate the standard error means.

Discussão geral

4 DISCUSSÃO GERAL

O tratamento de primeira escolha para a avulsão dentária é o reimplante. O ideal é que ele seja realizado o mais rápido possível; entretanto, na maioria das vezes, ocorre sob condições desfavoráveis, levando ao desenvolvimento de reabsorções radiculares que, em pouco tempo, podem ocasionar a perda do dente (FLORES et al., 2007; TROPE, 2011).

Para evitar isso, é fundamental que pesquisas sejam realizadas e o conhecimento atual seja ampliado em relação aos traumatismos alvéolo-dentários, objetivando a busca e identificação de substâncias anti-reabsortivas que atenuem ou, preferencialmente, eliminem, a ocorrência de sequelas indesejáveis, como as reabsorções radiculares.

Sabe-se que o desenvolvimento da reabsorção inflamatória externa decorre da combinação de microorganismos no interior do canal radicular e do dano causado ao cemento na superfície externa da raiz, resultando em um processo que pode ser bastante agressivo (TROPE, 2011).

Apesar da pasta de hidróxido de cálcio ser a medicação de escolha na prevenção e no tratamento de reabsorções inflamatórias, atualmente, não é a única a ser recomendada (TROPE, 2011). Ela apresenta como inconveniente a necessidade de trocas sucessivas, por um longo período de tempo, até que o processo inflamatório seja eliminado e o canal radicular possa ser obturado definitivamente (JACOBOVITZ e PONTES LIMA, 2009). Isso acarreta na necessidade de maior tempo despendido, tanto pelo profissional, quanto pelo paciente, bem como, em um maior custo do tratamento.

O MTA apresenta um mecanismo de ação muito semelhante ao do hidróxido de cálcio. Em razão disso e, na tentativa de simplificar o tratamento para dentes reimplantados tardiamente, é que este produto vem sendo utilizado como material obturador em relatos de caso da literatura, indicando efeitos bastante promissores. Também tem sido utilizado com o intuito de melhorar os resultados do reparo em áreas de reabsorção radicular, como material obturador e, induzir a apecificação; nesse caso, aplicado como um tampão apical (AGGARWAL e SINGLA, 2010; FELIPPE et al., 2006; GHAZIANI et al., 2007; CEHRELI et al., 2011; MOHAMMADI e DUMMER, 2011).

No entanto, os estudos recomendam sempre a utilização da pasta de hidróxido de cálcio previamente à obturação definitiva (TROPE, 2011; MOHAMMADI e DUMMER, 2011). Baseado nisso, um estudo *in vitro* demonstrou que a utilização da pasta de hidróxido de cálcio no interior do canal radicular durante o período de uma semana, previamente à aplicação de um tampão apical de MTA, resultou na melhora da adaptação marginal do MTA na região do ápice dentário, durante processo de apecificação. É provável que isso ocorra em decorrência da formação e deposição do carbonato de cálcio pelo hidróxido de cálcio, ocasionando uma redução da permeabilidade. No entanto, os autores sugerem a necessidade de estudos *in vivo* (BIDAR et al., 2010).

Outra excelente propriedade do MTA é a sua bioatividade, a qual caracteriza um dos efeitos benéficos deste material quando colocado nos tecidos. Neste caso, há precipitação do fosfato de cálcio que age como precursor durante a formação da apatita carbonada, a qual representa a fase mineral dos tecidos duros (osso, dentina e cemento). Essa precipitação promove o processo de biomineralização, que tem relação direta na capacidade de selamento e, consequentemente, na diminuição da infiltração (MARTIN et al., 2007; REYES-CARMONA et al., 2009). Provavelmente, em razão desta maior capacidade de deposição nos tecidos duros é que, em nosso estudo, os grupos WMTA e MTA Fillapex apresentaram maiores áreas de anquilose.

Todas essas propriedades também fazem com que o MTA possa ser utilizado como material de preenchimento do canal; porém, atenção deve ser dada a algumas limitações como o risco de extravasamento no periápice e de haver falha no preenchimento do canal, devido à dificuldade de manipulação (TORABINEJAD e PARIROK, 2010).

Com relação ao processo inflamatório desencadeado pelos materiais, Shahi et al. (2010) compararam o efeito do WMTA (MTA branco), GMTA (MTA cinza) e cimento Portland branco e cinza em relação às células inflamatórias, através de tubos de polietileno implantados em dorso de ratos. Os resultados demonstraram que o MTA produziu baixa reação tecidual, um processo que diminuiu com o passar do tempo. Em nosso estudo, destacamos a ocorrência de um processo inflamatório bastante agressivo quando houve extravasamento do cimento endodôntico MTA Fillapex para fora do canal radicular. Dessa forma, é possível sugerir que mais testes de biocompatibilidade são necessários, a fim de conhecer melhor as características biológicas deste material, recentemente lançado no mercado.

Nos casos de dentes reimplantados tardiamente, quando instalado o processo de reabsorção, seja ela, inflamatória ou por substituição, muitas vezes não é possível impedir o seu desenvolvimento e, este permanece ativo durante anos até a perda do dente. Nesta linha de pesquisa, a análise imunoistoquímica está em andamento, a fim de determinar a expressão das proteínas do sistema OPG/RANK/RANKL e, conhecer o período em que o mecanismo da reabsorção está mais ativo (MANFRIN, 2007). Apresentamos algumas imagens preliminares do estudo, identificando apenas o padrão de marcação das mesmas, o que permite sugerir que a ocorrência de remodelação dos tecidos está presente em maior ou menor intensidade, dependendo dos grupos experimentais, embora análises adicionais ainda se façam necessárias. (ANEXO E).

Com base no que foi exposto, o MTA apresenta resultados clínicos promissores, quando utilizado em dentes traumatizados, com necrose pulpar e rizogênese incompleta, em áreas de reabsorção e no preenchimento completo do canal radicular. Porém, ainda julgamos ser necessário um período maior de acompanhamento para confirmar o sucesso clínico frente aos diversos tratamentos necessários aos reimplantes dentários. Uma vez que o WMTA e o MTA Fillapex não foram capazes de impedir o desenvolvimento das reabsorções radiculares, novos estudos são necessários para melhor elucidar os mecanismos de ação destas substâncias no que diz respeito ao tratamento de dentes reimplantados tardiamente. É possível inferir que modificações químicas nos cimentos à base de MTA podem originar materiais com propriedades superiores àquelas observadas para os produtos que já estão no mercado.

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ANEXO A – Comprovante de submissão do Artigo 1

Dental Traumatology - Manuscript ID DT-06-11-RE-1342

dtoffice@qualitynet.net <dtoffice@qualitynet.net> 7 de junho de 2011 19:42
Para: bfvogt@ig.com.br

07-Jun-2011

Dear Miss Vogt:

Your manuscript entitled "Mineral trioxide aggregate applications in management of dental trauma: a literature review" has been successfully submitted online. Within the next few days your manuscript will be checked for its compliance with the journal's requirements. If anything does not comply with the Journal's requirements, you will be notified by email that your manuscript has been unsubmitted and returned to your author centre. The email will include detailed explanation of the changes to be made before your manuscript can be resubmitted and considered for publication in the Dental Traumatology.

Your manuscript ID is DT-06-11-RE-1342.

Please mention the above manuscript ID in all future correspondence. If there are any changes in your street address or e-mail address, please log in to Manuscript Central at http://mc.manuscriptcentral.com/dt and edit your user information as appropriate.

You can also view the status of your manuscript at any time by checking your Author Center after logging in to http://mc.manuscriptcentral.com/dt

Thank you for submitting your manuscript to the Dental Traumatology.

Sincerely,
Karin Andersson
Dental Traumatology Editorial Office

ANEXO B – Comprovante de submissão do Artigo 2

International Endodontic Journal - Manuscript ID IEJ-11-00324

iejeditor@cardiff.ac.uk <iejeditor@cardiff.ac.uk> 3 de junho de 2011 01:26
Para: bfvogt@ig.com.br

03-Jun-2011

Dear Ms. Vogt

Your manuscript entitled "Evaluation of two mineral trioxide aggregate formulations as intracanal medications in a rat model of delayed tooth replantation" has been successfully submitted online to the International Endodontic Journal.

Your manuscript ID is IEJ-11-00324.

Please mention the above manuscript ID in all future correspondence or when calling the Editorial Office for questions. If there are any changes in your postal or e-mail address, please log in to ScholarOne Manuscripts at http://mc.manuscriptcentral.com/iej and edit your user information as appropriate.

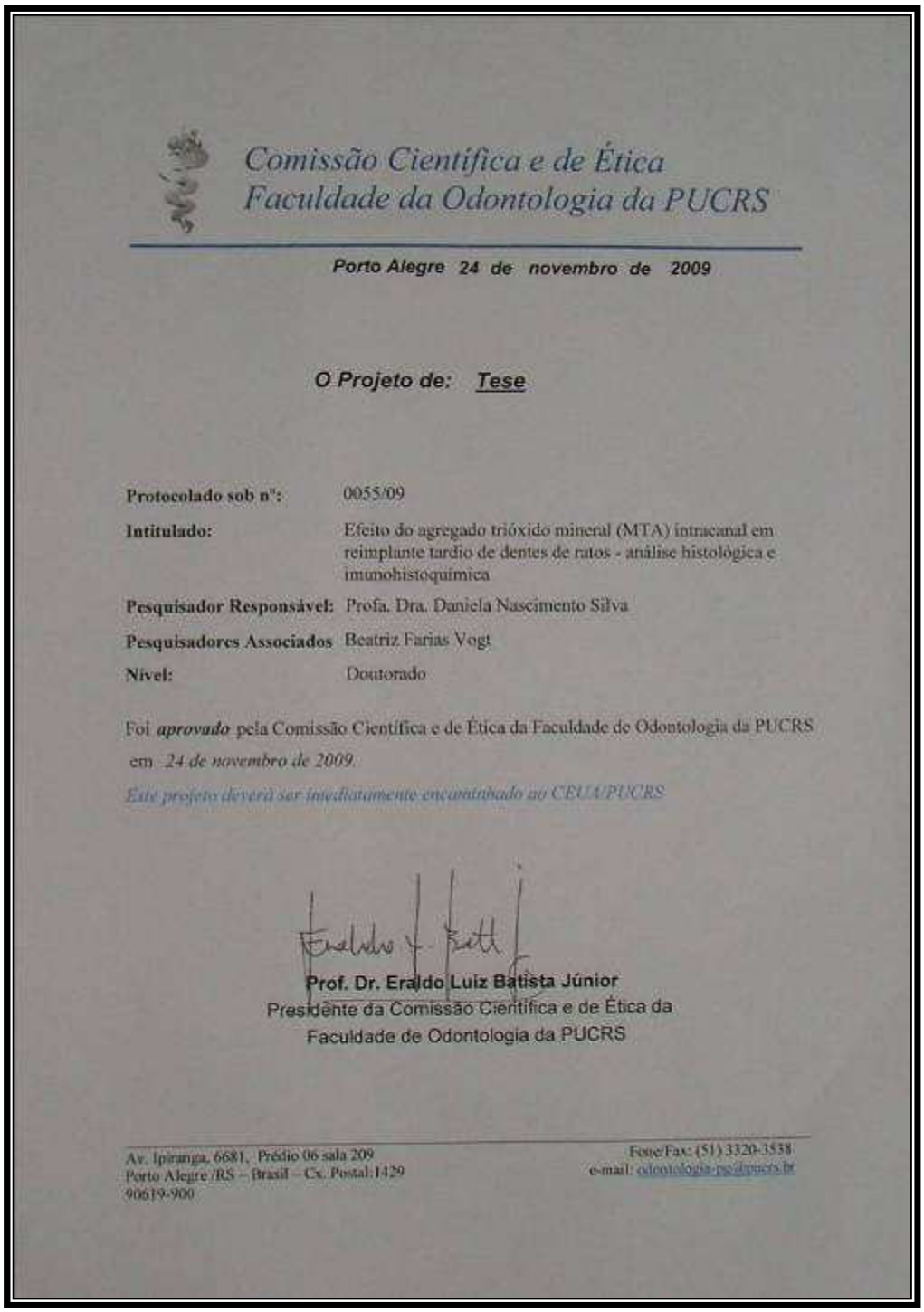
You can also view the status of your manuscript at any time by checking your Author Centre after logging in to http://mc.manuscriptcentral.com/iej .

Thank you for submitting your manuscript to the International Endodontic Journal.

Kind regards

Paul Dummer
Editor, International Endodontic Journal
iejeditor@cardiff.ac.uk

ANEXO C - Aprovação da Comissão Científica e de Ética da Faculdade de Odontologia da PUCRS



Comissão Científica e de Ética
Faculdade da Odontologia da PUCRS

Porto Alegre 24 de novembro de 2009

O Projeto de: Tese

Protocolado sob nº:	0055/09
Intitulado:	Efeito do agregado trióxido mineral (MTA) intracanal em reimplante tardio de dentes de ratos - análise histológica e imunohistoquímica
Pesquisador Responsável:	Profa. Dra. Daniela Nascimento Silva
Pesquisadores Associados	Beatriz Farias Vogt
Nível:	Doutorado

Foi *aprovado* pela Comissão Científica e de Ética da Faculdade de Odontologia da PUCRS em *24 de novembro de 2009.*

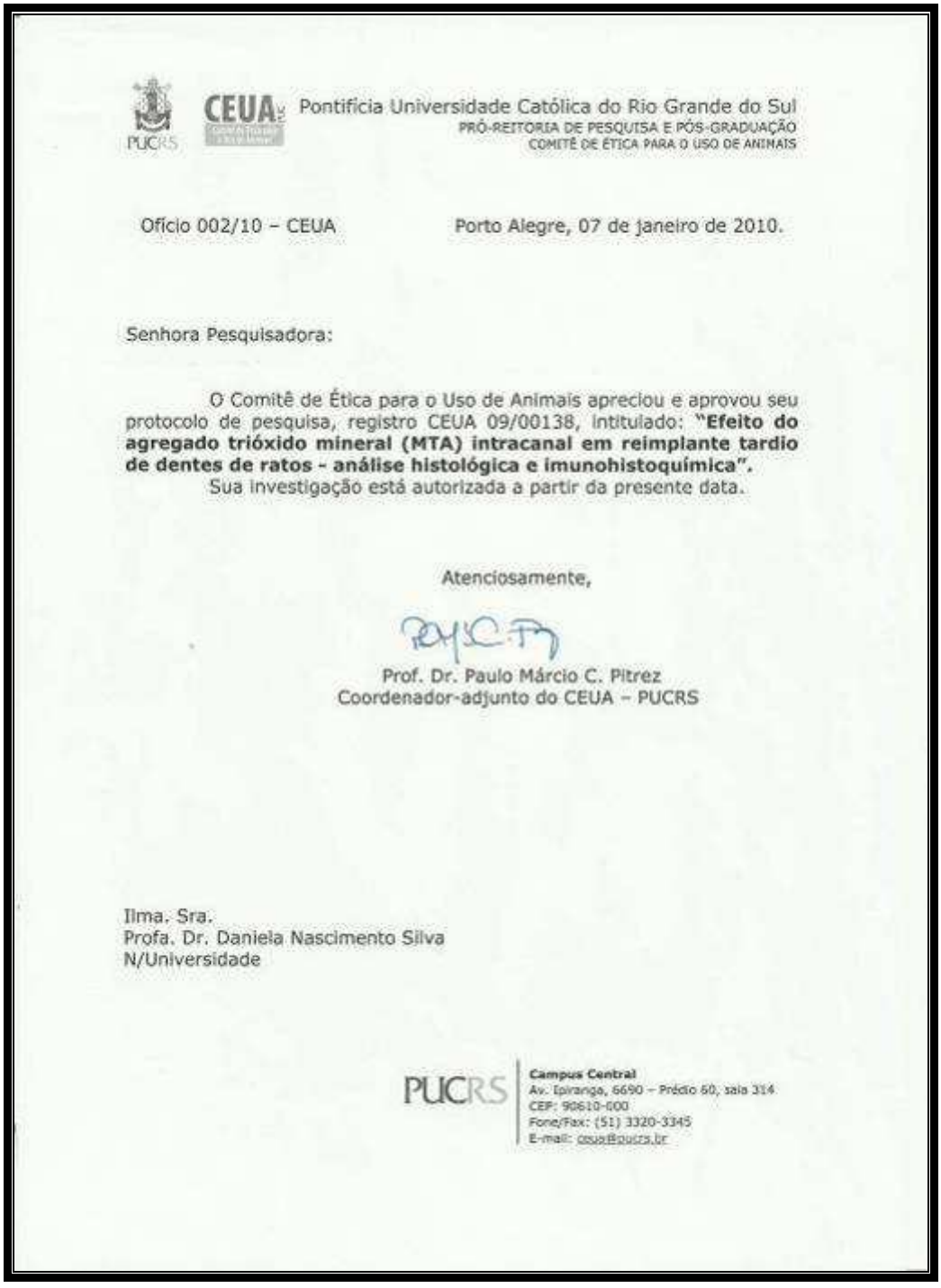
Este projeto deverá ser imediatamente encaminhado ao CEUA/PUCRS

Prof. Dr. Eraldo Luiz Batista Júnior
Presidente da Comissão Científica e de Ética da
Faculdade de Odontologia da PUCRS

Av. Ipiranga, 6681, Prédio 06 sala 209
Porto Alegre /RS – Brasil – Cx. Postal:1429
90619-900

Fone/Fax: (51) 3320-3538
e-mail: odontologia-pg@pucrs.br

ANEXO D - Aprovação do Comitê de Ética em Pesquisa da PUCRS



PUCRS CEUA Pontifícia Universidade Católica do Rio Grande do Sul
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
COMITÊ DE ÉTICA PARA O USO DE ANIMAIS

Ofício 002/10 – CEUA Porto Alegre, 07 de janeiro de 2010.

Senhora Pesquisadora:

O Comitê de Ética para o Uso de Animais apreciou e aprovou seu protocolo de pesquisa, registro CEUA 09/00138, intitulado: **"Efeito do agregado trióxido mineral (MTA) intracanal em reimplante tardio de dentes de ratos - análise histológica e imunohistoquímica".**
Sua investigação está autorizada a partir da presente data.

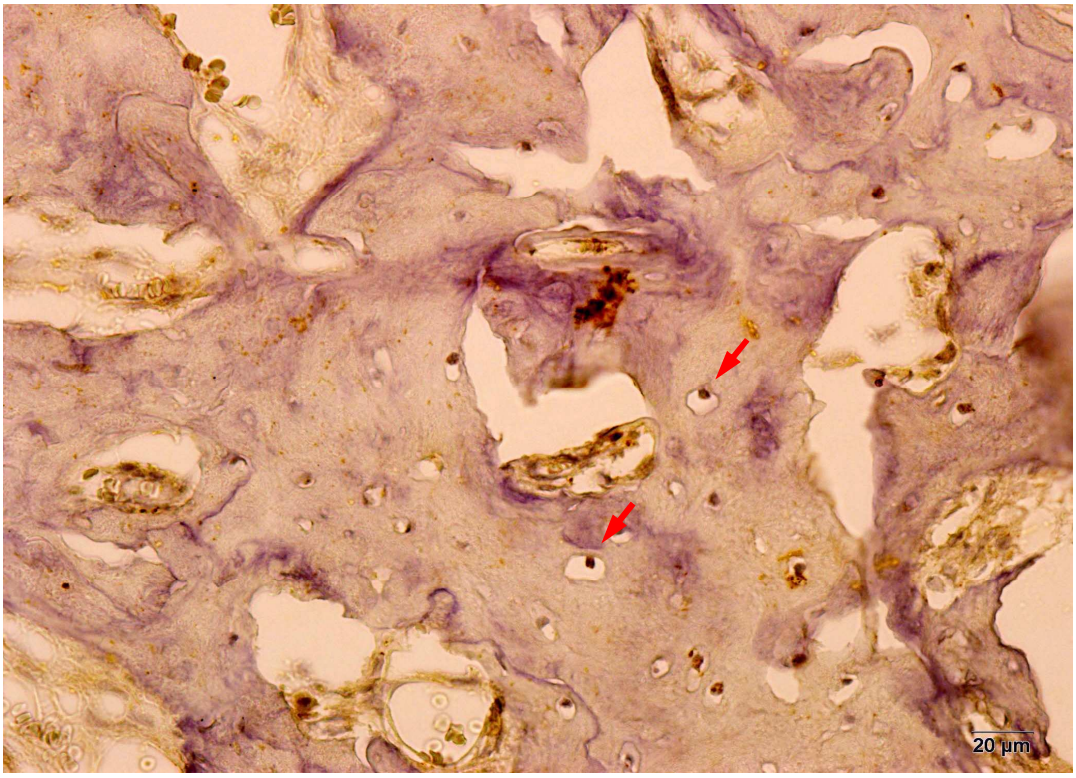
Atenciosamente,

Prof. Dr. Paulo Márcio C. Pitrez
Coordenador-adjunto do CEUA – PUCRS

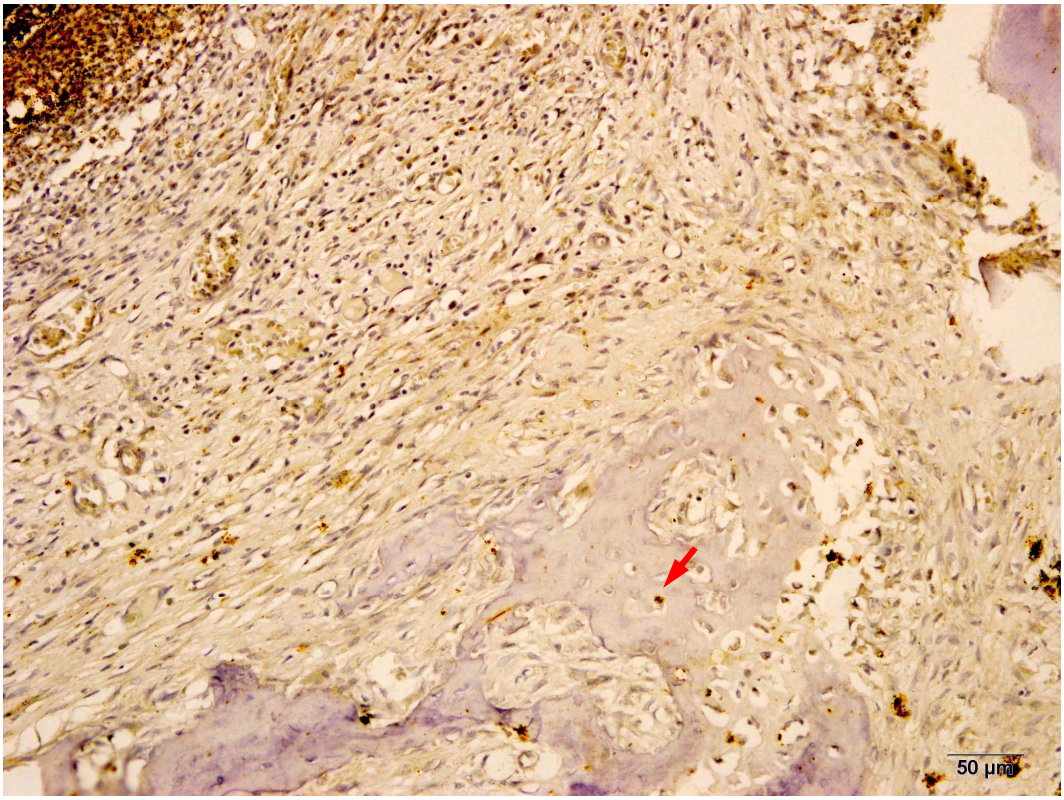
Ilma. Sra.
Profa. Dr. Daniela Nascimento Silva
N/Universidade

PUCRS **Campus Central**
Av. Ipiranga, 6690 – Prédio 60, sala 314
CEP: 90610-000
Fone/Fax: (51) 3320-3345
E-mail: ceua@pucrs.br

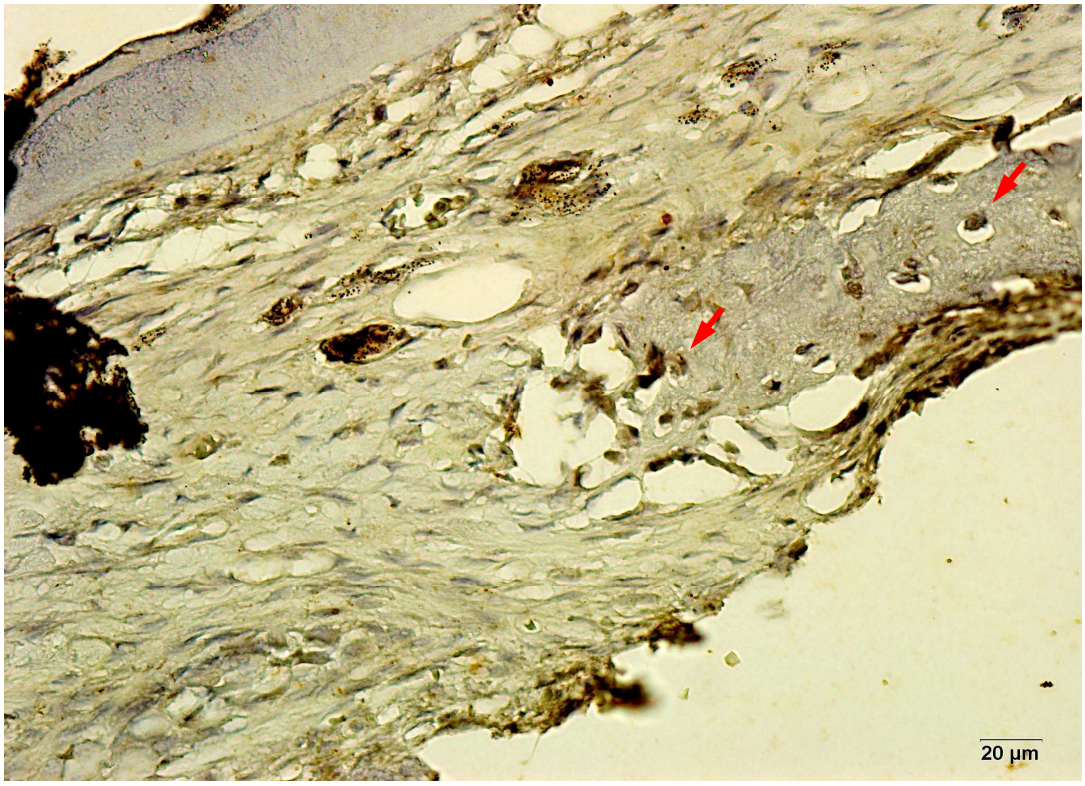
ANEXO E – Imagens demonstrando o padrão de marcação das proteínas OPG, RANK e RANKL



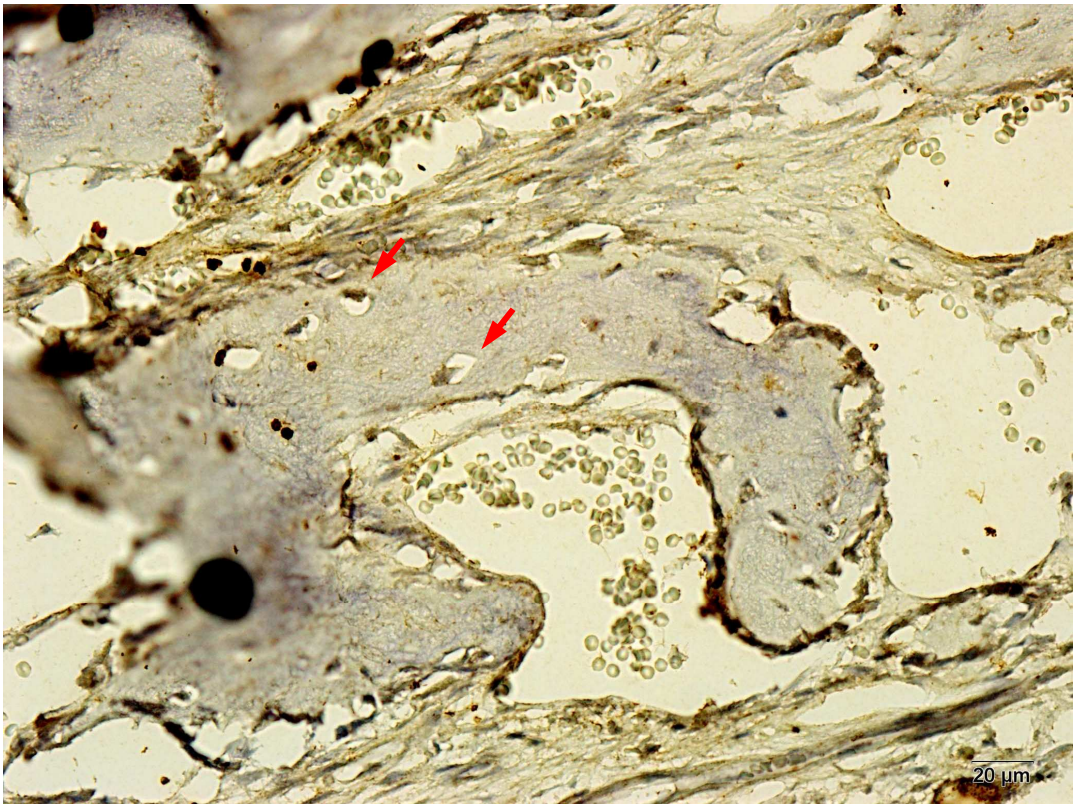
Expressão de OPG no tecido ósseo (setas) (imunoistoquímica; original 400x).



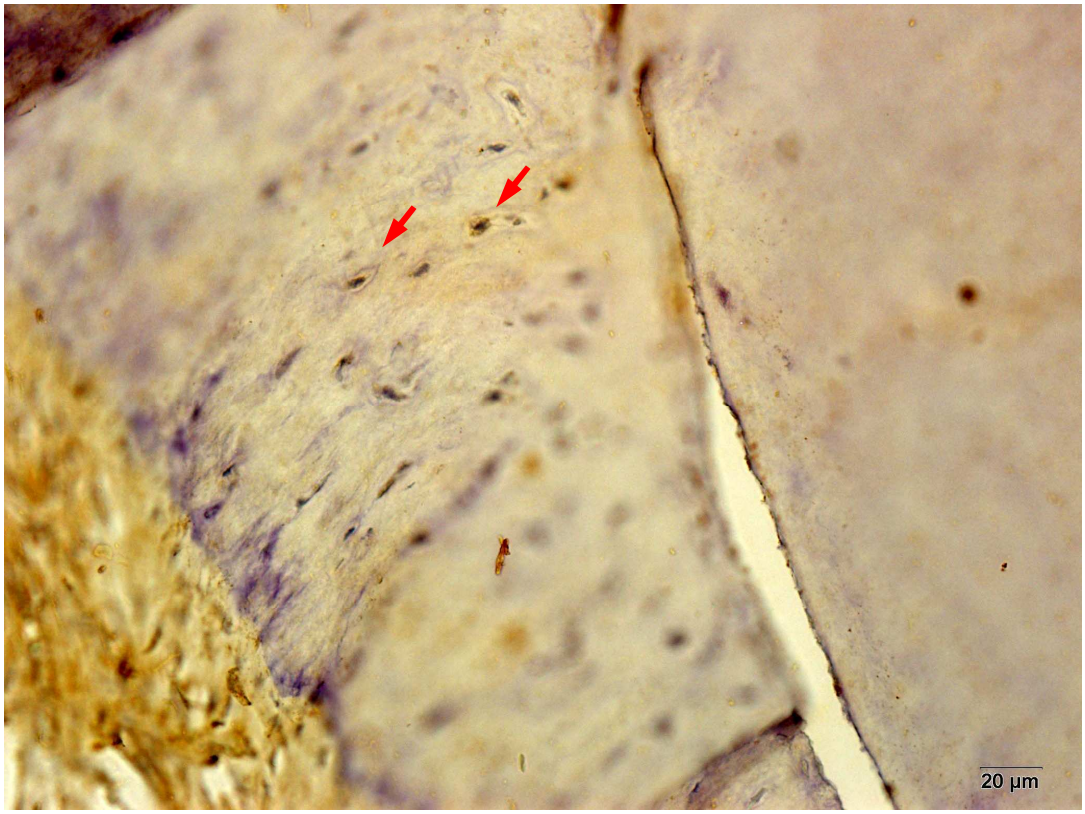
Expressão de OPG no tecido ósseo (setas) (imunoistoquímica; original 200x).



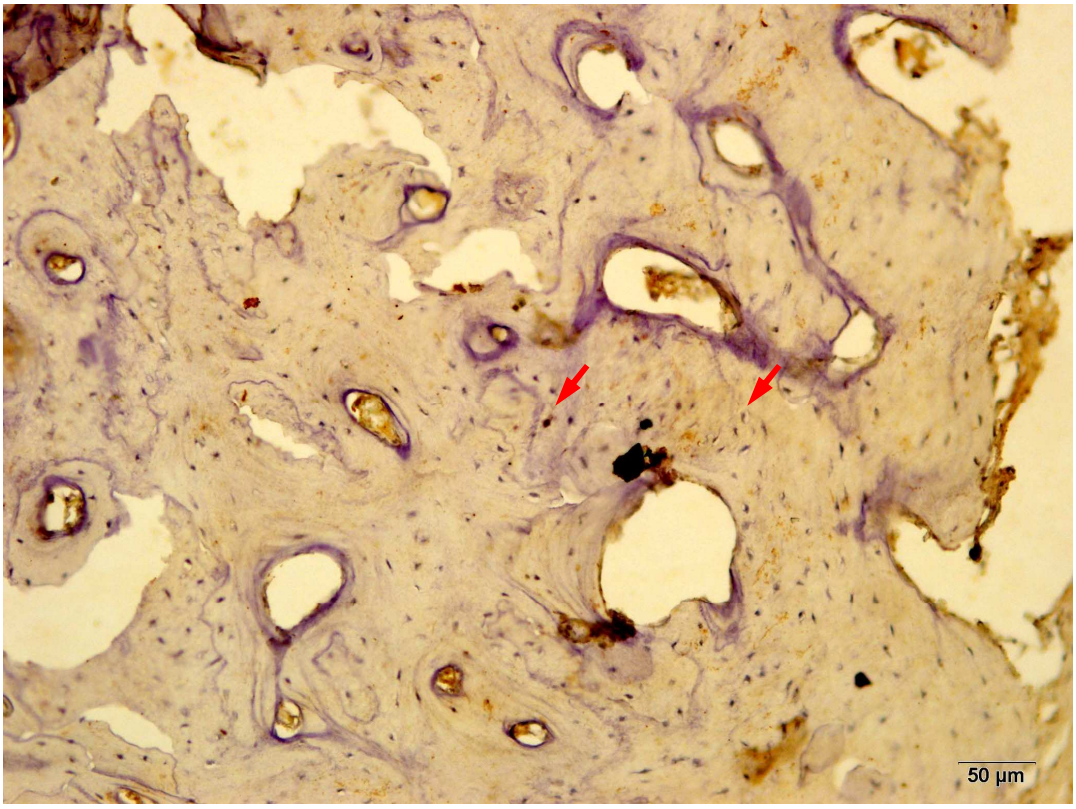
Expressão de RANK no tecido ósseo (setas) (imunoistoquímica; original 400x).



Expressão de RANK no tecido ósseo (setas) (imunoistoquímica; original 400x).



Expressão de RANKL no tecido ósseo (setas) (imunoistoquímica; original 400x).



Expressão de RANKL no tecido ósseo (setas) (imunoistoquímica; original 200x).