UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA
PROGRAMA DE PÓS GRADUAÇÃO EM ODONTOLOGIA – NÍVEL MESTRADO
ÁREA DE CONCENTRAÇÃO CLÍNICA ODONTOLÓGICA – ENDODONTIA

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CITOTOXICIDADE, BIOCOMPATIBILIDADE E PERFIL OSTEOINDUTOR DE UM NOVO MATERIAL A BASE DE MTA

Porto Alegre
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Dissertação de Mestrado apresentado ao Programa de Pós-Graduação em Odontologia da Universidade Federal do Rio Grande do Sul, como pré-requisito final para a obtenção do título de Mestre em Clínica Odontológica - Endodontia**.**

Linha de Pesquisa: Biomateriais e Técnicas Terapêuticas em Odontologia.

Orientador: Profª. Drª. Patrícia Maria Poli Kopper Móra

Porto Alegre

2017

CIP - Catalogação na Publicação

Hack Coelho, Fernanda
CITOTOXICIDADE, BIOCOMPATIBILIDADE E PERFIL OSTEOINDUTOR DE UM NOVO MATERIAL A BASE DE MTA / Fernanda Hack Coelho. -- 2018.
60 f.
Orientadora: Patrícia Maria Poli Kopper Móra.

Dissertação (Mestrado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Odontologia, Programa de Pós-Graduação em Odontologia, Porto Alegre, BR-RS, 2018.

1. Citotoxicidade. 2. Bicompatibilidade. 3. Cimento a base de MTA. 4. MTA Flow. I. Poli Kopper Móra, Patrícia Maria, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

Dedico este trabalho aos meus pais, Angelo Santos Coelho e Mônica Flocke Hack Coelho. Obrigada pelo apoio e incentivo incondicionais durante esses nove anos de dedicação pela minha formação na UFRGS.

AGRADECIMENTOS

À Profª. Drª. Patrícia Maria Poli Kopper Móra, orientadora deste trabalho. Obrigada pelos ensinamentos, apoio e tranqüilidade que me passastes durante esses dois anos de pós-graduação.

À Profª. Drª. Manoela Domingues Martins, por estar mais uma vez ao meu lado em um trabalho que aprimora minha formação acadêmica. O carinho e a admiração que tenho por ti se perpetuam por todos esses anos.

Aos professores do grupo da Endodontia da UFRGS: Prof. Dr. Régis Burmeister dos Santos, Prof. Dr. João Ferlini Filho, Prof. Dr. Marcus Vinícius Reis Só, Profª. Drª Fabiana Grecca Villela, Prof. Dr. Francisco Montagner, Profª. Drª Simone Bonato Luisi, Profª. Drª Renata Grazziotin, Profª. Drª Roberta Scarparo e Prof. Dr. Tiago Fontoura de Melo por terem me transmitido todo o conhecimento endodôntico que vou carregar ao longo da minha vida profissional. Tenho muito orgulho de ser formada por vocês.

À equipe da Unidade de Experimentação Animal do Hospital de Clínicas de Porto Alegre, especialmente a responsável pela unidade, Drª Marta Justina Giotti Cioato e a médica veterinária responsável Drª Daniela Campagnol. Graças ao profissionalismo e dedicação de vocês contamos com a execução da parte experimental dentro de critérios pré-estabelecidos e temos segurança dos nossos resultados. À equipe do serviço de pesquisa experimental do HCPA, especialmente a bióloga Emily Ferreira Salles Pilar e a técnica Flávia Rejane Giusti, pelo auxilio em toda confecção do material histopatológico que nos possibilitou a análise deste trabalho.

À Drª Marcia Martins Marques, coordenadora do Laboratório de Pesquisas Básicas do Departamento de Dentística da FOUSP e a Drª Maria Stella Moreira pelo auxílio imprescindível na realização da parte *in vitro* do nosso estudo.

À colega Alexandra de Mello pela ajuda na parte experimental no início do trabalho.

À ultradent pelo fornecimento dos materiais que nos possibilitou o desenvolvimento desta pesquisa.

A esta universidade, seu corpo docente, direção, administração, PPG da odontologia e seu coordenador Cassiano Kuchenbecker Rosing, que oportunizaram minha formação como mestra em clínica odontológica.

À Fundação de Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), do Ministério da Educação (MEC), pelo suporte financeiro durante o período de mestrado.

A todas amigas que fazem parte da minha vida, mas especialmente a Rafaela Zereu, por ter sido mais que uma amiga, ter se tornado uma Irmã para os momentos bons e ruins. Por sempre torcer pelas minhas conquistas como se fossem dela também.

Ao meu namorado, Diego Vinhas, por ter caminhado comigo durante essa trajetória final, entendendo algumas ausências e adversidades. Por dizer sempre o quanto se orgulha de mim, me incentivar a não desistir e me ensinar a sempre ver o lado positivo da vida.

RESUMO

COELHO, Fernanda Hack. **Avaliação da resposta de células tronco humanas do ligamento periodontal e resposta tecidual em tecido subcutâneo de ratos frente a um novo cimento a base de MTA.** 2017. 60 f. Dissertação de Mestrado apresentado ao Programa de Pós-Graduação em Odontologia da Universidade Federal do Rio Grande do Sul, área de concentração Clínica Odontológica/Endodontia – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2017.

Este estudo teve como objetivo avaliar a citotoxicidade, biocompatibilidade e perfil osteoindutor de um novo material a base de MTA (MTA Flow) comparado ao AH plus e MTA fillapex. A viabilidade celular foi avaliada em células-tronco do ligamento periodontal pelo teste MTT. A resposta inflamatória e o perfil osteoindutor foram avaliados em tecido conjuntivo de 24 ratos machos (*Rattus norvegicus albinus*, Rodentia, Mammalia da linhagem Wistar), pesando entre 300 e 350g. Os animais foram divididos em 4 grupos experimentais. Foram implantados 4 tubos de polietileno no dorso de cada animal, sendo um vazio e outros três preenchidos com um dos materiais. Após 7, 30 e 60 dias, 8 animais foram mortos com anestésico inalatório. O tubo e o tecido subcutâneo adjacente foram removidos e fixados em solução de formalina a 10%. O material foi analisado histológicamente quanto ao processo inflamatório, condensação fibrosa e presença de abscesso. Posteriormente, realizou-se a análise imunoistoquímica para identificação de colágeno I, osteopontina, sialoproteina óssea e proteína morfogentica óssea-4 (BMP-4). Os dados foram analisados estatisticamente pela ANOVA, complementado pelo teste de Tukey usando SPSS 15.0. O MTA Flow mostrou não ser citotóxico e ter excelente biocompatibilidade com menor reação inflamatória no tecido subcutâneo de ratos em comparação com AH Plus e MTA Fillapex. Além disso, o MTA Flow mostrou ser capaz de estimular o processo mineralização, sendo uma vantagem em relação aos outros materiais testados.

Palavras-chave: Endodontia, Biocompatibilidade, Cimentos Endodônticos, MTA

ABSTRACT

COELHO, Fernanda Hack. **Evaluation of the human periodontal ligament dental stem cell (hPDSC) and rat subcutaneous tissue response to a new based–MTA cement.** 2017. 60 p. Dissertation presented to the Postgraduate Program in Dentistry of the Federal University of Rio Grande do Sul, area of concentration: Dental Clinic/Endodontics– Faculty of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, 2017.

This study aimed to evaluate the cytotoxicity, biocompatibility and osteoinductive profile of a new MTA based material (MTA Flow) compared to AH plus and MTA fillapex. Cell viability was evaluated in periodontal ligament stem cells by the MTT test. The inflammatory response and the osteoinductive profile were evaluated in the connective tissue of 24 male rats (Rattus norvegicus albinus, Rodentia, Mammalia of the Wistar line) weighing between 300 and 350g. The animals were divided into 4 experimental groups. Four polyethylene tubes were implanted on the back of each animal, one empty and another three filled with one of the materials. After 7, 30 and 60 days, 8 animals were killed with inhaled anesthetic. The tube and adjacent subcutaneous tissue were removed and fixed in 10% formalin solution. The material was analyzed histologically for the inflammatory process, fibrous condensation and presence of abscess. Subsequently, the immunohistochemical analysis was performed to identify collagen I, osteopontin, bone sialoprotein and bone morphogenetic protein-4 (BMP-4). Data were analyzed statistically by ANOVA, complemented by the Tukey test using SPSS 15.0. MTA Flow was shown to be non-cytotoxic and to have excellent biocompatibility with lower inflammatory reaction in rat subcutaneous tissue compared to AH Plus and MTA Fillapex. In addition, the MTA Flow showed to be able to stimulate the mineralization process, being an advantage over the other materials tested.

Keywords: Endodontics, Biocompatibility, Endodontic Cements, MTA

SUMÁRIO

1 APRESENTAÇÃO

A presente dissertação teve como foco de estudo avaliar a citotoxicidade, a biocompatibilidade e o perfil osteoindutor de um novo cimento a base de MTA (MTA Flow) através de testes MTT e implantação em tecido subcutâneo de ratos com análise histopatológica e testes imunohistoquímicos.

Esta dissertação de mestrado está estruturada da seguinte maneira:

- Introdução: enfatizando os pontos principais deste estudo;
- Artigo: o desenvolvimento do trabalho está apresentado na forma de artigo científico de periódico em inglês, a ser enviado para publicação na revista International Endodontic Journal, fator de impacto 3.015 (Qualis A1, CAPES);
- Considerações finais.

2 INTRODUÇÃO

A completa obturação do sistema de canais radiculares constitui um fator chave para o sucesso da terapia endodôntica, tendo fundamental importância para o estabelecimento de um adequado reparo dos tecidos periapicais (Prinz 1912; Schilder 2006). Ao final do tratamento endodôntico objetiva-se uma resposta biológica do organismo caracterizada pelo selamento apical por meio da deposição de tecido mineralizado ou mesmo por tecido fibroso de reparação. A indução desse reparo está condicionada à correta execução de todas as fases do tratamento endodôntico, assim como a fatores inerentes à obturação, podendo-se citar o limite apical e as características do material obturador (Leonardo & Leal 2005).

Cimentos obturadores de canal são utilizados para selar o sistema de canais radiculares uma vez que somente a guta-percha não seria capaz de fazer isso. Esses materiais podem ser categorizados em diferentes grupos de acordo com sua composição química, o qual usualmente compreende hidróxido de cálcio, óxido de zinco e eugenol ou resina epóxi (Kim & Shin 2014).

De acordo com Soares e Goldberg (2001), a agressão aos tecidos periapicais suscita a instalação do processo inflamatório e os danos teciduais estão representados pela destruição dos tecidos moles e duros da região. Segundo estes autores, a ocorrência do processo de reparo passa pela eliminação dos fatores de agressão e pela reabsorção do exsudato inflamatório, seguido de neoformação vascular que fornecerá condições nutricionais para as neoformações teciduais que repararão os danos. No periápice espera-se que, após a remissão do processo inflamatório, haja a formação do ligamento periodontal apical e do tecido ósseo adjacente. Dessa forma, é imprescindível que o material obturador possua, além de adequadas propriedades físico-químicas, compatibilidade biológica com os tecidos apicais e periapicais.

Sabe-se que a biocompatibilidade está relacionada com a capacidade do material de promover reação inflamatória mínima ou inexistente além de, favorecer o reparo (Berbert *et al*. 2002). Portanto, são considerados apropriados, do ponto de vista biológico, cimentos obturadores que apresentem boa tolerância tecidual, sejam reabsorvidos no periápice em casos de extravasamento e permitam o reparo,

contribuindo para que se obtenha a menor agressão possível aos tecidos periapicais (Mendonça & Estrela 2001). Além destas características, é desejável que os materiais obturadores em contato com os tecidos periapacais estimulem a neoformação de tecido mineralizado neste local (Kim & Shin 2014, Cintra *et al*. 2017).

Como os materiais obturadores de canal podem ser colocados em contato íntimo com tecidos moles e duros ao redor do dente, sempre existe a possibilidade de que esses materiais influenciem biologicamente os tecidos vivos através de vias como túbulos dentinários, canais laterais, acessórios e forame apical (Kim & Shin 2014). A extrusão dos materiais acidentalmente pode ocorrer levando ao contato com os tecidos periapicais, principalmente em casos de ápice aberto, sobre-instrumentação e reabsorção patológica da raiz, levando a danos no ligamento periodontal e osso alveolar. Isso pode gerar problemas no resultado final do tratamento e piorar o prognóstico do caso (Kim & Shin 2014).

Sendo assim, a determinação do comportamento biológico de materiais obturadores faz parte do embasamento científico necessário para a realização de práticas terapêuticas bem fundamentadas (Costa 2001). Em busca de um cimento endodôntico que preencha os requisitos de um material ideal, conforme já descrito por Grossman, em 1958, novos materiais são constantemente introduzidos no mercado odontológico.

A aceitação do uso de um determinado produto deve ser baseada em trabalhos experimentais e laboratoriais que comprovem, entre outras propriedades, a sua baixa citotoxicidade e boa biocompatibilidade. Para isso, diversas metodologias têm sido desenvolvidas de modo a elucidar com maior clareza o potencial biológico dos materiais (Olsson *et al*. 1981).

A citotoxicidade é definida como a capacidade de um material impactar a viabilidade celular. Portanto, os testes de citotoxicidade são testes primários de biocompatibilidade que determinam o impacto das substâncias na lise e no crescimento celular além de outros efeitos observados nas células expostas aos materiais (Peters *et al*. 2013). Alguns desses métodos de teste foram combinados para estabelecer padrões (padrões ISO 10993). O objetivo foi melhorar a comparabilidade dos dados gerados em diferentes laboratórios de teste, bem como

economizar e reduzir o número de animais para testes posteriores, evitando ensaios duplicados (Collado-Gonzalez *et al*. 2017). Testes de citotoxicidade têm a vantagem de ser simples, reprodutíveis, bom custo-benefício, e adequados para avaliação de aspectos básicos (Kim & Shin 2014).

Como os cimentos são feitos de uma mistura de materiais em que ocorrem reações químicas, a liberação de material tóxico durante essa reação pode tornar o material menos biocompatível (Parirokh et al. 2015). A citotoxicidade de cimentos endodônticos tem sido testada de diferentes formas. O teste MTT é relativamente simples, reprodutível e possui acurácia (Kangarloo et al 2008, Collado-Gonzalez et al. 2017). Neste teste, diversos tipos de células-tronco podem ser utilizadas. Entre elas, as células-tronco do ligamento periodontal humano (hPDLSCs) são uma população de células multipotentes que são capazes de se diferenciar em células formadoras de cemento e colágeno, como já demonstrado em estudos *in vitro* e pré-clínicos (Collado-Gonzalez *et al*. 2017, Tour *et al.* 2012, Liu *et al.* 2015, Torii *et al.* 2015).

Uma segunda etapa para verificação da compatibilidade biológica de diferentes materiais pode ser realizada através da implantação de amostras dos materiais no tecido conjuntivo de pequenos animais. De acordo Olsson *et al.* (1981), tal modelo é adequado para avaliação da biocompatibilidade de materiais de uso endodôntico. As normas divulgadas pela American Dental Association (1972) e Federation Dentaire Internationale (1968) consideram os métodos de implante como testes válidos nas etapas preliminares de pesquisa da histocompatibilidade de diversos materiais.

A biocompatibilidade está diretamente relacionada com a composição do material e com os subprodutos que este produz (Cintra *et al.* 2017). A implantação de materiais endodônticos em tecido conjuntivo de ratos têm sido um dos métodos bastante utilizado para determinação da biocompatibilidade (Mittal et al. 1995, Silveira et al. 2011), devido a semelhança do genoma entre ratos e humanos (Kola 2004). A capacidade de gerar efeito inflamatório pode ser avaliada através do exame histopatológico do tecido que envolve os implantes (Zmener *et al*. 1988, Economides *et al*. 1995, Silveira *et al*. 2011).

Durante décadas, os cimentos endodônticos mais utilizados foram aqueles à base de óxido de zinco e eugenol, como o Endofill que, apesar de mostrarem desempenho satisfatório quanto às propriedades físico-químicas, não apresentam comportamento biológico favorável (Leonardo & Leal 2005). Scarparo *et al* (2010), comprovaram que estes cimentos são agressivos aos tecidos vivos. Devido a isso, já algum tempo, cimentos com diferentes composições têm sido desenvolvidos com o objetivo de melhorar propriedades biológicas: cimentos a base de resina epóxi (AH Plus, TopSeal), a base de MTA (MTA Fillapex), a base de silicato de cálcio (iRoot, Bioaggregate) e a base de resina metacrilato (EndoREZ, RealSeal) (Gandolfi *et al*. 2016).

O AH Plus (Dentsply - Maillefer, Suíça) foi desenvolvido e introduzido no mercado já há algum tempo. É um cimento a base de resina epóxi, considerado padrão-ouro graças as suas boas propriedades físicas, químicas e biológicas (Cintra *et al*. 2017, Kopper *et al*. 2003, Tavares *et al*. 2013, Assman *et al*. 2015). Ele foi criado a partir de modificações na fórmula do cimento AH 26 e tem sido estudado e amplamente utilizado. O AH Plus mantém as vantagens do AH 26 (radiopacidade, baixa solubilidade e pouca contração) diminuindo os índices de toxicidade do AH 26 que ocorrem provavelmente em decorrência da liberação de formaldeído, durante o processo químico (Kim & Shin 2014). Este cimento possui baixa citotoxicidade (Schwarze *et al*. 2002, Oztan *et al.* 2003) e tem apresentado bom comportamento biológico quando em contato com tecido conjuntivo (Leonardo *et al*. 1999, Batista *et al*. 2007, Scarparo *et al.* 2009, Tavares *et al.* 2013).

Cimentos a base de silicato de cálcio, também conhecidos por cimentos a base de MTA, mostraram propriedades físico-químicas e biológicas favoráveis quando utilizados em diversos procedimentos endodônticos como em casos de perfuração radicular e selamento após apicetomia (Sluyk *et al*. 1998, Main *et al*. 2004, Ferris & Baumgartner 2004, Lindeboom *et al*. 2005, Gupta & Goswami 2013). Esses materiais são biointerativos, preenchendo e tomando presa diante de ambiente com fluido e contaminado, sendo capaz de favorecer a remineralização dos tecidos e possuir boa vedação em avaliações de longos períodos (Gandolfi *et al*. 2016). Além disso, tem sido demonstrado que os cimentos de silicato de cálcio são capazes de liberar íons biologicamente relevantes (Ca e OH) que atuam como sinais

epigênicos em relação a várias células humanas envolvidas em processos de mineralização, incluindo células estaminais mesenquimais, osteoblastos e cementoblastos, e induzem uma resposta biológica positiva nos eventos reparadores de tecidos duros (Gandolfi *et al.* 2016).

O Agregado Trióxido Mineral (MTA) tem sido estudado e demonstrado possuir muitas propriedades de um material selador ideal. Ele ganhou popularidade nas últimas décadas devido a numerosos estudos *in vitro* e *in vivo* que confirmaram suas propriedades superiores como vedação, biocompatibilidade e bioatividade (Tawil 2015, Ha *et al*. 2017, Parirokh *et al*. 2017). O MTA é um pó que possui como principais componentes o silicato tricálcico, aluminato tricálcico, óxido tricálcico, óxido silicato e óxido de bismuto (Torabinejad *et al*. 1995). Sua capacidade de selamento em retrobturações, perfurações, tem sido superior a de outros materiais, bem como sua resposta histológica (Torabinejad *et al*. 1995a; Torabinejad *et al*. 1995b). Tem sido defendido como material de escolha para vários procedimentos clínicos, entre eles, reparo de perfurações, capeamento direto e indireto, pulpotomia, apicigênese e apicificação. Porém, a manipulação deste cimento, bem como seu emprego para obturação de canais radiculares é dificultada por suas características físico-químicas. Sendo assim, novos materiais, que contem MTA em sua composição, têm possibilitado seu emprego como material obturador (Kim & Shin 2014).

O MTA Fillapex (Ângelus, Brasil), por exemplo, é um cimento endodôntico que contém MTA. É um cimento de silicato de cálcio que começou a ser comercializado em 2011. Contém partículas de cálcio e dióxido de silicone. Mostrou fluidez satisfatória, bom selamento e baixa solubilidade (Gandolfi *et al*. 2016). Entretanto, além de conter MTA, este cimento é também composto por resinas, bismuto, sílica nanoparticulada e pigmentos. Sendo assim, apesar de conter MTA, este material tem apresentado resultados menos favoráveis em comparação com outros no que se refere a sua compatibilidade tecidual (Tavares *et al*. 2013, Assman *et al*. 2015). Cimentos baseados em componentes resinosos tem alguns efeitos tóxicos os quais diminuem ao longo do tempo a medida que a concentração do produto é reduzida (Parirokh et al. 2015).

Buscando não interferir nas propriedades biológicas do MTA, um novo material endodôntico foi proposto pela Ultradent (MTA Plus, Ultradent, EUA). De acordo com o fabricante, tal material contém apenas o MTA e radiopatizante, sem a presença do componente resinoso. Tendo o entendimento de que, para que um novo material seja considerado mais vantajoso que os já estabelecidos no mercado, são necessárias análises que permitam identificar suas características físicas e biológicas para que se possa comprovar sua eficácia clínica e justificar seu uso.

Além da análise da citotoxicidade e da biocompatibilidade, é necessário verificar a capacidade do novo material de estimular a formação de tecido mineralizado (dentinogênese e osteogênese). O processo de mineralização é altamente controlado e envolve a participação de proteínas colagênicas (colágeno tipo I) e não colagênicas tais como: osteonectina, osteocalcina, sialoproteina óssea (BSP) e proteínas morfogenéticas do osso (BMP), que são secretadas no fronte de mineralização tendo papel importante na indução de tecido mineralizado (Otsuka *et al*. 1984, Fisher & Termine 1985, Jundt *et al*.1987, Butler 1995; Reichert *et al*. 1992, Ingram *et al*. 1993, Goldberg & Lasfargues 1995).

Neste sentido, parece oportuno e de grande contribuição científica a realização de um estudo para investigar a citotoxicidade, o comportamento biológico e perfil de indução de formação de tecido mineralizado deste novo material, que contém MTA, quando em contato com tecido conjuntivo de ratos, em comparação com dois cimentos que já são comercializados (AH Plus e MTA Fillapex).

3 ARTIGO CIENTÍFICO

Title

Cytotoxicity, Biocompatibility and Osteoinductor Profile of a new MTA-Based material (MTA Flow).

Abstract

Introduction: The aim of this study was to evaluate the cytotoxicity, biocompatibility and potential to induce mineralization of a new MTA-hydrogel-based cement (MTA Flow). **Methods:** A cytotoxicity study using periodontal ligament stem cells was performed with the MTT test. Cell viability was verified when in contact with MTA Flow compared to two other sealers (AH Plus and MTA Fillapex). After that, the evaluation of the response in connective tissue of rats and the potential of mineralization induction was performed against the implantation of the same materials. Twenty-four male rats weighing between 300 and 350g, were divided into 4 experimental groups: Control, MTA Fillapex, AH Plus and MTA Flow. Four polyethylene tubes were implanted on the back of each animal, one empty and another three filled with one of the materials. After 7, 30 and 60 days, 8 animals were killed with inhaled anesthetic. The tube and adjacent subcutaneous tissue were removed and fixed in 10% formalin solution. The material was analyzed histologically for the inflammatory process, fibrous condensation and presence of abscess. Subsequently, the immunohistochemical analysis was performed to identify collagen I, osteopontin, bone sialoprotein and bone morphogenetic protein-4 (BMP-4). Data were analyzed statistically by ANOVA, complemented by the Tukey test using SPSS 15.0. **Results:** MTA Flow was shown to be non-cytotoxic and to have excellent biocompatibility with lower inflammatory reaction in the subcutaneous tissue in rats compared to AH Plus and MTA Fillapex. In addition, MTA Flow has shown to be able to stimulate the mineralization process in the immunohistochemical analysis. **Conclusions:** MTA Flow showed to be a material that can present advantages in the biocompatibility and induction of mineralized tissue compared to other cements already used.

Key Words: Endodontics, Biocompatibility, Endodontic Cements, MTA

Introduction

Several endodontic materials such as repair cements and sealers are frequently placed in close contact or extrude to vital tissue. These materials should be biocompatible with the host tissues, insoluble in tissue fluids, nontoxic, noncarcinogenic, nongenotoxic, dimensionally stable, easy to use and be radiopaque for recognition on radiographs to result in sealing ability and marginal adaptation (Torabinejad & Ford 1996, Ribeiro 2008). Looking for all these properties, in the 90´s, Mineral Trioxide Aggregate (MTA) was developed and since then, a strong interest in developing MTA-based endodontic materials could be observed in literature (Roberts *et al*. 2008, Parirokh & Torabinejad 2010, Ha *et al*. 2017, Parirokh *et al*. 2017, Torabinejad *et al*. 2017).

MTA is a bioactive calcium silicate cement, consisting of tricalcium silicate, dicalcium silicate and tricalcium aluminate. Several studies have been published about MTA's biocompatibility, chemical and physical properties, antibacterial activity and sealing ability (Silva *et al*. 2014, Parirokh & Torabinejad 2010, Torabinejad *et al*. 2017, Ha *et al*. 2017, Parirokh *et al*. 2017). It has been considered as main option for pulp capping, pulpotomy, apexogenesis, apical barrier formation in teeth with open apexes, repair of root perforations and as a root canal filling material (Roberts *et al*. 2008, Tawil *et al*. 2015, Lin *et al*. 2016). Scientists worldwide have been working to improve the handling characteristics and some physicochemical properties of MTA with the intention of expanding its applicability in endodontics. There are several products that contain MTA in its formulation with some variations in composition that could interfere in tissue response. However, in general, all MTA cements showed excellent biocompatibility, bioactivity, and osteoconductivity properties (Roberts *et al*. 2008, Scarparo *et al*. 2010, Tavares *et al.* 2013, Silva *et al*. 2014, Ha *et al*. 2017, Torabinejad *et al*. 2017, Salles *et al*. 2012).

Biocompatibility is considered one important step to clinical appliance of a new product. It is defined as the capability of a material coexistence with living tissues or organisms without causing harm. In other words, a material is considered biocompatible when in contact with the tissue fails to trigger an adverse reaction, such as inflammation, allergy, toxicity or carcinogenicity (Camilleri 2006, Roberts *et al.* 2008, Scarparo *et al*. 2010, Torabinejad & Parirokh 2010, Tavares *et al.* 2013,

Assmann *et al*. 2015, Tanomaru Filho *et al.* 2017). Among the various methodologies recommended to study the biocompatibility of endodontic products, cytotoxicity, using *in vitro* cell culture tests, is part of the initial protocols followed by tests in experimental animals according to accepted clinical protocols (International Organization for Standardization, 1997 and 2009).

Besides the biocompatibility analysis, it is important to verify if the new bioactive calcium silicate cement has the property to stimulate the formation of mineralized tissue (dentinogenesis and osteogenesis). MTA has been considered a useful bioactive material for bone and dentin repair in terms of inducing cell differentiation and mineralized matrix deposition (Gandolfi *et al*. 2011, Prati & Gandolfi 2015, Tani-Ishii *et al*. 2007, Rodrigues *et al*. 2017, Tanomaru-Filho *et al*. 2017). The mineralization process is highly controlled and involves the participation of several collagen proteins (collagen type I) and non-collagen proteins such as osteonectin, osteocalcin, bone sialoprotein (BSP) and bone morphogenetic proteins (BMPs), that are secreted on the front of mineralization (Chen *et al*. 1992, Ganss *et al*. 1999, Young 2003, Garcia *et al*. 2003, Ao *et al*. 2017).

New endodontic cements should be tested to verify their characteristics and to compare them with materials already evaluated. In 2016, MTA Flow was developed and is composed by bioactive powder that sets with hydrogel. The chemical-physical properties of this material were recently evaluated (Guimarães *et al*. 2017), but its biological characteristics are still unknown. So, the aim of this study was to evaluate the cytotoxicity, biocompatibility and potential to induce mineralization of a new MTA-hydrogel-based cement (MTA Flow).

Materials and Methods

In Vitro Study (cytotoxicity analysis)

Cytotoxicity evaluation was performed according to ISO 10993-5 specifications (International Organization for Standardization 2009). The research was approved by the Ethics Committee from University of São Paulo (CAAE: 40392214.5.0000.0075).

hPDLSCCell Line (hPDLSCs)

Human periodontal ligament dental stem cells (hPDLSCs) previously isolated and characterized from third molars were used. hPDLSCs between the 3rd and 6th passage were cultured in medium composed of alpha-minimum essential medium (a-MEM; Gibco, Grand Island, NY) supplemented with 10% fetal bovine *Serum* [MSC FBS; *Mesenchymal Stem Cell-qualified Fetal Bovine Serum*; (GIBCO, NY, EUA)], 100 µM ascorbic acid (Sigma-Aldrich, MO, EUA), 2 mM de L-glutamine (Gibco), penicillin (100 U/mL; Gibco), and streptomycin (100 mg/mL; Gibco). Cells were maintained in an incubator at 37°C in humid atmosphere containing 5% CO_2 and 95% air. Cell growth was monitored daily under a phase contrast microscope. Cell culture medium was changed each 2 or 3 days depending on the cell metabolism, and subculture was made whenever necessary. For the experiments the cells were harvested and plated into 96 wells culture plates.

hPDLSCs characterization

In the second passage (P2), the hPDLSCs were analyzed to confirm their stem cell nature. Briefly, an aliquot of cells was evaluated by flow cytometry, which revealed positive staining for surface markers of mesenchymal stem cells (STRO1, CD146 and CD105) and negative staining for markers of hematopoietic stem cells (CD14) (Figure 1).

Preparation of conditioned culture media with endodontic materials

AH Plus, MTA Fillapex and MTA Flow (in the putty consistency: 0,19g of powder to 1 drop of gel) were mixed according to the manufacturer's instructions for all the tests. The composition of the evaluated endodontic cements is shown in Table 1.

The conditioned media were obtained as recommended by the American Society For Testing Material (ASTM, 1992). Briefly, test tubes containing 1 gram of the different cements on the bottom surface were filled with 5 mL of alpha-minimum essential culture medium (a-MEM; Gibco, Grand Island, NY) resulting in a final

proportion of 0.2 grams per milliliter. Conditioning was carried out for 24 hours, at 37ºC. After this period, the conditioned medium of each tube was collected, centrifuged at 300x g for 30 seconds to remove fragments of the sealers and then filtered through 0.2 µm syringe filters to sterilize. These elutes were then diluted in fresh culture media to be placed in contact with cultured cells. Dilution used in the present study was 10% conditioned medium (Bruno *et al*. 2015, Cavalcanti *et al*. 2005, Candeiro *et al*. 2015).

Experimental groups

The experimental groups were as follows:

- Control: hPDSC grown in normal nutritional conditions (10% FBS).

- AH Plus: hDPSCs grown in medium conditioned with AHPlus.

- MTA Fillapex: hDPSCs grown in medium conditioned with MTA Fillapex.

- MTA Flow: hDPSCs grown in medium conditioned with MTA Flow.

Cell Viability Assay

For experiments, cells were plated ($1x10^4$ cells/well) in 96-well culture plates and maintained in a humified chamber at 37ºC. Twenty-four hours later the culture medium was replaced by the experimental conditioned medium. Control group received fresh culture medium. Then, the conditioned medium was exchanged by fresh medium and the cultures were incubated in a humified chamber at 37ºC for 24 hours. Three isolate experiments were done. Cytotoxicity was evaluated by mitochondrial activity as measured by the 3-(bromide, 4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium (MTT)-based cytotoxicity assay. This test is based on the ability of mitochondrial enzymes produced by metabolically active cells to reduce MTT (Invitrogen, Eugene, OR, USA) molecules to an insoluble salt of formazan, which can be detected at 562 nm of absorbance using a spectrophotometer (Amersham Biosciences, Biochrom Ltd., Cambridge, England).

In Vivo Study

This is an experimental, controlled and blind trial. The research was approved by the Research Committee of the Faculty of Dentistry of UFRGS and the Committee on Ethics in the Use of Animals (CEUA) of the HCPA and Research Committee (CEP) of the HCPA.

Animals

Twenty-four male rats (Rattus norvegicus albinus, Rodentia, Mammalia Wistar) weighing between 300 and 350g were used. The animals were kept in an acclimatized room with balanced diet and water *ad libitum,* temperature of 22 ± 2 ° C, light / dark cycle of 12 hours and relative air humidity between 40 and 60%.

The subcutaneous tissue reactions of the animals to MTA Fillapex, AH Plus and MTA Flow were evaluated after 3 experimental periods (7, 30 and 60 days, n=8 per period). A total of 96 polyethylene tubes (n=4 per animal), 10 mm long and 1.3 mm in internal diameter, were made from scalps (AbottLab do Brasil, São Paulo, SP, Brazil). The materials were handled according to the manufacturer's instructions and inserted into the tubes (n=24 per cement). An empty tube was used as the negative control (n=24).

Initially the rats were weighed and under aseptic conditions, animals were anesthetized with anintraperitoneal administration of ketamine (0.1 mL/100 g) and xylazine (0.05 mL/100 g). After anesthesia, the trichotomy was performed on the dorsum of the animals, involving an area of approximately 5x5 cm in the lumbar region. Antisepsis of these areas was performed with 2% chlorhexidine digluconate solution and four 0.5cm-long incisions were made in animal’s back 2 cm apart from one other. Using blunt-tipped scissors, the lateral tearing of subcutaneous tissue provided 4 cavities on the back of each animal. A polyethylene tube filled with a different material or an empty tube was introduced into each surgical cavity.

The suture was performed with single stitches using 4.0 mononylon yarn. Opioid drug, Tramadol 10mg / kg, was administered intraperitoneally during 12/12 hours for 3 days, and the first dose was administered intraoperatively. At the end of each experimental time (7, 30 and 60 days) eight animals were euthanized using

isoflurane inhalation diluted in oxygen at the highest possible concentration and the tubes and surrounding tissues were removed and fixed in 10% buffered formalin at pH 7.0 during 24h.

Histological Analysis

The material was processed and embedded in paraffin using routine histopathological procedures. Histological sections of 5µm were stained with hematoxylin-eosin and observed using an optical microscope. The analysis of the results was done with blind design, by three experienced and calibrated examiner (k=0.80). A descriptive and semi-quantitative analysis was made using scores from each experimental period and group. The presence of inflammatory cells, fibers and abscess was evaluated, based on pre-established criteria (Tavares *et al*, 2013), in order to quantify them according to representative scores for comparison through statistical tests. The inflammatory cellular component was determined by the presence of neutrophils, lymphocytes, eosinophils, macrophages and giant cells, being classified according to the following scores: (1) Absent: absent or inside inflammation of vessels; (2) Mild: cells present sparse or in small groups; (3) Moderate: cells present but without dominating the microscopic field; (4) Intense: cells present as an infiltrate close to the material used. The fibrous condensation was classified according to the following scores: (1) Absence of collagen fibers; (2) Presence of thin layer of collagen fibers; (3) Presence of thick layer of collagen fibers. Abscess formation was classified according to the following scores: (1) Absence of abscess; (2) Presence of abscess in contact with the surgical cavity where the material will be inserted; (3) Presence of abscess areas distant from the surgical cavity where the material will be inserted.

Immunohistochemical Analysis

Histological sections of 4µm, in silanized slides, were desparafinized in a 60 degree stove for 12 hours, followed by xylol baths and alcohol hydration. The slides were immersed in 0.3% hydrogen peroxide solution in methanol to block the

endogenous peroxidase. Next, the sections were submitted to antigenic recovery in a water bath. Anti-collagen I antibody (abcam-ab88147, Cambridge, UK), BMP-4 (Novocastra, Newcastle, Northumberland, UK), anti-BSP (abcam- ab125227, Cambridge, UK) and anti-Osteopontin (abcam-ab63856, Cambridge, UK) at 1:50 dilution for 12 hours at 4°C. The secondary antibody and streptavidin are part of the LSAB kit (Dako) which was used, and the disclosure was made with the DAB kit (DAB, DakoCytomation, USA), which contains 0.03% of 3-31-diaminobenzidine, and the Blades were scored with Harris hematoxylin solution. All reactions were followed by positive controls according to the manufacturer's instructions. For the negative control, the primary antibodies were replaced with bovine serum albumin (BSA) 1% diluted in TRIS-HCL buffer, pH 7.4. Only brown coloration, regardless of the intensity of the coloring, was considered as positive.

The slides were evaluated and photographed under an Olympus BX 50 microscope at a magnification of x40 and x1000. A positive cytoplasmic labeling assay was performed. All proteins analyzed showed stain present in the connective tissue matrix and cells. Each sample was evaluated and a score representing the percentage of positivity were attributed: negative (-, none), slight (+, 1%-50%) and strong (++,51%-100%).

Statistical analysis

Cytotoxicity activity data were analysed statistically using ANOVA complemented by the Tukey’s test, using the SPSS software 15.0. Histological analyses were evaluated in each experimental time by One-way ANOVA-multiple comparison using Prisma version 6. The significance level was set at 5%.

RESULTS

Cytotoxicity analysis

Data of cell viability in the presence of the different cements are shown in Figure 2b. At 24 hours, all tested cements showed high cell viability and were similar

to control group (P>0.05).

Histopathological analysis

Representative images of the subcutaneous connective tissue response of each group can be observed in Figure 3, 4 and 5.

At 7 days, Control and MTA Flow groups showed similar results with slight inflammatory reaction characterized by hyperemia and mild lymphocytes inflammatory infiltrate (P>0.05). AH Plus and MTA Fillapex groups showed significant higher lymphocytes inflammatory infiltrate compared to control group (P<0.05; P<0.01, respectively). Absence or thin layer of collagen fibers was detected in all groups (Figure 3).

At 30 days, Control and MTA Flow groups showed similar results considered as absence of inflammatory reaction (P>0.05). AH Plus and MTA Fillapex groups presented significantly higher lymphocytes inflammatory infiltrate (P<0.01, P<0.05, respectively). These groups also exhibited sparse macrophages infiltrate but no significant difference was detected compared to control and MTA Flow. These results were considered mild inflammatory reaction for both groups. All groups presented similar thin collagen fibers around the tube (Figure 4).

At 60 days, all groups showed reduction of inflammatory reaction and fiber deposition (P>0.05) (Figure 5).

Acute inflammatory reaction (neutrophils inflammatory infiltrate), eosinophils and giant cells were not observed in all groups at 7, 30 and 60 days.

Immunohistochemical analysis

Results from immunohistocehmical analysis are presented in Figures 6, 7, 8 and 9.

Collagen I analysis in all groups after 7, 30 and 60 days revealed mild collagen formation except by MTA Flow in 60 days that showed stronger collagen labeling (Figure 6).

BMP-4 labeling showed that, in day 7, only MTA Flow group exhibited mild expression of this protein. At 30 and 60 days, Control Group showed mild labeling and AH Plus, MTA Fillapex e MTA Flow exhibited stronger immunolabeling of BMP-4 (Figure 7).

The analysis of BSP indicated that only MTA Flow group presented a strong reactivity to this protein since day 7 until day 60. MTA Fillapex exhibited mild immunolabeling at 60 days. Control and AH Plus do not exhibited this protein (Figure 8).

Osteopontin (OP) analysis showed a strong reactivity to this protein for MTA Flow since day 7 until day 60. AH Plus and MTA Fillapex showed a mild reactivity in all experimental days. Control Group do not exhibited this protein (Figure 9).

DISCUSION

The American Association of Endodontists recommends that the use of a new material should be based on biological and clinical studies (American Association of Endodontists 2013). Endodontic materials must have their biological properties investigated by *in vitro* tests before their clinical use to minimize adverse effects. Moreover, materials that will be in permanent contact with the periapical tissues should exhibit low cytotoxicity and high biocompatibility and be able to induce a mineralized barrier (Cintra et al. 2017). Therefore, we decided to investigate the cytotoxicity, biocompatibility and osteoinductive properties of MTA Flow, a new MTA-hydrogel-based material recently launched in the market. Our results showed that MTA Flow proved to be non-cytotoxic, and biocompatible when in contact with rats' subcutaneous tissue. In comparison to MTA Fillapex and AH Plus, the new MTA cement presented better histological behavior and higher expression of bone matrix proteins expression.

MTA is an endodontic material that was first used as a root-end filing material and gained popularity over the last years because of numerous *in vitro* and *in vivo* studies that confirmed its good properties such sealing ability, biocompatibility, bioactivity and antibacterial activity (Parirokh & Torabinejad 2010 (a), Parirokh & Torabinejad 2010 (b), Torabinejad *et al*. 1993, Torabinejad & Watson 1993, Guzeler *et al*. 2010, Kim & Shin 2013). Due to some drawbacks of this cement as long setting time, difficult handling and possibility of tooth discoloration (Torabinejad *et al*. 1995, Camilleri 2014, Marciano *et al*. 2014), new cements formulations, containing MTA, have been proposed. MTA Flow is a bioactive material composed by a powder with different small particle size mineral components (less than 10 microns) and by a hydrogel. According to manufactory MTA Flow's powder/gel combination gives the clinician a variety of mixing options needed for an effective, non-gritty, easy-to-deliver MTA. It's mixing ratio is adaptable to every procedure, allowing the clinician the ability to achieve any desired consistency, from thin, to thick, to putty. In the present study we prepared the MTA Flow in the putty consistency as indicated to root end filling.

A key step when developing new endodontic materials for clinical use is an evaluation of their possible cytotoxicity. Here, the cytotoxicity was analyzed by the response of hPDLSC to substances leached from the three endodontic cements. We decided to use periodontal ligament stem cells because they represent a well-established human multipotent cell population that initiate their proliferation and differentiation aiming at healing of injured periodontal tissue (Ivanovski *et al*. 2006). Nowadays, it has been proposed that stem cells have potential application to tissue regeneration or tissue engineering. In the present study we isolated and characterized hPDLSC. After that, cell viability assay using MTT in short-term response (24h) of the cells to conditioned medium were performed. This strategy has been used by some authors to simulate the periodontal or pulp tissue response in contact with different materials (Candeiro *et al*. 2015, Cavalcanti *et al*. 2005, Bruno *et al*. 2016). Conditioned media method is important to test the effect of substances leached or dissolved during material setting especially when the product is applied in connective tissue in a humid environment making the study more clinically relevant. Other important aspect is that we used a diluted conditioned media. Some authors have been using conditioned media diluted to 10% (Candeiro *et al*. 2015, Cavalcanti

et al. 2005). They considered this dilution as appropriate, since in pulp tissue, the cell number is higher than in a culture dish. Also, they affirm that blood and lymphatic vessels are present in living tissue, diluting the substances. Our results showed that MTA Flow presented similar behavior when compared with these two well-studied cements and all the cements tested were similar to control, being not cytotoxic for periodontal stem cells. Some studies have shown that MTA Fillapex and AH Plus sealers can be cytotoxic for different types of cells (Eldeniz *et al*. 2007, Loushine *et al.* 2014, Silva *et al*. 2013, Camargo *et al*. 2014, Yoshino *et al*. 2013, da Silva *et al*. 2016, Collado-Gonzalez *et al*. 2017). It occurs especially with higher concentration of conditioned media. However, other studies investigated AH Plus and MTA Fillapex cytotoxicity and their findings corroborate with the present study, demonstrating a non-cytotoxic potential (Kangarloo 2008, Kim & Shin 2013, Cotti et al. 2014, Parirokh et al. 2015, Saygili et al. 2017, Teixeira et al. 2017, Cintra *et al*. 2017). Moreover, some authors affirm that cell response in cell culture models depends on many factors such as the cell types, time of experiments, use of a fresh or cured material, the use of direct contact or extract of MTA and the concentration of the conditioned media (Torabinejad & Parirokh 2010, Bin *et al*. 2012).

Histopathologic evaluation of subcutaneous tissue has been frequently used to compare the inflammatory response of MTA and other materials. In the present study MTA Flow showed scarce inflammatory reaction similar to histological aspects of control group in all days analyzed. Meanwhile, AH Plus and MTA Fillapex exhibited more chronic inflammatory infiltrate after 7 and 30 days. No abscess was detected in any group than, all material could be considered biocompatible however; MTA Flow presented less inflammatory reaction. Both AH Plus and MTA Fillapex are resin–based sealer with the difference that the latter have around 16% of MTA in it composition. Previous literature also showed that these materials have a potential for tissue irritation (Sousa *et al*. 2006, Kangarloo *et al*. 2008, Tavares *et al.* 2013, Kim & Shin 2014, Assman *et al*. 2015, Baldasso *et al.* 2016, Victoria-Escandell *et al.* 2017, Saygili *et al*. 2017, Teixeira *et al*. 2017). Authors support that resin component, especially epoxy, is responsible by tissue and cells injure caused by formaldehyde release from amines added to accelerate the epoxy polymerization (Eldeniz *et al*. 2007, Zhou *et al*. 2016), as well as the presence of bisphenol-A monomers (Soto & Sonnenschein 2010). The favorable biologic activity of MTA Flow could be explained

by absence of resin constituent associated with the fact that it is composed by small bioactive particles (less than 10 microns) that set with hydrogel acquiring smooth and easy dispensation. Also, the gel formulation makes the product more washout-resistant with less particle dispersion in contact with tissue.

Bone extracellular matrix is composed by 90% collagen proteins (97% collagen type I and 3% collagen type V) and 10% non-collagenous proteins (20% osteocalcin, 20% osteonectin, 12% of sialoprotein, 10% of proteoglycans, osteopontin, fibronestin, growth factors, BMPs and others), all of which are synthesized by osteoblasts (Young 2003, Anselme 2000, Katagiri & Takahashi 2002). To investigate a possible osteoinductive role of the endodontic materials, we performed immunohistochemical analyzes of bone matrix proteins such as collagen type I, osteopontin, BMP-4 and BSP. Interestingly, our results showed a higher expression of these proteins in MTA Flow group compared to the others since the 7 days after tube insertion. This was the first time that bone matrix proteins were studied during the healing in tissues associated to different endodontics material. Our results revealed that, beside it biocompatible properties, MTA Flow presented a capacity of induce bone matrix protein. MTA's biological integration is explained by the fact that calcium hydroxide releases calcium in contact with phosphate ions present in body (Cheng *et al*. 2010). Also, calcium ions are essential for the cell attachment, migration, differentiation, and proliferation of hard tissue-producing cells (Tanomaru-Filho *et al*. 2017). Research data regarding tissue reactions to MTA have demonstrated induction of hard tissue formation, bone formation, induction of connective tissue formation, among others (Torabinejad *et al*. 2017). In addition, the high pH activates and stimulates the expression of the enzyme alkaline phosphatase, supporting the formation and deposition of mineralized tissue and then allowing the formation of a fibrous capsule, with lower inflammatory reaction (Bin *et al*. 2012, Hauman & Love 2003, Ford *et al.* 1995).

Of all the bone matrix proteins analyzed, BSP, showed the most remarkably results. It is a multifunctional extracellular non-collagenous protein that has direct and indirect effects on cell activities and mineralization. The analysis of BSP indicated that only MTA Flow group presented a strong reactivity to this protein since day 7 until day 60. MTA Fillapex exhibited mild immunolabeling at 60 days. Control and AH Plus do not exhibited this protein. BSP and osteopontin shares a number of physical

and chemical properties (Chen *et al*. 1992). Osteopontin is another bone extracellular matrix glycoprotein that plays an important role in bone remodeling (Saito et al. 2016). Here, we observed that MTA Flow induced this marker since day 7 until day 60. AH Plus and MTA Fillapex showed a mild reactivity in all experimental days. Control group do not exhibited this protein. In summary, the increased concentration of the expression of these proteins in the MTA Flow may lead us to believe that this material has a greater osteoinductive potential compared to the other materials tested.

All groups, in different moments, presented some positivity for BMP-4. Despite this protein be important for bone and cartilage metabolism it has been associated with multiples effects in organisms. For example, it could be associated to inflammatory cells chemotaxis (Helbing *et al.* 2017). In the present study, the presence of this marker can be associated to healing process more than osteoinductive properties.

Based in the finding of the present study we can concluded that MTA Flow is not cytotoxic and presented excellent biocompatibility with less inflammatory reaction in subcutaneous tissue in rats compared to AH Plus and MTA Fillapex. Also, MTA Flow reflect a current requirement to have materials for endodontic therapy that are able to stimulate the healing process of periapical tissues by mineralized issue stimulation, instead of merely biocompatible or inert materials.

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Figure 2. Cytotoxicity assay. (A) hPDLSCs morphology. (B) Graph showing cell viability.

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Figure 4. Histological aspects of experimental groups on day 30 (Hematoxylin-eosin, x40 and x1000).

Figure 5. Photomicrographs of experimental groups on day 60 (Hematoxylin-eosin, x40 and x1000).

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Figure 8. Immunohistochemical analysis of BSP in the Control, MTA-fillapex, AH Plus and MTA Flow groups at 7, 30 and 60 days (LSAB, x1000).

Figure 9. Immunohistochemical analysis of osteopontin in the Control, MTA-fillapex, AH Plus and MTA Flow groups at 7, 30 and 60 days (LSAB, x1000).

Table 1. Cements tested and their Composition.

Commercial Name	Material Composition	Manufacturer
AH Plus	AH Plus Epoxy paste: diepoxy, calcium tungstate, zirconium oxide, aerosol, and dye Amine paste: 1-adamantane amine, N.N'dibenzyl-5 oxanonandiamine-1, 9, TCD-diamine, calcium tungstate, zirconium oxide, aerosol, and silicon oil	(Dentsply, Konstanz,Germany)
MTAFillapex	Salicylate resin, diluting resin, natural resin, bismuth oxide, nanoparticulated silica, MTA, pigments	Angelus (Londrina, PR, Brazil)
MTAFlow	*Powder Component* Tricalcium silicate, Dicalcium silicate, Tricalcium Aluminate, Bismuth Oxide, Tungsten, Calcium sulfate, Calcium hydroxide/ calcium carbonate, Calciumaluminoferrite, Silica *Gel Component* Water Polyvinyl alcohol Polyvinyl pyrrolidone	Ultradent (South Jordan, UT, USA)

Figure 1

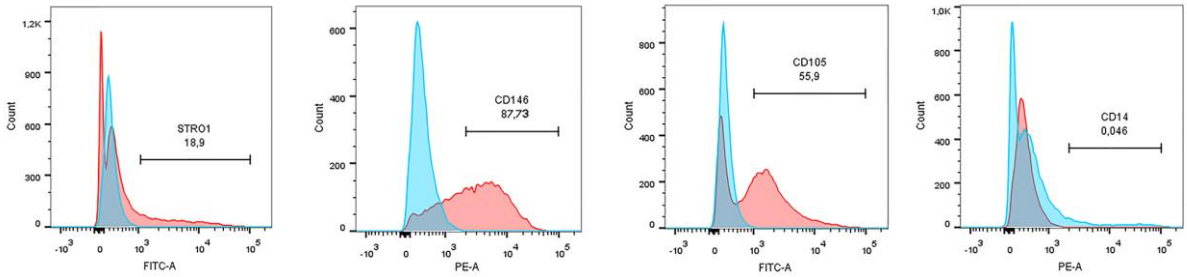
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Figure 2

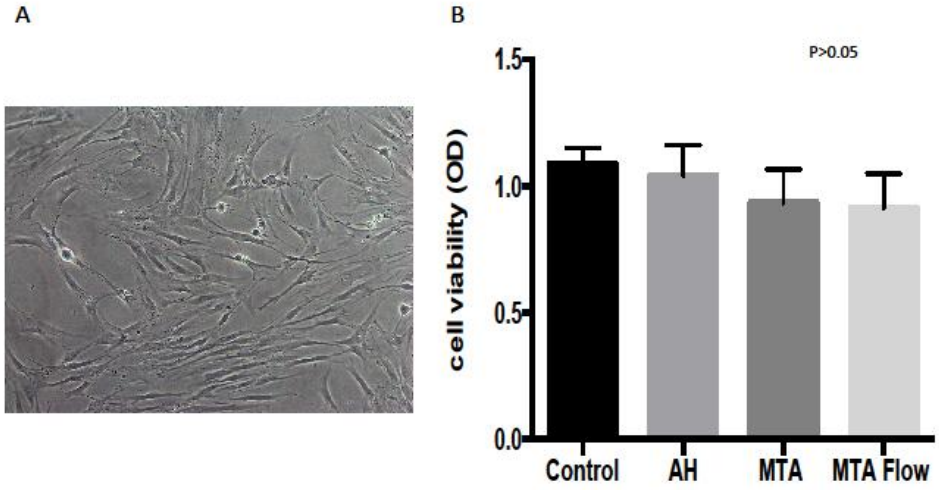
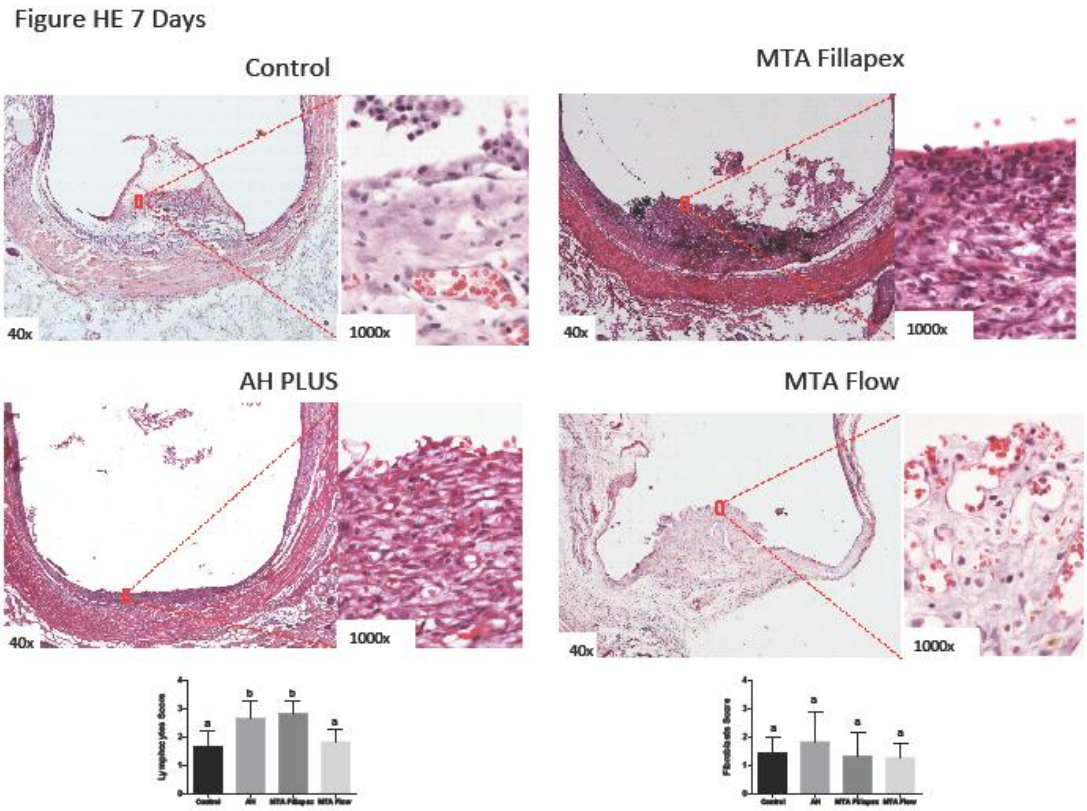


Figure 3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Control
MTA Fillapex
AH PLUS
MTA Flow
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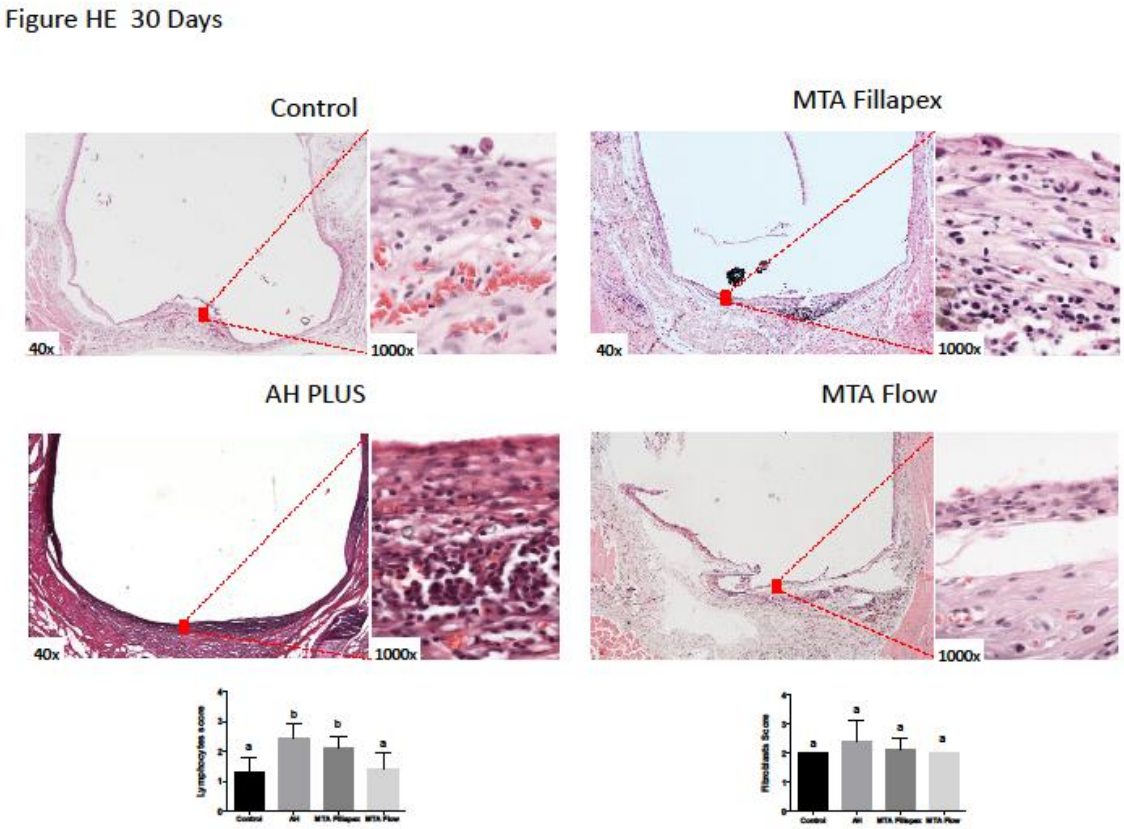
1000x
40x
1000x
40x
1000x
40x
1000x

Figure 4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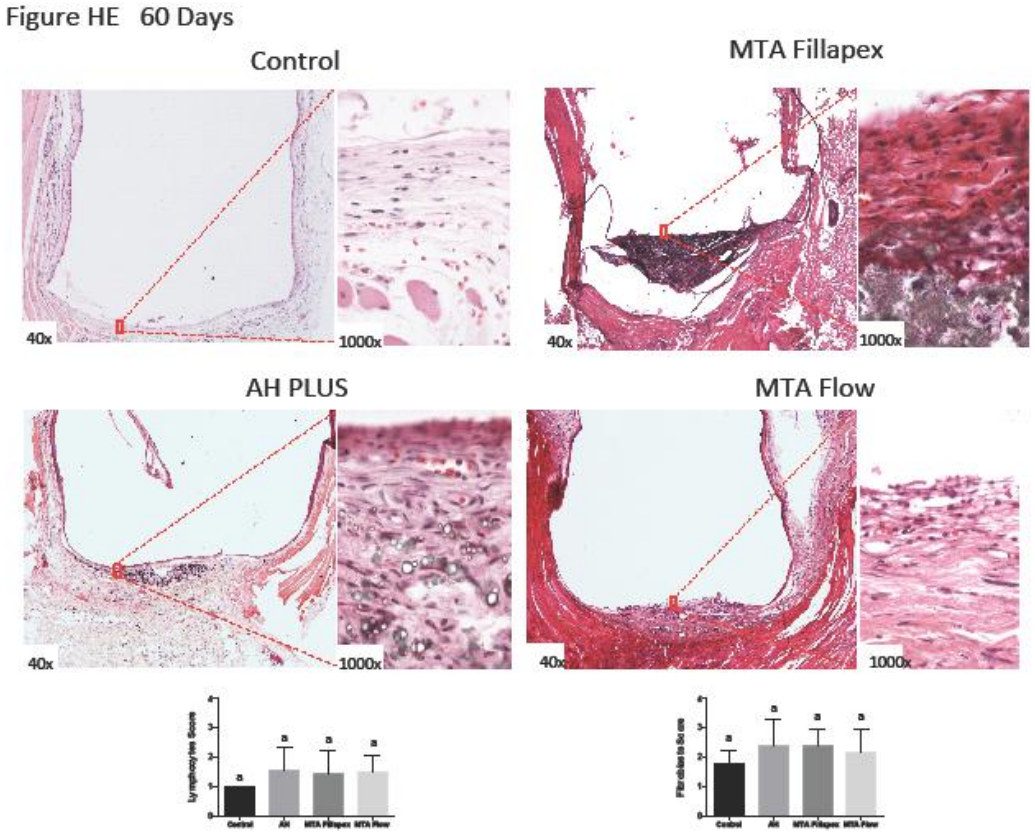
Control
MTA Fillapex
AH PLUS
MTA Flow
40x
1000x
40x
1000x
40x
1000x
1000x

Figura 5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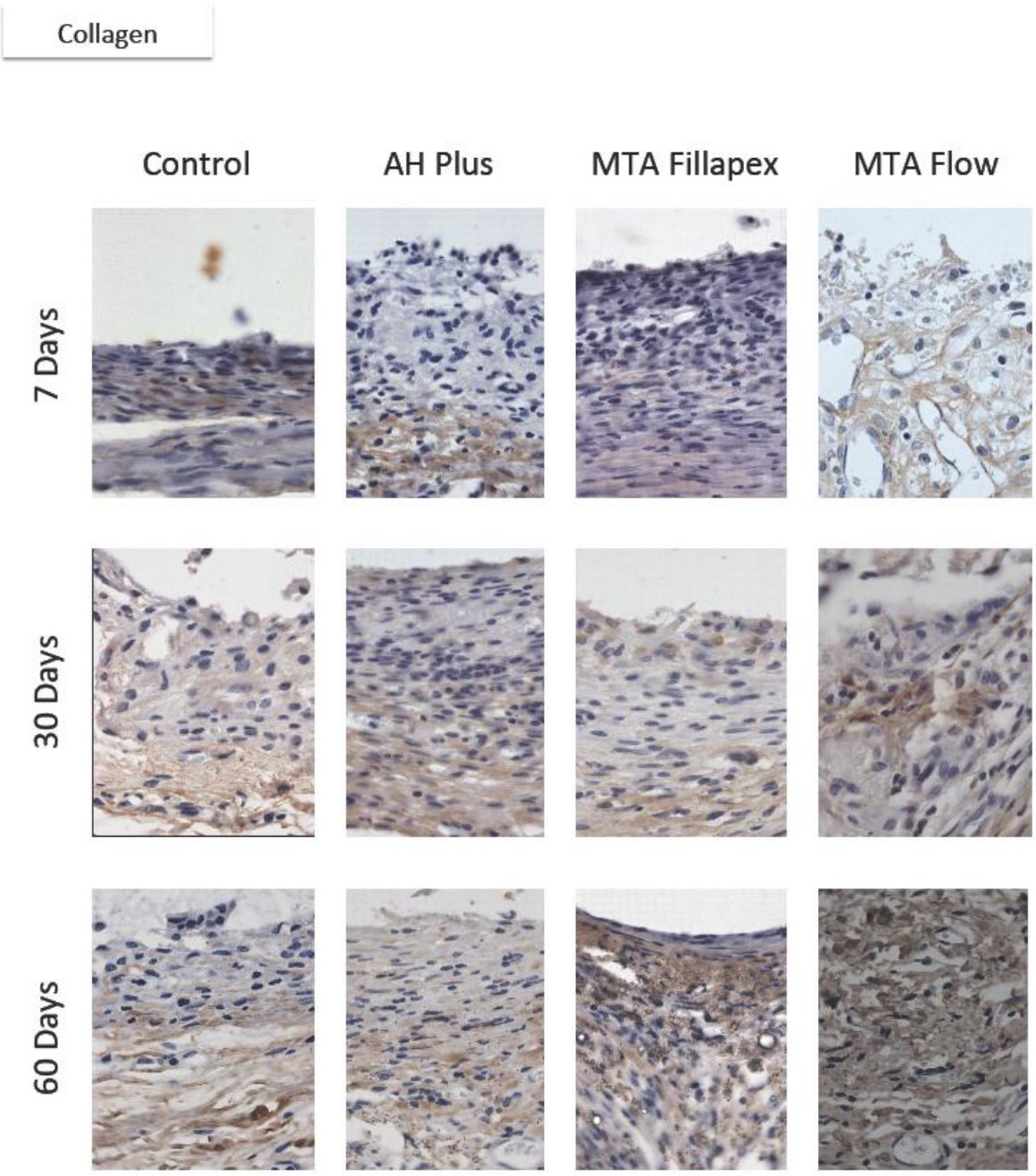
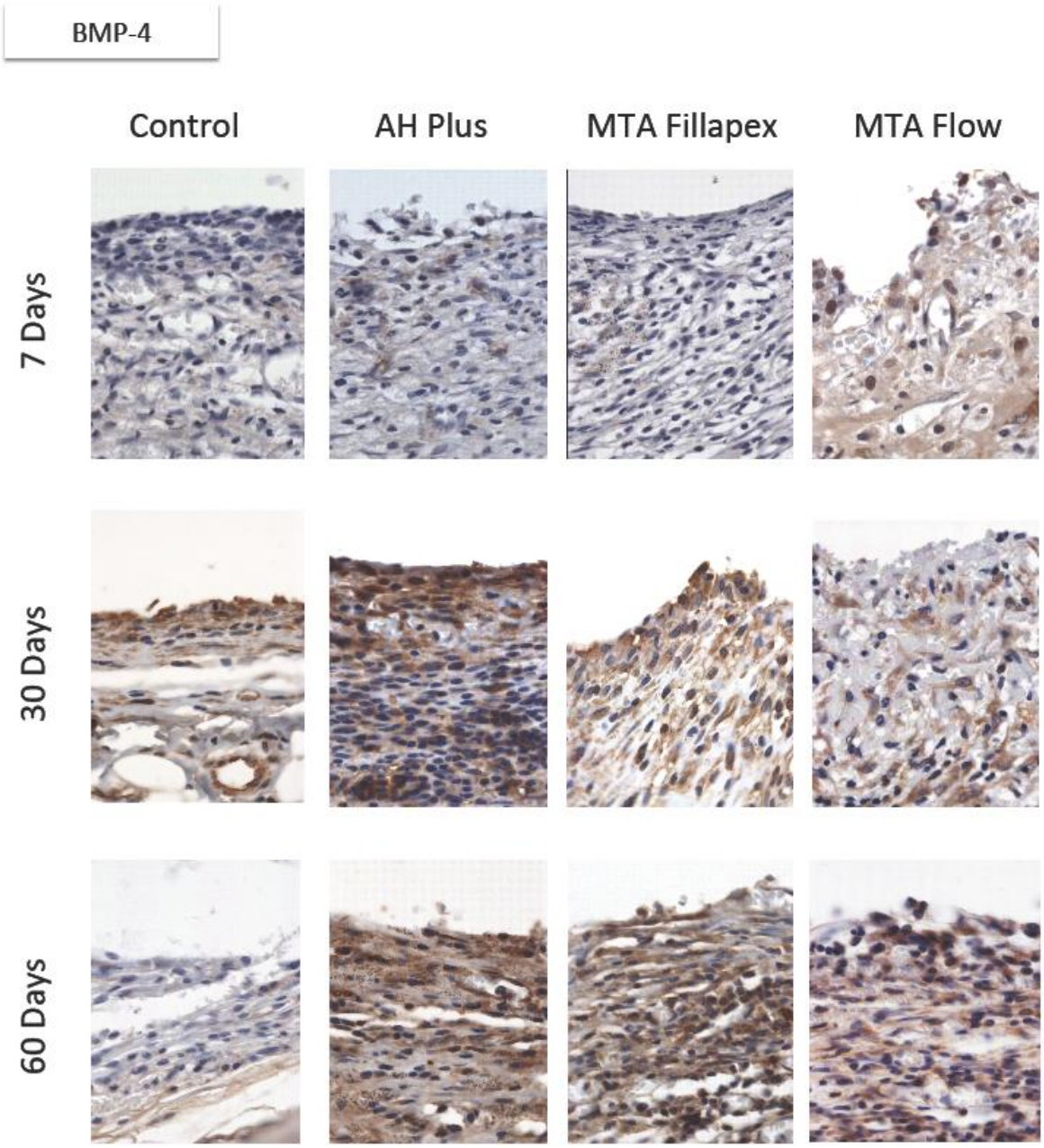
Control
MTA Fillapex
AH PLUS
MTA Flow
40x
1000x

Figure 6



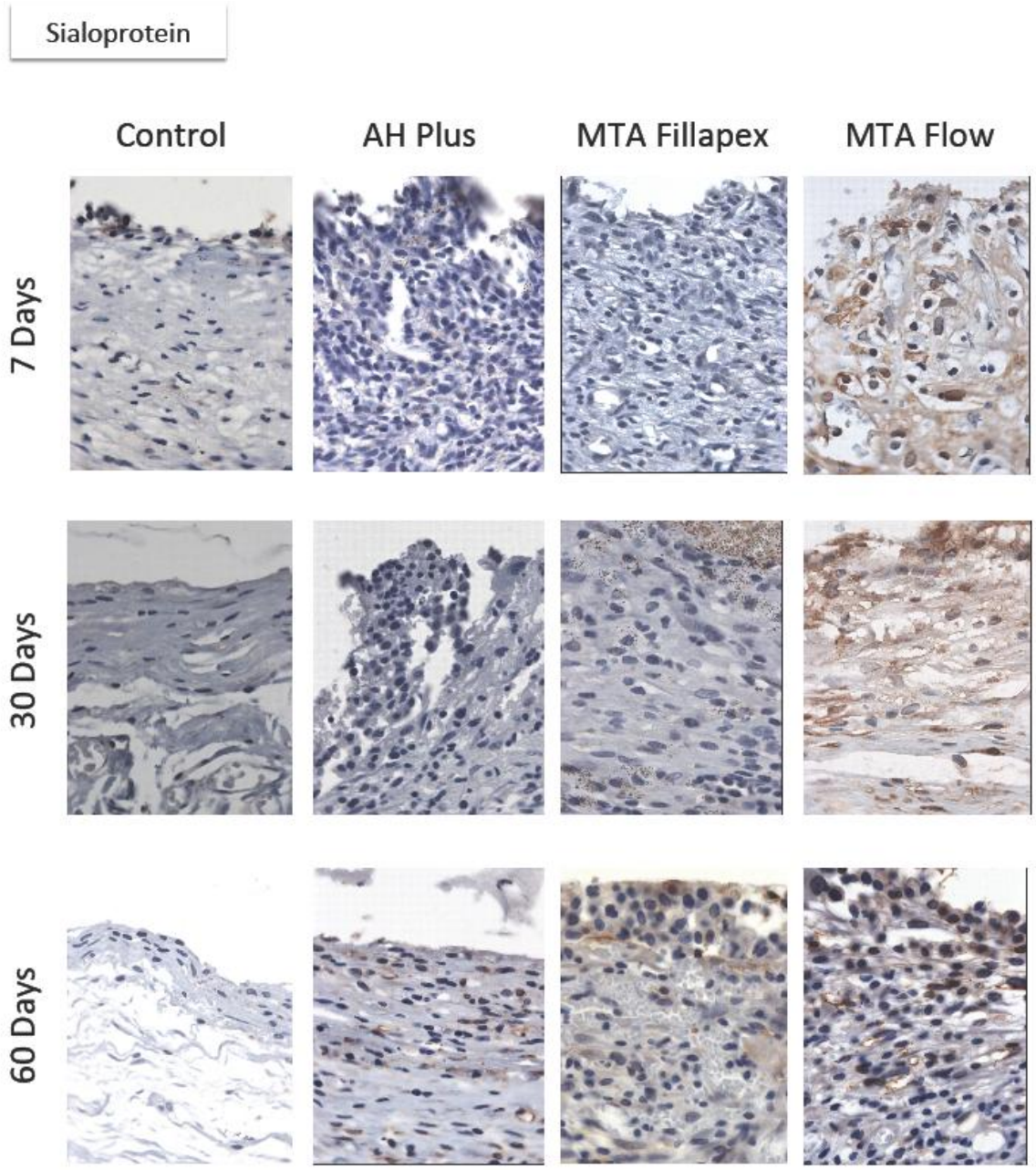
	Control	AH Plus	MTA Fillapex	MTA Flow
7 D	+	+	+	+
30 D	+	+	+	+
60 D	+	+	+	++

Figure 7



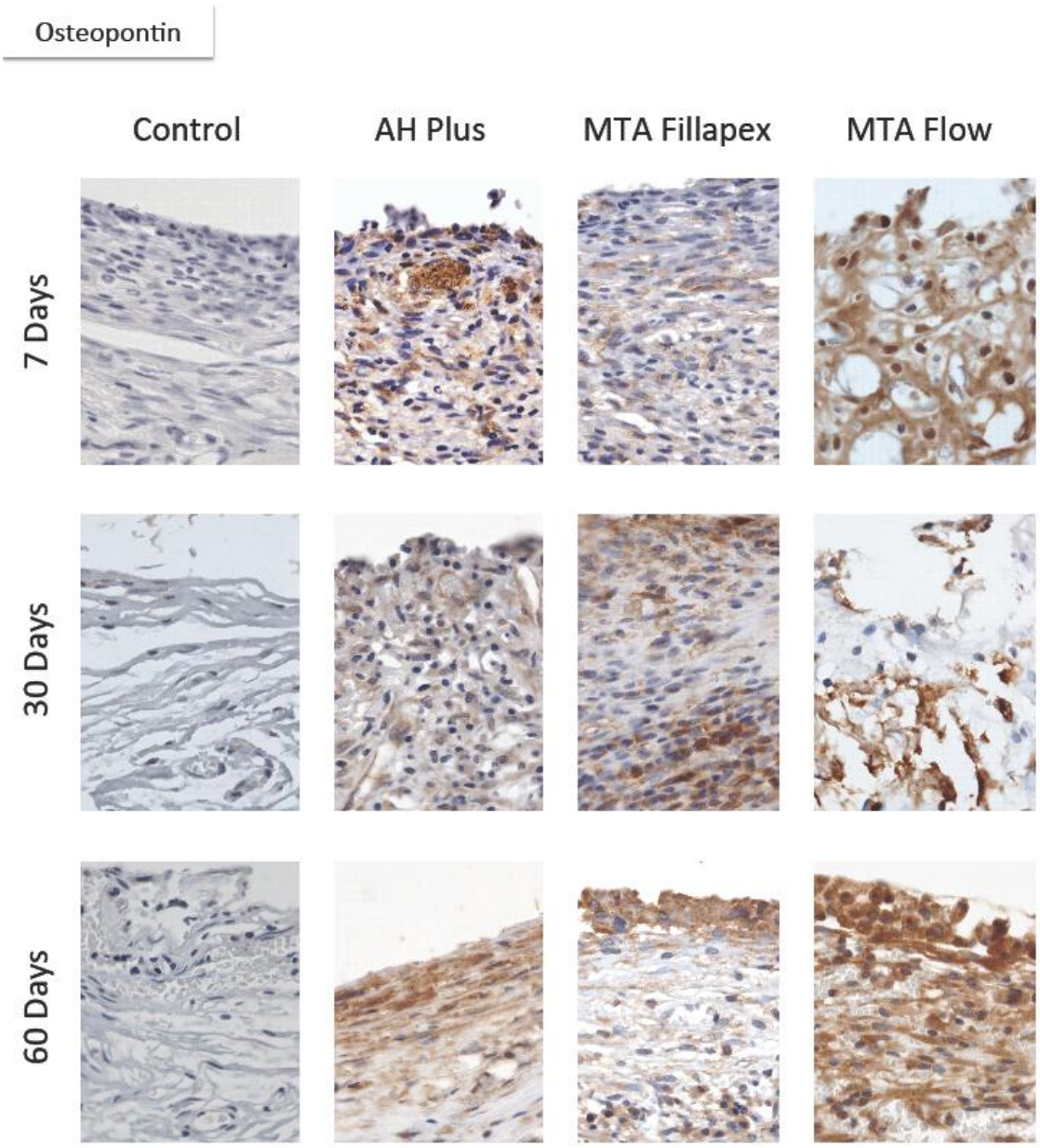
	Control	AH Plus	MTA Fillapex	MTA Flow
7 D	-	-	-	+
30 D	+	++	++	++
60 D	-	++	++	++

Figure 8



	Control	AH Plus	MTA Fillapex	MTA Flow
7 D	-	-	-	++
30 D	-	-	-	++
60 D	-	-	+	++

Figure 9



	Control	AH Plus	MTA Fillapex	MTA Flow
7 D	-	+	+	++
30 D	-	+	+	++
60 D	-	+	+	++

4 CONSIDERAÇÕES FINAIS

Considerando os resultados deste estudo, a partir da análise dos testes realizados, pode-se concluir que o novo cimento (MTA Flow) não é citotoxico e apresenta excelente biocompatibilidade e um perfil indutor de tecido mineralizado.

A análise da citotoxicidade mostrou que, na concentração testada, o MTA Flow manteve a viabilidade celular quando testado em células-tronco do ligamento periodontal sem diferença estatisticamente significativa com os outros grupos (controle, AH Plus e MTA Fillapex).

O ensaio de biocompatibilidade em tecido conjuntivo de ratos demonstrou que nos tempos experimentais de 7 e 30 dias o MTA Flow apresentou comportamento semelhante ao grupo controle, enquanto os outros grupos experimentais apresentaram maior resposta inflamação. Aos 60 dias, os quatro grupos experimentais apresentavam respostas teciduais semelhantes.

A análise imunoistoquímica mostrou uma maior marcação das proteínas Colágeno I, BMP-4, BSP e osteopontina para o grupo MTA Flow ao longo dos tempos experimentais, o que pode nos levar a acreditar que esse material estimula a formação de tecido mineralizado.

Novos materiais odontológicos estão sendo constantemente desenvolvidos para melhorar a qualidade dos tratamentos oferecidos à população. Cimentos que não são citotóxicos, que apresentam adequada compatibilidade biológica e demostram características osteoindutorasrepresentam um avanço importante na Endodontia. O MTA Flow mostrou ser um material promissor nesta área, sendo que estudos complementares a estedevem ser desenvolvidos, consolidando as informações aqui apresentadas.

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ANEXO A – PARECER DA COMPESQ

PARECER DA COMISSÃO DE PESQUISA

* Parecer aprovado em reunião do dia 24 de outubro de 2016 *

(idêntico ao parecer disponível no Portal Pesquisa)

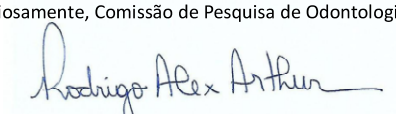
Prezada Pesquisadora Patrícia Maria Poli Kopper Mora,

A Comissão de Pesquisa da Faculdade de Odontologia da Universidade Federal do Rio Grande do Sul após análise do projeto 32008 - RESPOSTA TECIDUAL EM TECIDO CONJUNTIVO DE RATOS FRENTE AO IMPLANTE DE UM NOVO CIMENTO ENDODÔNTICO À BASE DE AGREGADO TRIÓXIDO MINERAL emitiu o seguinte parecer:

O presente estudo tem como objetivo avaliar a reação do tecido conjuntivo de ratos e o potencial de indução de mineralização de um novo cimento endodôntico a base de MTA (Ultradent) e comparar com outros cimentos preconizados na odontologia. Serão utilizados 24 ratos machos (Rattus norvegicus albinus, Rodentia, Mammalia da linhagem Wistar), pesando entre 300 e 350g. No dorso desses animais serão implantados tubos de polietileno (10 mm de comprimento e 1,3 mm de diâmetro) preenchidos com MTA Fillapex, AH plus, MTA (Ultradent) e tubos sem preenchimento (controle), sendo 1 tubo de cada grupo experimental por animal, totalizando 24 animais. Após 7, 30 e 60 dias serão mortos 8 animais com anestésico inalatório. O tubo e o tecido subcutâneo adjacente serão removidos e fixados em solução de formalina a 10%. O material será analisado morfologicamente quanto ao processo inflamatório, condensação fibrosa e presença de abscesso. Posteriormente, o material será submetido a análise imunoistoquímica para identificação de colágeno I, osteonectina, osteopontina, sialoproteina óssea e proteína morfogentica óssea-4 (BMP-4). Será realizada a análise semi-quantitativa por escore para cada marcador. As associações entre as diferentes variáveis serão estudadas pelo teste de Pearson. A comparação das diferenças entre os escores em grupo e período experimental tanto na análise semiquantitativa como na quantitativa será realizada utilizando o teste de Kruskal-Wallis. Todas as análises serão feitas no programa SAS for Windows, v.9.1.3 e no programa GraphPadPrism, v.4.0. O projeto encontra-se aprovado pelo CEUA do HCPA.

O projeto está bem delineado e apresenta mérito. O parecer dessa comissão é favorável para aprovação.

Atenciosamente, Comissão de Pesquisa de Odontologia



Prof. Dr. Rodrigo Alex Arthur

Coordenador da Comissão de Pesquisa ODONTOLOGIA UFRGS

ANEXO B – PARECER DO CEUA/HCPA

HCPA - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE
GRUPO DE PESQUISA E PÓS-GRADUAÇÃO

COMISSÃO DE ÉTICA NO USO DE ANIMAIS

A Comissão de Ética no Uso de Animais (CEUA/HCPA) analisou o projeto:

Projeto: 160146
Data da Versão do Projeto: 08/06/2016 **Versão do Projeto:** 08/06/2016

Pesquisadores:
MANOELA DOMINGUES MARTINS
ALEXANDRA MELLO
RENATO MIOTTO PALO
MARCIA MARTINS MARQUES
PATRÍCIA MARIA POLI KOPPER MORA

Título: RESPOSTA TECIDUAL EM TECIDO CONJUNTIVO DE RATOS FRENTE AO IMPLANTE DE UM NOVO CIMENTO ENDODÔNTICO À BASE DE AGREGADO TRIÓXIDO MINERAL

Este projeto foi APROVADO em seus aspectos éticos e metodológicos de acordo com as Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08/10/2008, que estabelece procedimentos para o uso científico de animais.

- Os membros da CEUA/HCPA não participaram do processo de avaliação de projetos onde constam como pesquisadores.
- Toda e qualquer alteração do Projeto deverá ser comunicada à CEUA/HCPA.
- O pesquisador deverá apresentar relatórios semestrais de acompanhamento e relatório final ao CEUA/HCPA.

Porto Alegre, 14 de junho de 2016.

Biol. Michael Everton Andrades
Coordenador CEUA/HCPA