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O MTA FORNECE UMA RESPOSTA HISTOLÓGICA MAIS FAVORÁVEL QUANDO COMPARADO A OUTROS MATERIAIS NO SELAMENTO DE PERFURAÇÃO DE FURCA? UMA REVISÃO SISTEMÁTICA DE ESTUDOS PRÉ-CLÍNICOS *IN VIVO*.

Lucas Siqueira Pinheiro

Porto Alegre
2021

LUCAS SIQUEIRA PINHEIRO

O MTA FORNECE UMA RESPOSTA HISTOLÓGICA MAIS FAVORÁVEL QUANDO COMPARADO A OUTROS MATERIAIS NO SELAMENTO DE PERFURAÇÃO DE FURCA? UMA REVISÃO SISTEMÁTICA DE ESTUDOS PRÉ-CLÍNICOS *IN VIVO*.

Tese apresentada ao Programa de Pós-Graduação em Odontologia da Universidade Federal do Rio Grande do Sul, como requisito final para a obtenção do título de Doutor em Odontologia, área de concentração Clínica Odontológica, ênfase em Endodontia.

Orientador (a): Profª. Drª. Fabiana Soares Grecca

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Todo Cambia

"Cambia lo superficial
Cambia también lo profundo
Cambia el modo de pensar
Cambia todo en este mundo

Cambia el clima con los años
Cambia el pastor su rebaño
Y así como todo cambia
Que yo cambie no es extraño

Cambia el más fino brillante
De mano en mano su brillo
Cambia el nido el pajarillo
Cambia el sentir un amante

Cambia el rumbo el caminante
Aunque esto le cause daño
Y así como todo cambia
Que yo cambie, no extraño
...
Cambia, el sol en su carrera
Cuando la noche subsiste
Cambia la planta y se viste
De verde en la primavera

Cambia el pelaje la fiera
Cambia el cabello el anciano
Y así como todo cambia
Que yo cambie, no es extraño
...
Y lo que cambió ayer
Tendrá que cambiar mañana
Así como cambio yo
Cambia, todo cambia"

Música escrita por Julio Numhause e interpretada por Mercedes Sosa (1984)

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PINHEIRO, L. S. **O MTA fornece uma resposta histológica mais favorável quando comparado a outros materiais no selamento de perfuração de furca? Uma revisão sistemática de estudos pré-clínicos *in vivo*.** 2021. 65f. Tese/Doutorado – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2021.

RESUMO

Introdução: Diferentes materiais seladores de perfuração radicular têm sido avaliados ao longo dos anos. Não existe um consenso sobre qual deles induz uma resposta histológica mais favorável. **Objetivos:** Essa revisão sistemática de estudos pré-clínicos *in vivo* fornece dados sobre os estudos disponíveis que comparam materiais reparadores e avalia se o Agregado Trióxido Mineral (MTA) fornece uma resposta histológica mais favorável que outros materiais quando utilizado no selamento de perfurações de furca. **Metodologia:** Essa revisão foi reportada de acordo com o checklist PRISMA. Os estudos *in vivo* incluídos testaram o uso de materiais no selamento de perfurações de furca e compararam ao MTA. Estudos que não estavam disponíveis para leitura foram excluídos. A busca eletrônica foi conduzida no EMBASE, PubMed, Scopus e Web of Science até dia 2 de setembro de 2020, sem restrição de idioma ou data de publicação. As ferramentas ARRIVE e SYRCLE foram utilizadas para avaliar a qualidade metodológica e risco de viés dos estudos. **Resultados:** Os vinte estudos incluídos na síntese qualitativa foram classificados com baixa qualidade e alta heterogeneidade metodológica, com alto risco de viés. MTA e Biodentine foram os materiais mais frequentemente avaliados, e a resposta histológica induzida foi adequada. **Discussão:** O nível de evidência da pesquisa pré-clínica é baixo devido às limitações inerentes a esse tipo de design de estudo. Além disso, a heterogeneidade, qualidade e risco de viés dos estudos incluídos indicam que a evidência de estudos que avaliam a resposta histológica induzida por materiais reparadores de perfuração de furca necessita melhoria. Portanto, o conhecimento gerado por essa revisão sistemática deve ser translacionado para a prática clínica com cautela. Essa revisão mostra que mudanças importantes devem ser realizadas na forma que os estudos pré-clínicos são conduzidos na área de materiais reparadores endodônticos. **Conclusões:** Guias para estudos em animais na endodontia devem ser utilizados. Apesar das limitações, os achados indicam que o MTA obteve o melhor comportamento biológico. O Biodentine obteve resultados adequados. Estudos clínicos

devem ser conduzidos para definir qual desses dois materiais devem ser a referência na prática clínica. **Financimento:** Bolsa de pesquisa concedida pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). **Registro:** PROSPERO (CRD42020181297).

Palavras-chave: Endodontia; Perfuração de furca; Resposta histológica; Estudos *in vivo;* Revisão sistemática.

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ABSTRACT

Background: A wide variety of root perforation sealing materials has been tested over the years, but no consensus has been reached about which yield a more favorable histological response. This Systematic review of preclinical studies provides an overview of the available studies comparing repair materials. **Objectives:** To evaluate whether Mineral Trioxide Aggregate (MTA) yields a more favorable histological response than other materials when used to seal furcal perforations. **Methods:** This review is reported according to PRISMA checklist. The *in vivo* studies included tested the use of materials used to seal furcal perforations, compared groups, one of which for MTA, and evaluated histological response. Studies whose full text was unavailable were excluded. An electronic search was conducted in EMBASE, PubMed, Scopus, and Web of Science up to September 2, 2020, with no language or publication date restrictions. The ARRIVE and SYRCLE tools were used to assess the methodological quality and the risk of bias of the studies. **Results:** The twenty studies included in the qualitative synthesis were classified as having a low methodological quality and a high risk of bias. They also had a high methodological heterogeneity. MTA and Biodentine were the materials most often compared, and their histological response was adequate. **Discussion:** Preclinical research is low in the scale of evidence because of the inherent limitations of this type of study design. Moreover, the heterogeneity, quality, and RoB of the studies included indicated that the evidence on furcal histological response to repair materials needs improvement. Therefore, the knowledge generated by this systematic review should be translated to clinical practice cautiously. This review shows that important changes have to be made to the way preclinical studies are conducted in the area of endodontic repair materials. **Conclusions:** Guidelines for animal studies in endodontics should be used. Despite study limitations, findings indicated that MTA had the most predictable biological behavior. Biodentine had positive results. Clinical trials should be conducted to define which of these two materials should be the reference standard for clinical

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Keywords: Endodontics; Furcal perforation; Histological response; In vivo studies; Systematic review.

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1 INTRODUÇÃO

A perfuração de furca pode ser definida como uma comunicação patológica ou mecânica entre o sistema de canais radiculares e a região externa do dente na zona de furca de dentes multirradiculares (AMERICAN ASSOCIATION OF ENDODONTICS, 2020; CARDOSO et al., 2018). As principais causas da perfuração de furca são reabsorções dentárias, lesões cariosas ou iatrogênicas, podendo também ocorrer durante procedimentos endodônticos ou restauradores (SELTZER; SINAI; AUGUST, 1969; SIEW; LEE; CHEUNG, 2015; VEHKALAHTI; SWANLJUNG, 2020). Vehkalati e colaboradores (2020) relatam que perfurações radiculares compreendem aproximadamente um terço das graves lesões que ocorrem durante o tratamento endodôntico.

A reação inflamatória induzida pela perfuração radicular tem sido estudada ao longo dos anos (AL-DAAFAS; AL-NAZHAN, 2007; ALADIMI et al., 2020; DA FONSECA et al., 2019; FORD et al., 1995; SELTZER; SINAI; AUGUST, 1969; SILVA et al., 2017). Quando estabelecida, a perfuração induz resposta inflamatória que afeta o tecido periodontal adjacente e, por consequência, ocorre perda óssea (SELTZER; SINAI; AUGUST, 1969). Dependendo do grau de severidade, a reação inflamatória pode resultar em proliferação epitelial e formação de tecido de granulação (SELTZER; SINAI; AUGUST, 1969). Além disso, tem sido demonstrado que o curso clínico da lesão é afetado pela contaminação da região exposta, pelo tamanho e localização da perfuração e pelo material utilizado para reparação (ASKERBEYLI ÖRS S et al., 2019; BEAVERS; HILL; CAROLINA, 1982; HOLLAND et al., 2007; SINAI, 1977).

O adequado tratamento da perfuração radicular é fator decisivo para o prognóstico do dente acometido (ESTRELA et al., 2018; MENTE et al., 2014; NG; MANN; GULABIVALA, 2011; VEHKALAHTI; SWANLJUNG, 2020). O tratamento não cirúrgico da perfuração radicular possui taxas de sucesso de aproximadamente 70% (SIEW; LEE; CHEUNG, 2015), diante disso, essa modalidade de intervenção deve ser considerada como primeira opção**.** O tratamento não cirúrgico consiste na inserção de um material reparador para selamento da cavidade formada pela perfuração e indução do reparo dos tecidos adjacentes (FORD et al., 1995; SIEW; LEE; CHEUNG, 2015; YILDIRIM et al., 2005). Para isso, um material reparador ideal deve possuir características físico-químicas

e biológicas adequadas (FORD et al., 1995; HOLLAND et al., 2007; RATHINAM et al., 2016).

Devido a problemas éticos, não existe nenhum ensaio clínico randomizado avaliando perfurações radiculares, somente alguns estudos observacionais (DE CHEVIGNY et al., 2008; FARZANEH; ABITBOL; FRIEDMAN, 2004; GORNI et al., 2016; GORNI; GAGLIANI, 2004; MENTE et al., 2010; NG; MANN; GULABIVALA, 2011). Dessa forma, estudos pré-clínicos em animais que simulam as condições clínicas são mundialmente utilizados para avaliar biocompatibilidade e bioatividade de materiais seladores de perfurações radiculares (CARDOSO et al., 2018). Estudos pré-clínicos utilizam métodos estandardizados (HOOIJMANS; RITSKES-HOITINGA, 2013) que favorecem a comparação entre materiais, reduzindo o risco de viés desses estudos. Devido a isso, os modelos animais de perfuração de furca podem guiar o clínico na escolha do material adequado para tratar perfurações radiculares. Embora uma variedade de materiais tenha sido desenvolvida e comparada ao MTA ao longo dos anos em estudos *in vivo* de perfuração de furca, não existe um consenso na literatura sobre qual material induz uma resposta histológica mais favorável.

Revisões sistemáticas de estudos pré-clínicos em animais criam uma visão geral sobre o que tem sido publicado e podem sugerir a possibilidade de translação do conhecimento para estudos clínicos (HOOIJMANS; RITSKES-HOITINGA, 2013). Além disso, esse tipo de estudo pode ajudar o pesquisador a identificar possíveis variáveis para serem analisadas em futuras pesquisas clínicas e identificar áreas de fraqueza metodológica. Dessa forma, esse modelo de revisão pode gerar mais transparência na pesquisa pré-clínica (HOWELLS; SENA; MACLEOD, 2014; SENA et al., 2014).

Embora a revisão sistemática de estudos experimentais em animais seja uma importante modalidade de pesquisa, a consciência do mérito desse tipo de estudo é recente e aumentou a partir de 2002 com o artigo publicado por Peter Sandercock e Ian Roberts na revista Lancet. Os autores citam as revisões sistemáticas publicadas por Horn e colaboradores (2000 e 2001) para justificar a crucial importância das revisões sistemáticas de estudos pré-clínicos em animais.

No ano de 2000, Horn e Limburg publicaram uma revisão sistemática de estudos clínicos com o objetivo de avaliar a influência da nimodipina, uma droga bloqueadora dos canais de cálcio, nos desfechos da isquemia cerebral focal. Foram incluídos 6468

pacientes de 22 estudos clínicos. Os pesquisadores concluíram poder descartar de forma confiável um efeito clinicamente importante dessa droga na isquemia cerebral focal. Um ano depois, Horn e colaboradores realizaram uma outra revisão sistemática de estudos experimentais, mas agora em animais, para verificar se a evidência desse estudo suportava ou não o começo de estudos clínicos utilizando a nimodipina em humanos diagnosticados com isquemia cerebral focal. Foram incluídos 20 estudos. Os pesquisadores verificaram que não haviam evidências suficientes para fundamentar a decisão de iniciar os estudos clínicos. Além disso, os autores observaram que nas duas revisões sistemáticas, o mesmo resultado foi obtido. Essas pesquisas demonstram a importância e necessidade da revisão sistemática de estudos experimentais em animais.

Apesar das semelhanças metodológicas entre a revisão sistemática de estudos clínicos e a revisão de estudos pré-clínicos em animais, existem diferenças que devem ser ressaltadas. As etapas de avaliação crítica dos estudos primários incluídos devem ser realizadas de acordo com checklists específicos para o modelo animal. A avaliação de qualidade metodológica e de risco de viés é peça-chave para uma revisão sistemática de estudos experimentais em animais, pois a confiança dos resultados depende da validade dos dados e dos resultados dos estudos incluídos (CHALMERS et al., 1983; HOOIJMANS et al., 2014; KILKENNY et al., 2009; MACLEOD et al., 2009; SCHULZ et al., 1995).

Em 2009, Kilkenny e colaboradores avaliaram a qualidade do desenho experimental, análise estatística e de reporte de pesquisas em animais publicadas entre 1995 e 2005. Os autores observaram que apenas 59% dos 271 artigos descreveram a hipótese ou objetivo do estudo, número e características dos animais utilizados. Além disso, verificaram que a maioria dos artigos avaliados não reportavam randomização (87%) ou cegamento (86%). Estudos relatam que omissões de informações, somadas às limitações do modelo de animal utilizado, se tornam uma barreira para chegar a uma conclusão adequada sobre a eficácia de uma droga ou intervenção (KILKENNY et al., 2009; SENA et al., 2010; WORP et al., 2010). Diante disso, em 2010, Kilkenny e colaboradores publicaram um guia de reporte de estudos pré-clínicos em animais denominado ARRIVE (Animals in Research: Reporting *in vivo* Experiments) guidelines.

O ARRIVE guideline, desenvolvido baseado no CONSORT (Consolidated Standards of Reporting Trials), é um checklist que descreve a mínima informação que toda publicação científica utilizando animais deve incluir, descrevendo a randomização

e alocação dos animais, cegamento dos investigadores, cuidadores e avaliadores e demais itens considerados importantes no protocolo. Desde sua publicação, o ARRIVE tem sido utilizado por mais de mil periódicos (DU SERT et al., 2020a). Apesar disso, estudos recentes demonstram que informações importantes como randomização, cegamento, justificativa do tamanho amostral e características dos animais, ainda estão ausentes na maioria das publicações (AVEY et al., 2016; LEUNG et al., 2018; MACLEOD et al., 2015). Diante desses resultados, compreendeu-se a necessidade de realizar alterações na forma de apresentação e de modificar/incluir determinados itens nesse checklist. Neste sentido, em 2020, o ARRIVE 2.0 foi publicado (DU SERT et al., 2020a, 2020b).

Diante da crescente consciência da importância de revisões sistemáticas de estudos em animais, em 2014, o Systematic Review Centre for Laboratory animal Experimentation (SYRCLE) desenvolveu a ferramenta SYRCLE para avaliar o risco de viés destes estudos (HOOIJMANS et al., 2014). Essa ferramenta foi desenvolvida com base no checklist da Cochrane (HIGGINS et al., 2011) para avaliação de risco de viés de ensaios clínicos randomizados. Os principais objetivos da utilização do SYRCLE são aumentar a eficiência da translação do conhecimento da pesquisa animal para a prática clínica e conscientizar a comunidade científica sobre a necessidade de melhorar a qualidade metodológica dos estudos em animais.

Na Endodontia, as revisões sistemáticas de estudos pré-clínicos em animais são escassas e têm sido realizadas com diferentes objetivos, como avaliar o comportamento dos tecidos dentais e perirradiculares quando em contato com determinados materiais ou técnicas, identificar modelos de animais adequados para protocolos de pesquisa e compreender a patogênese da periodontite apical (AL-HEZAIMI et al., 2013; ALTAII; RICHARDS; ROSSI-FEDELE, 2017; BENETTI et al., 2018; CARDOSO et al., 2018; DA ROSA et al., 2017; FAWZY EL-SAYED et al., 2019; JAVED et al., 2017; KATSAMAKIS et al., 2013). Apesar dos diferentes objetivos e protocolos dos estudos primários, a maioria das revisões sistemáticas têm alertado para a alta heterogeneidade metodológica, baixa qualidade de reporte e risco de viés nos estudos incluídos (DA ROSA et al., 2017; FAWZY EL-SAYED et al., 2019; KATSAMAKIS et al., 2013). Recentemente, o Preferred Reporting Items for Animal Studies in Endodontology (PRIASE) 2021 guidelines (NAGENDRABABU et al., 2021) foi criado, integrando o guia ARRIVE 2.0 (DU SERT et al., 2020a, 2020b) e o

guia de reporte de imagens clínicas e laboratoriais em publicações (CLIP guidelines) (LANG; TALERICO; SIONTIS, 2012). Esse guia pode melhorar o reporte de futuros estudos em animais na Endodontia (NAGENDRABABU et al., 2019).

Neste contexto, diversos materiais tem sido propostos para selamento de perfurações dentárias e avaliados em estudos pré-clínicos. O agregado trióxido mineral (MTA), primeiro material à base de silicato de cálcio desenvolvido para fins endodônticos, surgiu no começo da década de 1990 (LEE; MONSEF; TORABINEJAD, 1993)com o nome comercial de ProRoot MTA (Tulsa Dental Products, Tulsa, OK, USA). É um material composto basicamente de silicato tricálcio, aluminato tricálcio, óxido tricálcio, óxido de silicato e óxido de bismuto (TORABINEJAD et al., 1995). A adição do radiopacificador óxido de bismuto é o componente diferencial deste produto para o cimento Portland (FUNTEAS; WALLACE; FOCHTMAN, 2003). Inicialmente, o MTA foi desenvolvido para ser utilizado apenas como material retro-obturador, porém, devido às suas características de biocompatibilidade, bioatividade, ação antimicrobiana e capacidade seladora, esse material começou a ser indicado para diversas finalidades clínicas como, apicificação, apicigênese, pulpotomia, capeamento pulpar e selamento de perfurações dentárias (CAMILLERI, 2015; PRATI; GANDOLFI, 2015).

A capacidade antimicrobiana, de biocompatibilidade e bioatividade do MTA têm sido comprovadas ao longo dos anos. Quando avaliado como material selador de perfurações de furca em cães, a resposta inflamatória regrediu ao longo do tempo e observou-se a formação de tecido duro e reparo dos tecidos perirradiculares (KATSAMAKIS et al., 2013). Esses fenômenos podem ser explicados pois materiais à base de silicato de cálcio liberam íons cálcio e induzem a alcalinização do meio associada a formação de hidróxido de cálcio (CAMILLERI, 2007; CAMILLERI; SORRENTINO; DAMIDOT, 2013; DUARTE MAH et al., 2018).

Katsamakis e colaboradores, em 2013, conduziram uma revisão sistemática para avaliar a resposta histológica dos tecidos periodontais adjacentes ao contato com o MTA. Foram incluídos 24 estudos em animais. Os autores observaram que esse material induziu adequada resposta biológica e formação de tecido duro, compatível com o reparo das estruturas do dente e dos tecidos perirradiculares. Diante dos achados, os autores sugeriram a avaliação do MTA em estudos clínicos prospectivos.

Apesar das adequadas características do MTA, esse material possui algumas desvantagens. Camilleri (2014), ao colocar o ProRoot MTA (Dentsply Tulsa Dental, Johnson City, TN) em contato com a solução irrigadora hipoclorito de sódio, observou formação de pigmentação marrom escura devido a interação do óxido de bismuto com a solução. Além disso, esse cimento possui baixa radiopacidade, longo tempo de presa, risco de *wash-out* em ambientes úmidos e difícil manipulação e manuseio (CAMILLERI, 2015; MOORE; HOWLEY; CONNELL, 2011; PARIROKH; TORABINEJAD, 2010). Diante disso, outros materiais têm sido desenvolvidos e comercializados objetivando melhor performance. Mudanças na composição química de materiais reparadores podem afetar a resposta biológica dental e dos tecidos perirradiculares (ALADIMI et al., 2020).

Estudos pré-clínicos em animais utilizando o modelo de perfuração de furca têm sido publicados para avaliar as características de biocompatibilidade e bioatividade desses novos materiais. No entanto, apesar do aumento de estudos com esse design, não está claro na literatura se esses materiais equivalem ou superam as características biológicas do MTA. Dessa forma, a presente revisão sistemática tem como objetivo avaliar se o MTA, quando utilizado para selamento de perfuração de furca em estudos experimentais em animais, induz resposta histológica mais favorável em comparação a outros materiais.

2 OBJETIVOS

Objetivo geral

Avaliar se o MTA fornece uma resposta histológica mais favorável que outros materiais quando utilizado no selamento de perfurações de furca.

Objetivos Específicos

Avaliar a qualidade metodológica dos estudos primários incluídos através do ARRIVE guideline.

Avaliar o risco de viés dos estudos primários incluídos através da ferramenta SYRCLE.

3 ARTIGO CIENTÍFICO

Title: Does MTA provide a more favorable histological response than other materials in furcal perforation sealing? A systematic review.

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Running Title: Perforation repair materials. **Keywords**: Endodontics; Furcal perforation; Histological response; In vivo studies; Systematic review.

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Abstract

Background: A wide variety of root perforation sealing materials has been tested over the years, but no consensus has been reached about which yield a more favorable histological response. This Systematic review of preclinical studies provides an overview of the available studies comparing repair materials.

Objectives: To evaluate whether Mineral Trioxide Aggregate (MTA) yields a more favorable histological response than other materials when used to seal furcal perforations.

Methods: This review is reported according to PRISMA checklist. The *in vivo* studies included tested the use of materials used to seal furcal perforations, compared groups, one of which for MTA, and evaluated histological response. Studies whose full text was unavailable were excluded. An electronic search was conducted in EMBASE, PubMed, Scopus, and Web of Science up to September 2, 2020, with no language or publication date restrictions. The ARRIVE and SYRCLE tools were used to assess the methodological quality and the risk of bias of the studies.

Results: The twenty studies included in the qualitative synthesis were classified as having a low methodological quality and a high risk of bias. They also had a high methodological heterogeneity. MTA and Biodentine were the materials most often compared, and their histological response was adequate.

Discussion: Preclinical research is low in the scale of evidence because of the inherent limitations of this type of study design. Moreover, the heterogeneity, quality, and RoB of the studies included indicated that the evidence on furcal histological response to repair materials needs improvement. Therefore, the knowledge generated by this systematic review should be translated to clinical practice cautiously. This review shows that important changes have to be made to the way preclinical studies are conducted in the area of endodontic repair materials.

Conclusions: Guidelines for animal studies in endodontics should be used. Despite study limitations, findings indicated that MTA had the most predictable biological behavior. Biodentine had positive results. Clinical trials should be conducted to define which of these two materials should be the reference standard for clinical practice.

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Introduction

A furcal perforation is defined as a pathologic or mechanical communication between the root canal system and the external tooth surface in the interradicular region of multirooted teeth (Cardoso *et al*. 2018a, American Association of Endodontists 2020). Accidental perforations may account for up to 29% of all serious injuries during an endodontic treatment (Vehkalahti *et al.* 2020).

Root perforations may lead to inflammatory reactions affecting periodontal tissue and leading to alveolar bone loss (Seltzer *et al*. 1969). Inflammation may result in epithelial proliferation and granulation tissue formation, depending on its severity (Seltzer *et al*. 1969). In some cases, a periodontal pocket may develop, and a polypoid lesion may form, extending through the coronal portion (Seltzer *et al*. 1969). If not treated correctly, a root canal perforation may potentially become a prognostic factor for tooth loss (Ng *et al*. 2011, Mente *et al*. 2014, Estrela *et al*. 2018, Vehkalahti *et al*. 2020).

Nonsurgical treatment of perforations has a success rate of more than 70%, and may, therefore, be the approach of choice (Siew *et al.* 2015). In addition to the patient's clinical characteristics, the material used for the repair may affect the biological response of a perforated tooth and its surrounding tissues (Pitt-Ford *et al.* 1995, Yildirim *et al*. 2005, Siew *et al.* 2015). To avoid environmental contamination, the repair material should have adequate physicochemical properties and induce periradicular tissue regeneration (Holland *et al*. 2007, Pitt-Ford *et al*. 1995, Rathinam *et al.* 2016).

For years, materials such as amalgam and zinc oxide–eugenol-based cements have been used as repair materials. Amalgam has potential disadvantages, such as a higher cytotoxicity, moisture sensitivity, mercury and tin contamination, initial leakage and a need for an undercut in the cavity preparation (Gartner & Dorn 1992, Eley *et al*. 1993, Torabinejad *et al.* 1993, Badr 2010). Because of these drawbacks, zinc oxide-eugenol-based cements have been recommended. These materials, however, have some potential disadvantages: irritation of vital tissue, solubility and moisture sensitivity (Gartner & Dorn 1992, Torabinejad *et al.* 1993).

Mineral trioxide aggregate (MTA) (Dentsply, Tulsa, OK) was the first calcium silicate-based material introduced into the market (Lee *et al*. 1993). Despite its good biological, antibacterial, and bioactive properties (Parirokh & Torabinejad 2010a, Prati

et al. 2015, Gomes-Cornélio *et al*. 2017, Pinheiro *et al.* 2018;), it has poor handling properties, low radiopacity, discoloration when in contact with sodium hypochlorite, a long setting time and a risk of wash-out in humid environments (Parirokh & Torabinejad 2010b, Moore *et al*. 2011, Camilleri 2015). Therefore, calcium silicate-based materials, with different components and a supposedly better performance, such as Biodentine (Septodont, Saint-Maur-de-Fossés, France) and Bioaggregate (Innovative BioCeramix, Vancouver, BC, Canada) have been developed (Parirokh *et al.* 2018). As MTA, these materials are biocompatible and bioactive (Parirokh *et al.* 2018, Pinheiro *et al.* 2018, Quintana *et al*. 2019).

Materials with different compositions affect the response of teeth and their surrounding tissues (Aladimi *et al*. 2020). Although randomized clinical trials are the reference standard for the comparison of materials and methods, few observational studies (Farzaneh *et al*. 2004, Gorni & Gagliani 2004, de Chevigny *et al.* 2008, Gorni *et al.* 2016, Mente *et al.* 2010, Ng *et al*. 2011) and no randomized clinical trials have investigated root canal perforations, because of ethical issues and difficulties in standardizing procedural accidents. Therefore, experimental *in vivo* models, which may simulate the periradicular tissues and clinical conditions, are widely used to evaluate the biocompatibility and bioactivity of sealing materials (Cardoso *et al*. 2018). These studies use standardized methods (Hooijmans & Ritskes-Hoitinga 2013), which favors comparison between materials and reduces the risk of bias (RoB). These models have provided most of the scientific evidence available to guide clinicians in their choice of an adequate material to treat root canal perforations. A wide variety of materials has been tested over the years, but no consensus has been reached about which yield a more favorable histological response.

No systematic review has been conducted to compare sealing materials used in furcal perforation models and to assess RoB, methodological quality of primary studies, and body of existing evidence. This review was conducted to evaluate whether MTA yields a more favorable histological response than other materials when used to seal furcal perforations.

The research question framed for this systematic review was: Does MTA used to seal furcal perforation in experimental animal studies provide a more favorable histological response than other materials?

Methods

Protocol and registration

This systematic review is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (Page *et al*. 2020). It was registered in the International Prospective Register of Ongoing Systematic Reviews (PROSPERO) under the registration number CRD42020181297 entitled "Biological response to furcal perforation sealing materials: a systematic review". The clinical question was formulated and organized using the Population, Intervention, Comparison, Outcome and Study (PICOS) strategy.

Eligibility criteria

Two independent reviewers (LSP and RMQ) screened the full texts of the studies to define whether they met inclusion criteria. *In vivo* studies were included if they tested the use of materials to seal furcal perforations in animals, compared groups, one of which for MTA, and evaluated histological response. Studies whose full text was unavailable were excluded.

Information sources, search, study selection, data collection

Two independent reviewers conducted an electronic search in EMBASE (https://www.embase.com), PubMed (MEDLINE) (https://pubmed.ncbi.nlm.nih.gov/), Scopus (http://www.scopus.com) and Web of Science (https://www.webofknowledge.com) up to September 2, 2020, with no language or publication date restrictions. The search strategy adapted terms for each database and followed their syntax rules (Supplementary table 1). After identification in the database, the studies were imported into the EndNote Web software® (https://www.myendnoteweb.com), and duplicates were removed. The studies were initially selected according to their title and abstract. After a full-text screening, studies were included in the review if they met inclusion criteria. The reference lists of the selected studies were also screened manually.

In cases of disagreement at any stage of the search, the reviewers met for discussion, and a consensus was defined by two senior investigators (PMPK, RKS).

Supplementary Table S1 Search strategy used and results for each electronic database (Embase, PubMed, Scopus, Web of Science).

Databases	Search	Query	Items found
Embase	#1	'endodontics'/exp OR 'tooth root canal'/exp	30,813
	#2	'perforation'/exp OR 'furcal perforation' OR 'furcation perforation'	100,085
	#3	#1 AND #2	95
PubMed	#1	(endodontics [MeSH Terms]) OR (“root canal”)	42,930
	#2	((perforation) OR ("furcal perforation")) OR ("furcation perforation")	102,549
	#3	#1 AND #2	754
Scopus	#1	ALL (endodontics OR “root canal”)	134,518
	#2	ALL (perforation OR "furcal perforation" AND "furcation perforation")	676
	#3	#1 AND #2	655
Web of Science	#1	ALL= (endodontics OR "root canal")	29,078
	#2	ALL= (perforation OR 'furcal perforation' OR 'furcation perforation')	57,045
	#3	#1 AND #2	486

Data extraction

The first reviewer (LSP) extracted data independently into a standardized data spreadsheet in Microsoft Office Excel® 2016 (Microsoft Corporation, Redmond, WA). Data extracted were: authors, publication year, species, number of animals, number and type of teeth, groups evaluated, perforation diameter/bur number, contamination status, sealing period, observation time points, evaluation methods, characteristics evaluated and significant results. One study in Chinese was translated into English using Google translator (https://translate.google.com.br). The second reviewer (RMQ) double-checked extracted data.

Methodological quality and RoB assessment

The two independent reviewers (LSP, RMQ), previously calibrated by discussing each checklist item, evaluated interobserver agreement for the methodological quality (kappa = 0.75) and the RoB (kappa = 0.8) of the studies included. Any disagreement after the evaluation of methodological quality and RoB was decided as described above. Methodological quality was evaluated using the 21-item checklist of the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) 2.0 guidelines (du Sert *et al*. 2020a, du Sert *et al*. 2020b): (1) study design, (2) sample size, (3) inclusion and exclusion criteria, (4) randomization, (5) blinding, (6) outcomes measure, (7) statistical methods, (8) experimental animals, (9) experimental procedures, (10) results, (11) abstract, (12) background, (13) objectives, (14) ethical statement, (15) housing and husbandry, (16) animal care and monitoring, (17) interpretation/scientific implications, (18) generalizability/translation, (19) protocol registration, (20) data access and (21) declaration of interests.

A pre-defined grading system described by Schwarz *et al*. (2012) and adapted for the ARRIVE 2.0 guidelines was used to assign scores to each item, as following: items 1 to 12, 14 to 18 and 21 received a score ranging from 0 to 2: 0 = clearly inaccurate or not reported; 1 = possibly accurate, unclear, or incomplete; 2 = clearly accurate. The other items (13, 19, and 20) received a score of 0 or 1: 0 = inaccurate, not concise, or not reported; 1 = accurate, concise, or reported. Differences between ARRIVE (Kilkenny *et al*. 2010) and ARRIVE 2.0 guidelines were discussed by two reviewers, who then assigned scores to the modified items. The sum of the scores ranged from zero to 39 points. The result of the division of quality score by maximum score generated three possible quality coefficients: 0.8–1, excellent; 0.5–0.8, average; and <0.5, poor (Delgado-Ruiz *et al*. 2014).

Bias was evaluated using the RoB tool for animal studies of the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) (Hooijmans *et al*. 2014). This tool, based on the Cochrane RoB tool, assesses RoB for 10 types of bias/domains: (1) selection bias/sequence generation; (2) selection bias/baseline characteristics; (3) selection bias/allocation concealment; (4) performance bias/random housing; (5) performance bias/blinding; (6) detection bias/random outcome assessment; (7) detection bias/blinding; (8) attrition bias/incomplete outcome data; (9) reporting bias/selective outcome reporting; and (10) other sources of bias. RoB for each item in

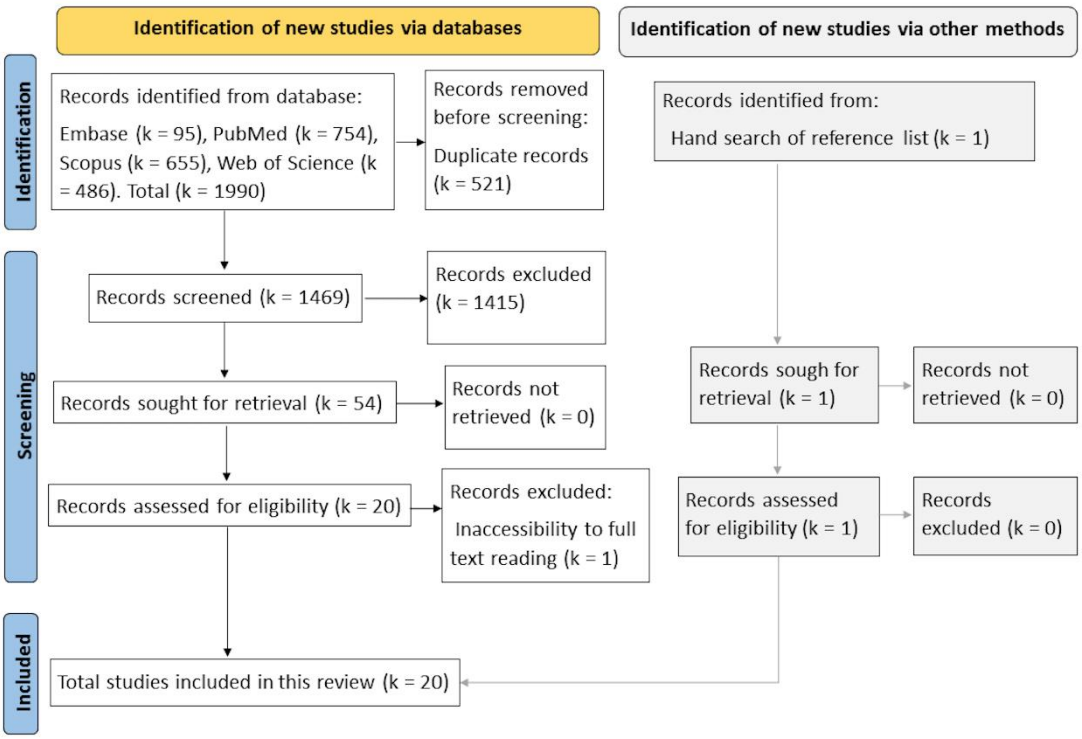
the selected studies was classified as low, high, or unclear using the RevMan 5.4 software (The Cochrane Collaboration, Denmark). If no checklist item had a RoB, the study was classified as having a low RoB; if RoB was unclear for any item, the RoB of the study was unclear; and if any item had a high RoB, RoB was classified as high for that study.

Results

Study selection

Initial screening in all databases yielded 1990 studies: 95 in Embase, 754 in PubMed, 655 in Scopus, and 486 in Web of Science. After discarding duplicates, 1469 were eligible for title reading, and, after that, 54 were selected for abstract analysis. Thirty-four were excluded because they did not meet inclusion criteria, and 20 remained for full-text assessment. One was excluded because the full text was not available for reading (Vladimirov *et al*. 2007), and one was added after reference screening (Zhu *et al*. 2003). Finally, 20 studies were included in the qualitative synthesis (Figure 1).

Figure 1 PRISMA flow diagram of screening and selection processes.



Characteristics of the studies included

The main characteristics of the included studies are described in Tables 1 and 2. Experimental designs were highly heterogeneous. Publication dates ranged from 1995 to 2020. Only three studies (#6, #7, #8) tested materials in rats, whereas all the others used dogs. The sample size in studies with rats was 60 animals, and in those with dogs, from one (#10, a pilot study) to 12 animals (#1, #17). Permanent molars and premolars were used for tests in almost all studies, and only one (#1) tested materials in canine primary molars.

MTA was compared with different materials: calcium silicate-based cements (n = 9), calcium phosphate-based material (n = 1), calcium and phosphate enriched material (n = 1), calcium hydroxide cement (n = 1), glass ionomer cement (n = 2), zinc oxide-eugenol-based sealer (n = 2), amalgam (n = 2), and bioactive molecules (n = 1). In addition to the material used for the repair, some studies also added basement materials: one added either stem cells loaded onto treated dentine matrix or a tricalcium-phosphate-based cement (#4); one included platelet-rich plasma and platelet-rich fibrin (#17); and four added a calcium sulphate barrier (#2, #3, #10, #11).

Intact teeth and perforations without sealing were used as negative and positive control groups in six studies. Five had only one type of control group. MTA was used as a control material in four studies (#10, #11, #15, #19), and five studies did not include a control group. Furcal perforation diameter varied from 0.25 mm to about 1 mm in rat models. Perforation diameters varied widely in dog studies, from 1 mm to 2.5 mm, but 1 mm was the most frequent measure (n = 5). Eight studies (#9, #10, #11, #13, #15, #16, #17, #18) did not provide the exact perforation diameter.

Most of the studies did not evaluate tissue repair in a contaminated environment (n = 16). Two (#13, #17) compared biological responses between non-contaminated and contaminated perforation sealing, and two (#3, #9) evaluated response only in a contaminated site. In the studies that evaluated repair in a contaminated environment (n = 4), tooth cavities were left open, without any perforation sealing, for four (#3, #9, #17) or six (#13) weeks.

The stains used for histologic evaluation were Masson's trichrome, picrosirius, Mallory’s trichrome, and Brown and Brenn stain (#3, #6, #13, #16, #19) in addition to hematoxylin-eosin. Some studies also conducted immunohistochemical,

immunofluorescence, radiographic and micro-CT analyses (#5, #6, #7, #12, #15, #16, #17). Only one study did not evaluate inflammatory reaction (#16). The other characteristics evaluated more frequently were new bone formation (n = 16), bone resorption (n = 12), cementum formation (n = 12) and epithelium proliferation (n = 9). However, many different qualitative, semi-quantitative, or quantitative methods were used to evaluate these characteristics. Three studies did not use any statistical methods to analyze the comparison of materials (#10, #13, #20).

Results of the studies included

A meta-analysis was not conducted because of the wide variation in study methodologies. Therefore, study results were analyzed qualitatively (Table 2).

Although a variety of materials for furcal perforation repair were tested and compared with MTA, Biodentine (Septodont, Saint-Maur-de-Fossés, France) was the most frequent (n = 6 studies). In the initial evaluation periods, the use of this material led to higher interleukin-6 expression and number of inflammatory cells in dogs (#6) and higher inflammation scores and number of Osterix-immunolabeled osteoblasts in rats (#6, #8). In addition, there were no statistically significant differences in inflammatory reactions between groups at 30, 60, 90, and 120 days (#1, #15), although Cardoso and colleagues (#5) found that Biodentine induced mild inflammation at 120 days. Moreover, in the same period, Biodentine induced greater cementum formation (#5), but less and thinner mineralized tissue (#5, #15). There were no statistical differences in hard tissue resorption and epithelium formation between materials at the different evaluation time points.

The comparison of other calcium silicate-based materials with MTA revealed that Endo-CPM sealer induced less inflammation at 7, 15, and 60 days and reduced width of the periodontal space at 7, 15, and 30 days (#7). Inflammatory infiltrate, hard tissue and epithelium formation at 7, 30, and 90 days were not statistically different between Bioaggregate and MTA (#9). No differences were found between Portland cement and MTA when a calcium sulfate barrier was used (#11). The biological response to furcal perforations treated with nano-filled resin-modified glass ionomer (Nano-FRMGI) or MTA was likewise not affected by the use of a calcium sulfate barrier (#2). In contrast, Al-Daafas & Al-Nazhan (2007) (#3) found that a calcium sulfate artificial floor used with

MTA or amalgam induced more severe inflammation and greater new bone formation than materials used alone.

Evaluation at all time points and in all environments revealed that, when MTA was used as an artificial floor for platelet-rich plasma (PRP) or platelet rich fibrin (PRF), these materials induced less inflammation than when MTA was used alone. Moreover, radiolucency indicated that bone loss was higher in the MTA group in a contaminated environment at 30 and 90 days (#17).

Tricalcium phosphate (TCP) induced greater inflammation than MTA, treated dentine matrix (TDM), TDM scaffold impregnated with dental pulp stem cells (DPSCs), and TCP scaffold impregnated with DSPCs. TDM scaffold impregnated with DSPCs induced more bone resorption than the other experimental materials. Cementum formation and epithelium proliferation were similar in all groups (#4).

The formation of new bone (#12, #14) and epithelium (#14) was not statistically different between groups treated with one of two experimental materials - calcium-enriched cement (CEC) or calcium phosphate cement (CPC) - and MTA at all evaluation time points.

MTA and basic fibroblast growth factor (bFGF) induced more new bone formation than a zinc and eugenol-based cement (IRM) at 21 days. In the same period, transforming growth factor β1 (TGFβ1) induced less new bone formation than MTA. At 56 days, IRM and TGFβ1 induced more severe inflammation than MTA. Insulin growth factor-I and TGFβ1 induced more epithelium proliferation than MTA (#19).

Table 1 Summary of the included studies characteristics.

#	Authors/year	Sample size: Animal number/Specie	Sample size: Number/type of evaluated teeth	Groups evaluated	Perforation diameter (mm)/bur number	Contamination status	Sealing period	Observation time points (days)
1	Abdelati *et al*. (2018)	12 dogs	96 primary M	• MTA* • Biodentine • +CT	1/NA	No	perforation period	30, 60 and 90
2	Aladimi *et al*. (2020)	6 dogs	96 PM/M	• MTA* • MTA* + CS basement • Nano-FRMGI • Nano-FRMGI + CS basement	1/#2	No	perforation period	30, 90 and 180
3	Al-Daafas & Al-Nazhan (2007)	9 dogs	72 PM	• MTA** • MTA** + CS basement • Amalgam • Amalgam + CS basement • +/- CT	1.4/#4	Yes	4 weeks after the perforation period	120
4	Bakhtiar *et al*. (2017)	5 dogs	32 PM	• MTA* • TDM • TCP • TDM scaffold + DPSCs • TCP scaffold + DPSCs • +/- CT	2/#2	No	perforation period	90

5	Cardoso *et al*. (2018)	5 dogs	50 PM	• MTA*** • Biodentine • +/- CT	1.2/#012	No	perforation period	120
6	da Fonseca *et al*. (2019)	60 rats	80 M	• MTA**** • Biodentine • +/- CT	0.25 /#¼	No	perforation period	7, 15, 30 and 60
7	da Silva *et al*. (2011)	60 rats	120 M	• MTA**** • Endo-CPM-Sealer • ZOE • -CT	0.25/#¼	No	perforation period	7, 15, 30 and 60
8	de Sousa Reis *et al*. (2019)	60 rats	54 M	• MTA**** • Biodentine • Biodentine + biodentine (restoration) • +/- CT	About 1/#1011	No	perforation period	14 and 21
9	Hassanien *et al*. (2015)	6 dogs	72 PM	• MTA**** • Bioaggregate • +CT	1.4/#4	Yes	4 weeks after the perforation period	7, 30 and 90
10	Neto *et al*. (2010)	1 dog	12 teeth: 11 PM and 1 M	• MTA$^{\#}$ + CS • PC type II + CS basement • PC type V + CS basement • White PC + CS basement	NA/#1016	No	perforation period	120
11	Neto *et al*. (2012)	10 dogs	80 PM	• MTA**** + CS basement • PC type II + CS basement • PC type V + CS basement • White PC + CS basement	NA/#1016	No	perforation period	120

12	Noetzel *et al*. (2006)	6 dogs	24 PM	• MTA** • Experimental CPC	NA/#012	No	perforation period	120
13	Pitt-Ford *et al*. (1995)	7 dogs	28 PM	• MTA***** • Amalgam	NA/#014	2 groups: contaminated and non-contaminated	2 groups: perforation period and 6 weeks after the perforation period	90
14	Samiee *et al*. (2010)	4 dogs	34 PM	• MTA** • Experimental CEC • +/- CT	1/NA	No	perforation period	90
15	Silva *et al*. (2017)	3 dogs	30 PM	• MTA* • Biodentine • +CT	NA/#1012	No	perforation period	120
16	Silva *et al*. (2019)	3 dogs	30 PM	• MTA* • Biodentine • +CT	NA/#1012	No	perforation period	120
17	Tawfik *et al*. (2016)	12 dogs	192 PM/M	• MTA*** • MTA*** + PRP • MTA*** + PRF • +/- CT	NA/#4	2 groups: contaminated and non-contaminated	2 groups: perforation period and 4 weeks after the perforation period	7, 30 and 90
18	Yildirim *et al*. (2005)	9 dogs	90 PM/M	• MTA# • Super EBA • -CT	NA/#014	No	One week after the obturation procedure	30, 90 and 180

19	Zairi *et al*. (2012)	6 dogs	74 PM/M	• MTA*** • IRM • OP-1 + BM • TGFβ1 + BM • bFGF + BM • IGF-I + BM • GFR Matrigel Matrix	1.4/#4	No	perforation period	21 and 56
20	Zhu *et al*. (2003)	3 dogs	42 PM/M	• MTA****** • Dycal • GIC	2 groups: 1/#010 and 2.5 /#025	No	perforation period	120

NE, not evaluated. NA, information not available. +CT, positive control. -CT, negative control. +/- CT, positive and negative controls. PM, premolars. M, molars. MTA, mineral trioxide aggregate. *, ProRoot White MTA (Dentsply Tulsa Dental, Tulsa, OK, USA). **, ProRoot Grey MTA (Dentsply Tulsa Dental, Tulsa, OK, USA). ***, ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK, USA). ****, white MTA (Angelus, Londrina, Brazil). ***** MTA (Loma Linda University, California). ******, Deng Sibo company. #, manufacturer not informed. CS, calcium sulfate. Nano-FRMGI, nano-filled resin modified glass ionomer. CPM, Endo-CPM-Sealer. TDM, treated dentine matrix. TCP, tricalcium phosphate. DPSCs, dental pulp stem cells. CPM, Endo-CPM-Sealer. ZOE, zinc oxide-eugenol cement. CPC, calcium phosphate cement. CEC, calcium enriched cement. PC, Portland cement. PRP, platelet-rich plasma. PRF, platelet-rich fibrin. EBA, ethoxybenzoic acid. IRM, zinc-oxide-eugenol-based cement. OP-1, osteogenic protein-1. BM, basement membrane. TGFβ1, transforming growth factorβ1. bFGF, basic fibroblast growth factor. IGF-I, insulin growth factor-I. GRF, growth-factor-reduced. GIC, glass ionomer cement.

Table 2 Summary of characteristics and results of the included studies showing significant differences between evaluated groups.

#	Evaluation methods	Evaluated characteristics	Significant results (time points - days)
1	Histological: H&E	Inflammatory infiltrate	
		• Presence/Absence	NO
		• Severity: none, mild, moderate, severe	MTA, Biodentine < +CT (30, 60, 90)
		Bone resorption	
		• Presence/Absence	MTA, Biodentine < +CT (30, 60, 90)
		Bone apposition	
		• Presence/Absence	NO
		Cementum formation	
		• Presence/Absence	NO
		Epithelium and granulation tissue formation	
		• Presence/Absence	NO
		Abscess formation	
		• Presence/Absence	MTA, Biodentine < +CT (NA)
2	Histological: H&E	Inflammatory infiltrate	
		• Presence/Absence	MTA + CS basement (30 < 180)
		• Severity (scores): (1) none, (2) mild, (3) moderate, (4) severe	NO
		Bone resorption	
		• Presence/Absence	NO
		Bone deposition	
		• Presence/Absence	NO
		Cementum deposition	
		• Presence/Absence	MTA (30 < 180); MTA + CS basement (30 < 180)
		Epithelium tissue formation	
		• Presence/Absence	NO
		Fibrosis	
		• Presence/Absence	NO
		Periodontal ligament reorientation	
		Presence/Absence	NO
3	Histological: H&E and Masson's trichrome	Inflammatory infiltrate	
		• Presence/Absence	NO
		• Severity: (0) none, (1) mild, (2) moderate, (3) severe	

		Bone resorption • Presence/Absence	MTA < Amalgam < MTA + CS basement < Amalgam + CS basement
		Bone deposition • Presence/Absence	NO
		Cementum formation • Presence/Absence	MTA < Amalgam < Amalgam + CS basement < MTA + CS basement (120)
		Epithelium tissue formation • Presence/Absence	NO
4	Histological: H&E	Inflammatory infiltrate • Inflammatory cell count: Score 0 (<10%), 1 (10-30%), 2 (30-50%), 3 (>50%) • Type: chronic/acute inflammatory cells	MTA, Amalgam > +CT TCP > MTA, TDM, TDM scaffold + DPSCs, TCP scaffold + DPSCs NO
		Bone formation • Counting	TDM scaffold + DPSCs > MTA, TCP, TCP scaffold + DSPCs
		Cementum formation • Type: cellular/acellular • Continuity: complete/incomplete	NO NO
		Dentine formation • Presence/Absence • Type: osteodentine/regular dentine	NO NO
		Connective tissue • Fibro vascular/granulation	NO
		Foreign body reaction • Presence/Absence of macrophages or giant cells	NO
5	Histological: H&E Radiographic Micro-CT	Inflammatory infiltrate • Severity (scores): (1) none, (2) mild, (3) moderate, (4) severe	Biodentine < MTA; MTA, Biodentine < +CT; -CT < MTA
		Hard tissue resorption • Presence/Absence • Development/increase of radiolucency	NO NO
		Hard tissue repair • Presence/Absence	NO
		Cementum repair	MTA < Biodentine; MTA, Biodentine > +CT

		• Scores: (1) totally repaired, (2) repair up to half of furcation, (3) repair up to a quarter of furcation, (4) no repair	
		Extruded material	
		• Volume quantification	Biodentine < MTA
6	Histological: H&E and picrosirius Immunohistochemical: IL-6, TRAP and Osterix	Inflammatory infiltrate	
		• IL-6 counting	+CT > Biodentine > MTA > -CT (7, 15); +CT > Biodentine, MTA > -CT (30); +CT > other groups (60); Biodentine (7, 15 > 30, 60); MTA (7,15 > 30,60); +CT (7 > 15 > 30, 60)
		• VvIC: volume density of inflammatory cells	+CT > Biodentine > MTA > -CT (7); +CT > Biodentine, MTA > -CT (15, 30, 60); Biodentine (7 > 15 > 30 > 60); MTA (7 > 15 > 30 > 60); +CT (7 > 15 > 30 > 60)
		• VvO: volume density of blood vessels, erythrocytes, extracellular matrix, extracellular spaces and material particles	NA
		Bone resorption	
		• Loss of the alveolar process	NA
		• Number of TRAP-positive osteoclasts	+CT > MTA, Biodentine > -CT (7, 15); +CT > MTA, Biodentine, -CT (30, 60); Biodentine (7, 15 > 30, 60); MTA (7, 15 > 30, 60)
		Bone deposition	
		• Number of osterix-immunolabeled osteoblasts	Biodentine > MTA, -CT > +CT (7); Biodentine, -CT > MTA > +CT (15); Biodentine > MTA > -CT > +CT (30); Biodentine, MTA > -CT > +CT (60); MTA (15 < 7 < 30 < 60); +CT (7 > 60)
		Fibrosis	
		• VvFb: volume density of fibroblasts	-CT > Biodentine, MTA, +CT (7); MTA > +CT (7); -CT > Biodentine, MTA > +CT (15, 30, 60); Biodentine (7 < 15 < 30, 60); MTA (7 < 15 < 30, 60); +CT (7, 15 < 30, 60). -CT > Biodentine, MTA, +CT (7, 15, 30, 60); MTA > Biodentine (30, 60)
		Content of birefringent collagen in the periodontal ligament	Biodentine, +CT > MTA > -CT (7); MTA, Biodentine, +CT > -CT (15); +CT > MTA, Biodentine > -CT (30, 60)

		Width of periodontal space	Biodentine (7, 15 > 30, 60); MTA (7, 60 < 15); +CT (7 < 15 < 30 < 60)
7	Histological: H&E Immunohistochemical: TRAP	Inflammatory infiltrate • Inflammatory cell count	ZOE > MTA > Endo-CPM-Sealer (7, 15, 60); ZOE > MTA, Endo-CPM-Sealer (30); MTA (7 > 15 > 30 > 60); Endo-CPM-Sealer (7 > 15 > 30 > 60); ZOE (7 > 15 > 30 > 60)
		Bone resorption • Number of TRAP-positive osteoclasts	-CT < Endo-CPM-Sealer < MTA < ZOE (7); -CT < MTA, Endo-CPM-Sealer < ZOE (15); -CT < Endo-CPM-Sealer < MTA, ZOE (30); -CT, MTA, Endo-CPM-Sealer < ZOE (60); MTA (7, 15, 30 > 60); Endo-CPM-Sealer (15> 7, 30 > 60); ZOE (7 > 15 > 30 > 60)
		Width of periodontal space	-CT < Endo-CPM-Sealer < MTA < ZOE (7, 15, 30); -CT < Endo-COM-Sealer, MTA < ZOE (60); MTA (7, 15, 30 > 60); Endo-COM Sealer (7, 16, 60 < 30); ZOE (7 > 15, 30 > 60)
8	Histological: H&E	Inflammatory infiltrate • Intensity (scores): (1) absence, (2) sparse mononuclear cells, (3) mononuclear cells infiltrate and/or sparse neutrophils and eosinophils, (4) neutrophilic and/or eosinophilic infiltrate, areas of abscess	+CT > Biodentine + Biodentine (restoration) > MTA, Biodentine (14); +CT > Other groups (21)
		• Extent (scores): (1) absence, (2) restricted to furcal exposure, (3) occupying up to half of furcal bone area, (4) occupying more than half of furcal bone area	+CT > Other groups (21).
		Bone resorption • Scores: (1) Totally repaired, (2) Restricted to perforation area, (3) occupying up to half of furcal bone area, (4) occupying more than half of furcal bone area	+CT > Other groups (21).
		Cementum repair • Scores: (1) totally repaired, (2) until half of furcation cemental area, (3) until a quarter of furcation cemental area, (4) No repair	NO
9	Histological: H&E	Inflammatory infiltrate • Inflammatory cell count	MTA, Bioaggregate < +CT (7, 30, 90)
		Hard tissue formation • Scores: (0) absence, (1) presence	MTA, Bioaggregate > +CT (30)
		Epithelium tissue formation	

		•Scores: (0) absence, (1) presence	MTA, Bioaggregate > +CT (7)
10	Histological: H&E	Inflammatory infiltrate	
		•Count of points with Inflammatory infiltrate	NA
		Newly-formed bone	
		•Count of points with bone formation	NA
11	Histological: H&E	Inflammatory infiltrate	
		•Count of points with Inflammatory infiltrate	NO
		Bone formation	
		•Count of points with bone formation	NO
12	Histological: H&E Radiographic	Inflammatory infiltrate	
		•Severity (scores): (1) none, (2) mild, (3) moderate, (4) severe	MTA < Experimental CPC (120)
		•Type of inflammation: acute/Chronic/Recidivating	NA
		Bone reorganization	
		•Intensity (scores): (1) none, (2) mild, (3) moderate, (4) severe	NO
		•Radiolucency (scores): (1) no indication of bone loss, (2) minor bony defects, (3) medium bony defect, (4) major bony defect	NA
		Deposition of connective tissue	
		•Intensity (scores): (1) none, (2) mild, (3) moderate, (4) severe	NO
13	Histological: H&E, Masson's trichrome and Brown and Brenn stain	Inflammatory infiltrate	
		•Severity: none, few, moderate, severe	NA
		•Extension	NA
		Cementum formation	
		•Presence/Absence	NA
14	Histological: H&E	Inflammatory infiltrate	
		•Inflammatory cell count (scores): (0): none, (1) inflammatory cells < 25, (2) =25 – 50, (3) = 51 – 75, (4) > 75	NO
		Hard tissue formation	
		•Presence/Absence	NO
		•Continuity/discontinuity	NO
		Epithelium formation	
		•Presence/Absence	NO

15	Histological: H&E Immunofluorescence: RUNX2	Inflammatory infiltrate • Inflammatory cell count	Biodentine < +CT (120)
		Bone resorption • Scores: (0) absent, (1) present	MTA, Biodentine > +CT (120)
		Newly formed mineralized tissue • Scores: (0) absent, (1) partial, (2) complete	MTA > Biodentine > +CT (120)
		• Thickness	MTA > Biodentine (120)
		• Area of the formed mineralized tissue	MTA > Biodentine (120)
		• RUNX2 expression	Biodentine > +CT (120)
16	Histological: H&E and Masson's trichrome Immunohistochemical: TRAP, OPN, ALP, Immunofluorescence: BMP-2, BSP, OCN, CAP, CEMP-1	Bone resorption • Number of TRAP-positive osteoclasts	NO
		Newly mineralized tissue • Scores: (0) Absence, (1) Presence	MTA, Biodentine > +CT (120)
		• Positive OPN and ALP immunolabeling (scores): (1) weak, (2) moderate, (3) strong	OPN: MTA > Biodentine > +CT; ALP: MTA > Biodentine > +CT (120)
		• BMP-2, BSP, OCN, CEMP-1, CAP: Presence/Absence	NA
		Collagen fibers • Presence/absence and reorientation	NA
17	Histological: H&E Radiographic	Inflammatory infiltrate • Inflammatory cell count	NC: -CT > MTA > PRP, PRF > +CT (7, 30); -CT > MTA > PRF > PRP > +CT (90) C: -CT > MTA > PRP, PRF > +CT (7, 30, 90)
		Bone resorption • Scores: (0) no osteoclasts, (1) few, (2) moderate, (3) many	NC and C: +CT > MTA, PRP, PRF > -CT (7, 30, 90)
		• Radiolucency: bone loss	NC: +CT > MTA, PRP, PRF > -CT (30, 90) C: +CT > MTA > PRP, PRF > -CT (30, 90)
		New bone formation • Scores: (0) no osteoblasts or osteoid, (1) slight osteoblastic rimming with some osteoid, (2) moderate osteoblastic rimming with some osteoid, (3) heavy osteoblastic rimming with abundant osteoid	NC: MTA, PRP, PRF > -CT, +CT (7, 30, 90) C: MTA, PRP, PRF > -CT, +CT (30, 90)
		Cemental deposition • Scores: (0) absence, (1) deposition of newly formed cementum on lateral walls or close to it, (2) partial, (3) complete barrier	NC: MTA, PRP, PRF > -CT, +CT (30) C: PRF > -CT, +CT (30); MTA, PRP, PRF > -CT, +CT (90)
		Epithelial proliferation	

		• Scores: (0) absence, (1) presence	NC: +CT > MTA, PRP, PRF, -CT (7, 30, 90) C: +CT > PRP, PRF, -CT (7); +CT > MTA, PRP, PRF, -CT (30, 90)
18	Histological: H&E and Masson's trichrome	Inflammatory infiltrate	
		• Scores: (0) absent, (1) mild, (2) moderate, (3) severe	NA
		Hard tissue healing	
		• Soft tissue	NA
		• Hard tissue	NA
19	Histological: H&E, Mallori's trichrome and Brown and Brenn stain	Inflammatory infiltrate	
		• Absent, slight, moderate, severe	TGFβ1 + BM, IRM > MTA (56).
		Hard tissue resorption	
		• Yes/No	NO
		Bone formation	
		• Yes/No	MTA, bFGF + BM > IRM (21); MTA > TGFβ1 + BM (21); MTA, bFGF + BM, TGFβ1 + BM > IRM (56)
		Cementum resorption	
		• Yes/No	OP-1 > MTA, IRM (21)
		Cementum formation	
		• Yes/No	bFGF + BM > IRM (56)
		Epithelium proliferation	
		• Absent, partially organized, organized	IGF-I + BM > MTA (21); TGFβ1 + BM, bFGF + BM, IGF-I + BM > MTA (56)
		Bacterial presence	
			NO
20	Histological: H&E	Inflammatory infiltrate	
		• Absence, mild, moderate, severe	NA
		Bone deposition	
		• Presence/Absence	NA
		Epithelium tissue formation	
		• Presence/Absence	NA

NO, not observed. NA, information not available. H&E, hematoxylin and eosin. Micro-CT, micro-computed tomography. TRAP, tartrate-resistant acid phosphatase. IL-6, Interleukin-6. RUNX2, runt-related transcription factor 2. OPN, osteopontin. ALP, alkaline phosphatase. BMP-2, bone morphogenetic protein 2. BSP, bone sialoprotein. OCN, osteocalcin. CAP, cementum attachment protein. CEMP-1, cementum protein 1. MTA, mineral trioxide aggregate. +CT, positive control. -CT, negative control. CS, calcium sulfate. TCP, tricalcium phosphate. TDM, treated dentine matrix. DPSCs, dental pulp stem cells. CPM, Endo-CPM-Sealer. ZOE, zinc oxide-eugenol cement. CPC, calcium phosphate cement. NC, non-contaminated. C, contaminated. PRP, platelet-rich plasma. PRF, platelet-rich fibrin. BM, basement membrane. TGFβ1, transforming growth factorβ1. IRM, zinc-oxide-eugenol-based cement. bFGF, basic fibroblast growth factor. OP-1, osteogenic protein-1. IGF-I, insulin growth factor-I.

Methodological quality of the studies included

Tables 4 and Supplementary File 2 show the scores and the percentages of studies according to the different reporting categories of the ARRIVE 2.0 checklist. Studies were scored as described above.

No study had a protocol registration (item 19) or provided data access (item 20). The lowest score was predominant in the category 15 (housing and husbandry), as 85% of the studies had a score of 0 for this category. Participant blinding (item 5) was not reported or reported inaccurately in half of the studies, and the other publications were unclear on this topic. For item 10 (results), almost half (45%) of the studies scored 0, while 25% received a score of 1, and the remaining studies scored the maximum grade. Most studies received a score of 1 for sample size (100%), experimental animals (100%), objectives (100%), animal care and monitoring (100%), background (95%), outcome measures (90%), experimental procedures (75%), statistical methods (80%), declaration of interests (65%), inclusion and exclusion criteria (65%) randomization (60%) and study design (55%). The percentages of studies that received a score of 2 for checklist items 11 (abstract), 18 (generalizability/translation) and 17 (interpretation/scientific implication) were 5%, 70% and 60%.

Only two categories received excellent scores and achieved coefficients of 0.8–1: (13) objectives and (17) interpretation/scientific implication. Nine categories had intermediate grades, with coefficients of 0.5–0.8: (1) study design, (2) sample size, (8) experimental animals, (9) experimental procedures, (11) abstract, (14) ethical statement, (16) animal care and monitoring, (18) generalizability/translation. Finally, ten categories had scores that indicated a poor quality, with coefficients <0.5: (3) inclusion and exclusion criteria, (4) randomization, (5) blinding, (6) outcome measures, (7) statistical methods, (10) results, (15) housing and husbandry, (19) protocol registration, (20) data access and (21) declaration of interest.

Table 3 The scores of quality assessment according to Animal Research Reporting *In Vivo* Experiment (ARRIVE 2.0) guidelines of the included studies.

#	Author/year	Items																					
		Essential-10										Recommended Set											
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	T
1	Abdelati *et al*. (2018)	2	1	1	1	1	0	1	1	2	0	1	1	1	1	2	1	2	2	0	0	1	22
2	Aladimi *et al*. 2020	1	1	1	0	0	1	1	1	1	0	1	1	1	1	0	1	2	2	0	0	1	17
3	Al-Daafas & Al-Nazhan (2007)	2	1	1	1	1	1	1	1	2	0	0	1	1	1	0	1	2	2	0	0	1	20
4	Bakhtiar *et al*. (2017)	2	1	1	1	1	1	1	1	2	1	1	1	1	1	0	1	2	2	0	0	1	22
5	Cardoso *et al*. (2018)	2	1	1	1	1	1	1	1	1	0	1	2	1	2	0	1	2	2	0	0	1	22
6	da Fonseca *et al*. (2019)	2	1	0	0	1	1	1	1	1	2	1	1	1	2	1	1	2	2	0	0	1	22
7	da Silva *et al*. (2011)	1	1	0	0	0	1	1	1	1	2	1	1	1	1	0	1	1	0	0	0	1	15
8	de Sousa Reis *et al*. (2019)	2	1	0	0	1	1	1	1	1	2	2	1	1	1	0	1	2	2	0	0	1	21
9	Hassanien *et al*. (2015)	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	2	0	0	0	17
10	Neto *et al*. (2010)	1	1	1	1	0	1	1	1	1	1	1	1	1	1	2	1	1	0	0	0	1	18
11	Neto *et al*. (2012)	1	1	1	1	1	1	0	1	1	2	1	1	1	1	0	1	1	0	0	0	1	17
12	Noetzel *et al*. (2006)	1	1	1	1	0	1	1	1	2	0	1	1	1	1	0	1	2	2	0	0	0	18
13	Pitt-Ford *et al*. (1995)	1	1	1	0	0	0	1	1	0	1	1	1	1	0	0	1	2	2	0	0	0	14
14	Samiee *et al*. (2010)	2	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	2	2	0	0	1	20
15	Silva *et al*. (2017)	2	1	0	1	1	1	2	1	1	2	1	1	1	2	0	1	1	0	0	0	2	21
16	Silva *et al*. (2019)	1	1	0	1	1	1	1	1	1	1	1	1	1	2	0	1	1	0	0	0	2	18
17	Tawfik *et al*. (2016)	2	1	0	0	0	1	0	1	1	2	1	1	1	1	0	1	1	2	0	0	1	17
18	Yildirim *et al*. (2005)	1	1	1	0	0	1	1	1	1	0	1	1	1	1	0	1	2	2	0	0	1	17
19	Zairi *et al*. (2012)	1	1	1	0	0	1	1	1	1	0	1	1	1	1	0	1	2	2	0	0	0	16
20	Zhu *et al*. (2003)	1	1	1	1	0	1	0	1	1	0	1	1	1	0	0	1	1	0	0	0	0	12
	Category Score	30	20	13	12	10	18	17	20	24	17	20	21	20	22	5	20	32	28	0	0	17	
	Maximum Score Expected	40	40	40	40	40	40	40	40	40	40	40	40	20	40	40	40	40	40	20	20	40	
	Ratio Quality Score	0.75	0.5	0.32	0.3	0.2	0.47	0.42	0.5	0.6	0.42	0.5	0.52	1	0.55	0.12	0.5	0.8	0.7	0	0	0.42	

(1) study design, (2) sample size, (3) inclusion and exclusion criteria, (4) randomization, (5) blinding, (6) outcomes measure, (7) statistical methods, (8) experimental animals, (9) experimental procedures, (10) results, (11) abstract, (12) background, (13) objectives, (14) ethical statement, (15) housing and husbandry, (16) animal care and monitoring, (17) interpretation/scientific implications, (18) generalisability/translation, (19) protocol registration, (20) data access and (21) declaration of interests. (T) Total: represents total score obtained by each study out of a maximum of 39 points.

Supplementary Table S2 Percentage publications (n = 20) in different categories per ARRIVE 2.0 checklist item.

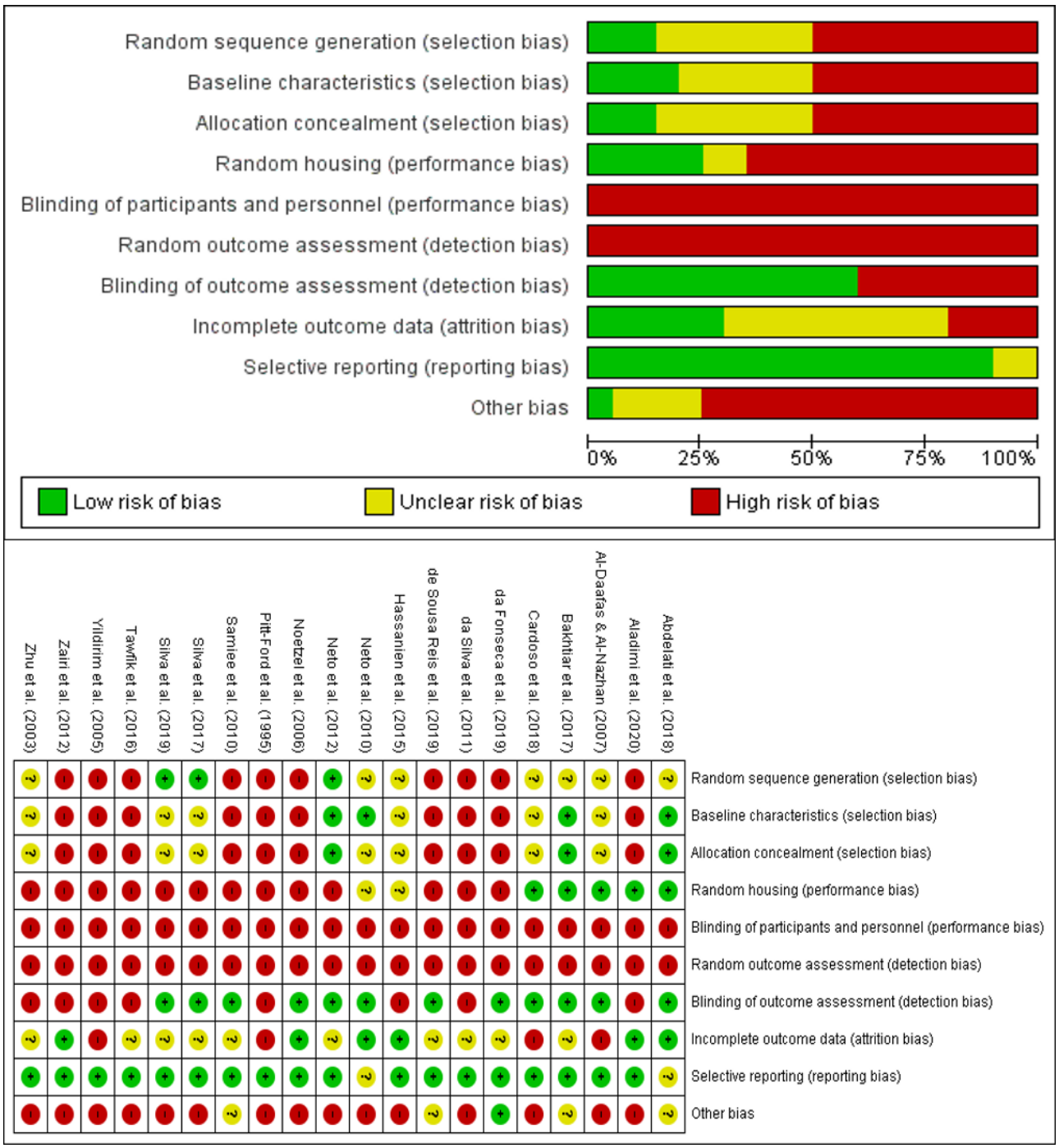
	Grading		
Item	0 (%)	1 (%)	2 (%)
1	0	55	45
2	0	100	0
3	35	65	0
4	40	60	0
5	50	50	0
6	10	90	0
7	15	70	5
8	0	100	0
9	5	75	20
10	40	30	30
11	5	90	5
12	0	95	5
13	0	100	-
14	10	20	70
15	85	5	10
16	0	100	0
17	0	40	60
18	30	0	70
19	0	0	-
20	0	0	-
21	25	65	10

(0) Clearly inaccurate or not reported, (1) possibly accurate/unclear, (2) clearly accurate

Risk of bias in the studies included

The results of RoB assessment according to the SYRCLE RoB tool (Hooijmans *et al.* 2014) are showed in Figure 2. All studies had a high RoB for “blinding of participants and personnel” and “random outcome assessment”. Half of the studies (50%) had a high RoB for “random sequence generation”, “baseline characteristics” and “allocation concealment”. RoB was also high for most studies in two other categories: “other bias” (75%) and "random housing" (65%). In contrast, most studies had a low RoB for "blinding of outcome assessment" (60%) and "selective reporting" (90%). Half of the studies (50%) had an unclear RoB for “incomplete outcome data”, because detailed information was missing. In general, RoB of all included studies was high.

Figure 2 Risk of bias according to categories: evaluation by review authors described as percentages across all studies and for each study included.



Discussion

Systematic reviews of preclinical studies provide an overview of what has been published and suggest the possibility of translating the knowledge generated to humans (Hooijmans & Ritskes-Hoitinga 2013). This is especially relevant for the investigation of

protocols to treat root perforations, as scientific evidence based on clinical studies with humans is limited by ethical issues and methodological difficulties. Therefore, the results of this review should help define the goal priorities for clinical studies about root perforations.

This systematic review evaluated the response induced by MTA in comparison with alternative materials to seal perforations. Most of the *in vivo* studies included in this review were conducted to define which material was the most adequate using a dog model, and only a few used a rat model. Both models are suitable for histological evaluations of the processes induced by materials to seal furcal perforations (Silva *et al*. 2009, Cardoso *et al*. 2018). Despite their similar physiology, the larger canine dental anatomy offers good visibility and accessibility, which explains the common use of this species for furcal perforation studies (Al-Daafas & Al-Nazhan 2007, Zairi *et al*. 2012, Cardoso *et al.* 2018). However, the relationship between bone margin and the furcal area in dogs is not directly comparable with that of the human tooth (Yildirim *et al.* 2005). Root furcation in dogs is often as close as 1-2 mm to the cementoenamel junction, whereas furcation lies deeper in humans, and the epithelization and formation of connective tissue is less common (Salman *et al*. 1999). Thus, the procedures that produced favorable outcomes in dogs may be expected to have a better effect in humans, whose distance from the furcal area to the cementoenamel junction is greater (Salman *et al.* 1999).

Rats have a smaller mouth and teeth, which makes the procedures more difficult, and the inflammatory processes in rodents are different from those observed in humans. (Genco *et al*. 1998, Weinberg & Bral, 1999, Scarparo *et al*. 2011) However, the choice of this model may be explained by the fact that the periodontal anatomy, histopathology of the periodontal lesion and basic immunobiology of these animals is similar to that found in humans (Klausen 1991, Genco *et al.* 1998, Silva *et al*. 2009).

Repair materials, in addition to having adequate physicochemical and antimicrobial properties, should be biocompatible and bioactive, so that they may reestablish periodontal and dental architecture and ensure a successful treatment outcome (Andreasen & Rud 1972, Zairi *et al*. 2012). The reaction induced by a material in contact with tissues should be reduced to a minor or mild inflammation along time (Hauman & Love 2003), and a mineralized tissue barrier should form (Zairi *et al*. 2012).

In the included studies, MTA fulfills the role of material to repair furcal perforations properly, which is confirmed by the results of a systematic review that evaluated the histological response of the periodontium to MTA (Katsamakis *et al*. 2013). Moreover, the individual analysis of the included studies revealed that the biological results of this material were better than or equivalent to those of other tested materials.

Unfortunately, methodological heterogeneity across the included studies precluded an adequate comparison between materials. Their protocols for outcome assessment differed in, for example, observation time points and outcome assessment methods. Many studies assessed these characteristics by means of scores (Noetzel *et al.* 2006, Al-Daafas & Al-Nazhan 2007, Samiee *et al.* 2010, Bakhtiar *et al.* 2017, Cardoso *et al*. 2018, de Sousa Reis *et al*. 2019, Silva *et al.* 2019, Aladimi *et al.* 2020) or cell counting (Neto *et al.* 2010, da Silva *et al.* 2011, Neto *et al.* 2011, Hassanien *et al.* 2015, Tawfik *et al.* 2016, Silva *et al.* 2017, da Fonseca *et al.* 2019). Even those studies that analyzed the outcomes by means of scores evaluated different characteristics in each score. Moreover, several materials were compared with MTA, including amalgam, eugenol oxide-based cements, calcium hydroxide-based cements and glass ionomer cements, as well as experimental pastes, bioactive molecules and nano-filled resin-modified glass ionomer. Most of these materials were evaluated only once, which makes it difficult to discuss and draw conclusions that are different from those reported in that individual study. However, since its introduction in the 1990s (Lee *et al*. 1993), there have been attempts to develop repair materials that supersede MTA.

MTA, Biodentine, Bioaggregate, and Endo-CPM sealer are calcium silicate-based materials. During setting in contact with tissues, this class of materials releases calcium ions and induces medium alkalinization associated with the formation of calcium hydroxide (portlandite) (Camilleri 2007, Camilleri *et al.* 2013, Duarte *et al.* 2018). These mechanisms play an important role in the antimicrobial activity, biocompatibility, and bioactivity of the materials in the studies included in this systematic review.

Apart from MTA, Biodentine was the material evaluated most frequently in furcal perforation studies. Biodentine, introduced in 2009, contains tricalcium silicate, dicalcium silicate, calcium carbonate, zirconium oxide (radiopacifier) and iron oxide. Its mixing liquid contains calcium chloride, a setting accelerator, a water-soluble polymer and a water-reducing agent (Grech *et al.* 2013, Haapasalo *et al*. 2015). In comparisons

with MTA in furcal perforation models, Biodentine induced a more severe inflammatory reaction in the initial evaluation periods, which decreased over time (Silva *et al.* 2017, Abdelati *et al.* 2018, da Fonseca *et al.* 2019; de Sousa Reis *et al.* 2019).

This intense initial inflammation may be explained by the release of calcium ions and a high pH (Dawood *et al.* 2015, Gandolfi *et al.* 2015, Li *et al.* 2017). The presence of the calcium chloride and pure tricalcium silicate, a high solubility, and calcium hydroxide formation may explain the physicochemical and biological behaviors of this material (Camilleri 2014a, Camilleri *et al.* 2014b, Gandolfi *et al.* 2015). In addition, calcium chloride reduced viability of MG-63 human osteosarcoma cells when added to MTA (Kang *et al.* 2013). All studies found hard tissue formation, but the comparisons with MTA produced conflicting results, which might be explained by the differences in study methodologies.

Despite the biological characteristics of repair materials, their extrusion to the surrounding tissues may compromise the success of furcal perforation sealing (Al-Daafas & Al-Nazhan 2007, Mente *et al*. 2010). Several studies evaluated the use of calcium sulfate as an artificial floor/barrier on the perforated floor to control placement and avoid material extrusion (Al-Daafas & Al-Nazhan 2007, Neto *et al*. 2010, Neto *et al*. 2012, Bakhtiar *et al.* 2017, Aladimi *et al.* 2020). Most of these studies found that the addition of this barrier did not improve treatment outcomes. Moreover, Al-Daafas & Al-Nazhan (2007) found that the addition of a calcium sulphate barrier induced more severe inflammation.

Type of repair material, location, perforation size and environment contamination may affect the outcome of perforation treatments (Sinai 1977, Askerbeyli *et al.* 2019). Few studies evaluated the effect of a contaminated environment, and perforation site was not standardized in the studies included. These methodological variations preclude comparisons between perforation sealing materials.

To our knowledge, this is the first systematic review that used ARRIVE 2.0 guidelines (du Sert *et al.* 2020a; du Sert *et al*. 2020b) and the SYRCLE RoB tool (Hooijmans *et al*. 2014) to assess reporting quality and RoB of *in vivo* studies of materials to seal furcal perforations.

The ARRIVE guidelines ensure the transparent reporting of study methods and findings, essential components of reproducibility (du Sert *et al.* 2020b). These guidelines

are divided into two groups: the Essential-10 items, which are the minimum reporting requirements for a reliable assessment of findings by reviewers and readers; and the Recommended Set, with categories that complement the essential categories and add context to the study. In this review, the scores of most of the Essential-10 categories indicated a poor quality, as important information about randomization, participant blinding, primary outcome definition, details of statistical methods and results were not adequately reported in the studies included. Moreover, most of the Recommended Set categories received intermediate scores. The lack of the minimum information required in most Essential-10 categories indicates that reporting was inadequate. Therefore, the studies could not be properly analyzed, and their reproducibility was poor. Although the first ARRIVE guidelines were introduced in 2010 (Kilkenny *et al*. 2010), studies using furcal perforation models have not adhered to this checklist. In general, important information described in the ARRIVE guidelines is still missing in recent studies in the area of preclinical research (Avey *et al.* 2016, Leung *et al.* 2018). Recently, the Preferred Reporting Items for Animal Studies in Endodontology (PRIASE) 2021 guidelines (Nagendrababu *et al*. 2021) have been created, integrating ARRIVE 2.0 guidelines (du Sert *et al*. 2020b) and a guideline for documenting clinical and laboratory images in publications (CLIP guidelines) (Lang *et al*. 2012). This guideline may improve future reporting of animal studies in Endodontics (Nagendrababu *et al*. 2019).

The SYRCLE RoB tool was developed in 2014 (Hooijmans *et al*. 2014) as an adapted version of the Cochrane RoB tool. In this review, random outcome assessment and blinding of the investigators had a high RoB. The studies did not describe whether animals were selected at random for outcome assessment, and researchers did not describe measures used, if any, to blind researchers from knowing which intervention each tooth received. In contrast, most studies described blinding of the outcome assessor and pre-specified the outcomes evaluated. The individual analysis of studies revealed that all had a high RoB. Although Katsamakis *et al*. (2013) did not use the SYRCLE checklist, they also found a RoB in preclinical histological studies that included MTA.

This study included specific methodological steps to increase transparency and strength: the review protocol was registered *a priori*; the study was based on an extremely comprehensive review of furcal perforation sealing studies in four major

databases; and the methodological quality and RoB of the included studies was assessed using the ARRIVE 2.0 and SYRCLE tools. Therefore, this review should help define the goal priorities for clinical studies about root perforation, as it demonstrated that MTA and Biodentine should be compared in clinical trials.

This systematic review has some limitations. The studies included had high methodological heterogeneity, low reporting quality and high RoB. In addition, preclinical research, although fundamental for the understanding of biological mechanisms (Sena *et al*. 2014), is low in the scale of evidence because of the inherent limitations of this type of study design. Therefore, the level of evidence produced by this systematic review might be low. The knowledge generated by this systematic review should be translated to clinical practice cautiously. Despite these limitations, this review shows that important changes have to be made to the way preclinical studies are conducted in the area of endodontic repair materials. We strongly suggest that standardized protocols and reporting guidelines should be used for animal studies in Endodontics to standardize research procedures and, consequently, decrease RoB and improve reporting quality. The continuous update of scientific evidence generated by *in vitro* studies that use, for example, physicochemical, cell cytotoxicity and bioactivity tests should be considered when designing well-controlled experiments and defining priorities for *in vivo* studies (Hauman & Love 2003).

Conclusions

Studies that used furcal perforation models to compare repair materials had a high methodological heterogeneity, low methodological quality and a high RoB. The guidelines now available, together with knowledge generated by biological studies about material characterization, should be used during the development and reporting of animal studies in Endodontics.

Although a variety of alternative repair materials has been developed over the years, several have been evaluated only one or very few times using furcal perforation models. Despite their methodological limitations, these studies have exhaustively studied MTA and demonstrated that this material has the most predictable and adequate biological behavior. Biodentine also had favorable results. Clinical trials

comparing MTA and Biodentine should be conducted to assess which material should be the reference standard for clinical practice.

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Supporting information

Supplemental Table S1: Search strategy used and results for each electronic database (Embase, PubMed, Scopus, Web of Science).

Supplemental Table S2: Percentage of publications (n = 20) in different categories per ARRIVE 2.0 checklist item.

3 CONSIDERAÇÕES FINAIS

A presente revisão sistemática teve como objetivo principal comparar os materiais reparadores quanto à resposta histológica induzida por eles em modelos experimentais de perfurações de furca em animais. Esse questionamento surgiu a partir do reconhecimento das limitações metodológicas e éticas em comparar os materiais em situações clínicas de perfurações radiculares e, além disso, do crescente desenvolvimento de materiais e publicações científicas utilizando este modelo para avaliar propriedades biológicas dos novos cimentos.

Além da comparação entre materiais, essa revisão avaliou a qualidade metodológica e o risco de viés dos estudos incluídos de acordo com duas ferramentas estabelecidas na literatura, ARRIVE e SYRCLE. A avaliação crítica dos estudos incluídos possibilita apontar futuras melhorias na qualidade metodológica e de reporte das publicações que avaliam materiais reparadores em modelos *in vivo* de perfuração de furca. Diante dos resultados, podemos observar que os estudos incluídos demonstraram alta heterogeneidade e baixa qualidade metodológica, com alto risco de viés. Portanto, o conhecimento gerado por essa revisão sistemática deve ser translacionado para a prática clínica com cautela. A partir dessas informações, sugere-se que os guias disponíveis na literatura devem ser utilizados durante o desenvolvimento e escrita de estudos em animais na Endodontia. A utilização desses guias visa garantir acurácia, reprodutibilidade, comparabilidade e transparência dos estudos em animais, dessa forma, influenciando diretamente nos desfechos das revisões sistemáticas desses estudos.

Apesar da variedade de materiais reparadores desenvolvidos ao longo dos anos, foi observado que muitos deles foram avaliados apenas uma ou poucas vezes em modelos de perfuração de furca. Embora existam limitações metodológicas, pode se observar que o MTA é um material que tem sido exaustivamente avaliado e demonstrou adequado e previsível comportamento biológico quando comparado aos outros materiais. Além do MTA, o Biodentine foi o segundo material mais avaliado e também apresentou adequadas características biológicas nos estudos incluídos. Diante do exposto, compreende-se a necessidade da comparação do MTA e Biodentine em estudos clínicos para avaliar qual material pode ser referência na prática clínica.

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