Universidade de Trás-os-Montes e Alto Douro

Bioceramics in Tissue Regeneration in Furcation Perforations in a Canine Model

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PhD Thesis in Veterinary Sciences – Biomedical Sciences

Tese de Doutoramento em Ciências Veterinárias – Ciências Biomédicas

Miguel Agostinho Beco Pinto Cardoso

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Carlos Alberto Antunes Viegas

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Vila Real, 2017

I declare that the contents of this Thesis are my own work and that they have not been presented to any University other than the University of Trás-os-Montes and Alto Douro.

To my Family and Friends

“Sempre chegamos ao sítio aonde nos esperam.”
(José Saramago)

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Abstract

Furcation perforations (FP) are pathological conditions of complex treatment and, currently, bioceramics are good options for FP repair, with ProRoot™ MTA as a gold standard. However, it is not devoid of drawbacks, prompting the development of new calcium silicate cements. Among these, Biodentine™ has gained popularity and has been widely used in many settings since its approval by the FDA in 2009.

In the present Thesis, the main objectives were to evaluate the role of Biodentine as FP repair material and to improve both the methodology of experimental studies of FP and approach for canine endodontics.

In order to systematize the experimental FP models described in literature and to determine if there is evidence that a model is superior to others, a review of the literature was undertaken (Chapter II). The available data established that, although there is no model without disadvantages that can be stated as ideal and clearly higher than others for FP studies, dogs appear to present the most appropriate characteristics.

To systematically summarize what is known about Biodentine´s results when used in FP repair, compared with currently used materials, a contemporary literature review was performed (Chapter III). As noteworthy findings, first, we found no human studies or case reports in the literature. Biodentine's performance in FP repair has been scarcely addressed and available results were scattered and disorganized. Studies support that Biodentine is a good FP repair material, with overall better or equivalent performance when compared with currently used materials. However, research on Biodentine for repair of FP remains incomplete and larger clinical studies are still lacking.

The experimental part of our work consisted of an *in vivo* study comparing the histological, radiographic and micro-computed tomographic outcomes four months after FP repair with Biodentine or ProRoot MTA in dogs' teeth (Chapter V). Biodentine presented tissue compatibility and allowed for tissue regeneration, with mineralized tissue formation with similar morphology and integrity but greater cementum formation and lesser extrusion than ProRoot MTA. We concluded that, using an established large animal model, Biodentine's *in vivo* bioactivity was at least equivalent to the gold standard.

Improving the methodology of the experimental study, a new line of clamps specifically designed by the authors to better fit the dog's teeth and improve the isolation with rubber dam was developed for application in veterinary endodontics (Chapter IV). Once a detailed description of the method of different rubber dam placement techniques has been given for the

isolation of the dog's teeth, this could facilitate the spread of its use in veterinary endodontic treatment and research.

The use of Biodentine in FP has not previously been addressed in literature regarding the human use, as demonstrated by the review of literature focused on case reports of its clinical use in human dentistry (Chapter VI). Literature search was complemented by a description of a series of different applications of Biodentine to dentistry clinical practice selected from our personal experience, including a clinical case of Biodentine use for FP repair. In this latter report, we confirmed that Biodentine can also be used successfully for FP repair.

In summary, the work presented under the scope of this Thesis consolidates investigation in the field of bioceramics and furcation perforation repair and enhances Biodentine's potential in dentistry.

Keywords: Bioceramics; Biodentine; Dog model; Endodontics; Furcation perforation repair; *in vivo*.

Resumo

As perfurações de furca (PF) são condições patológicas de tratamento complexo. Atualmente, as biocerâmicas são boas opções para reparar PF, sendo o ProRoot MTA® o material de eleição. No entanto, este não é desprovido de inconvenientes, levando à necessidade do desenvolvimento de novos cimentos de silicato de cálcio. Entre estes, o Biodentine® ganhou popularidade e, desde a sua aprovação pela FDA em 2009, tem sido empregue em diversas aplicações de uso clínico.

Os principais objetivos da presente Tese foram avaliar o papel do Biodentine como material de reparação de PF e melhorar a metodologia de estudos experimentais de PF e a abordagem na endodontia canina.

Uma revisão da literatura foi realizada para sistematizar os modelos de PF experimentais descritos na literatura e determinar se há evidência de que um modelo seja superior aos outros (Capítulo II). A análise dos dados disponíveis permitiu concluir que, embora não existam modelos que possam ser declarados como ideais e claramente superiores aos demais para estudos de PF, o modelo canino parece apresentar as características mais apropriadas.

Para resumir sistematicamente os resultados do Biodentine quando usado na reparação de PF, em comparação com os materiais atualmente utilizados, foi realizada uma revisão da literatura (Capítulo III). Como resultados a realçar, primeiro, não foram encontrados estudos ou relatos de casos em humanos. O desempenho do Biodentine na reparação de PF foi pouco estudado e os resultados disponíveis encontram-se dispersos e desorganizados. Os estudos sugerem que o Biodentine é um bom material de reparação de PF, maioritariamente com desempenho melhor ou equivalente quando comparado com os materiais atualmente utilizados. No entanto, o uso de Biodentine para reparação de PF necessita de maior validação, nomeadamente por estudos clínicos.

A parte experimental do nosso trabalho consistiu num estudo *in vivo* que comparou os resultados de histologia, radiografia e microtomografia computorizada quatro meses após a reparação de PF com Biodentine ou ProRoot MTA em dentes de cães (Capítulo V). O Biodentine apresentou biocompatibilidade e permitiu a regeneração tecidular, com formação de tecido mineralizado com morfologia e integridade semelhante, mas com maior formação de cemento e menor extrusão de material do que o ProRoot MTA. Concluímos que, utilizando um modelo animal estabelecido, a bioatividade *in vivo* do Biodentine é pelo menos equivalente ao material padrão atual.

Com o objetivo de melhorar a metodologia do estudo experimental, desenvolveu-se uma nova linha de grampos especificamente desenhada pelos autores para melhor adaptação aos dentes do cão, permitindo um melhor isolamento com dique de borracha, para aplicação em endodontia veterinária (Capítulo IV). Foi realizada uma descrição detalhada do método de diferentes técnicas de colocação do dique de borracha para o isolamento dos dentes do cão, com o objetivo de poder facilitar a disseminação do seu uso em tratamentos clínicos e estudos experimentais em endodontia veterinária.

O uso de Biodentine na reparação de PF em humanos não foi abordado anteriormente na literatura, como demonstrado pela revisão da literatura focada em relatos de casos do seu uso clínico em medicina dentária humana (Capítulo VI). Como complemento da pesquisa feita é apresentada uma série de casos de diferentes aplicações de Biodentine na prática clínica de medicina dentária selecionada da nossa experiência pessoal, incluindo um caso clínico de uso de Biodentine para reparação de PF. Neste último, confirmámos que o Biodentine também pode ser usado com sucesso para reparação de PF.

Assim, o trabalho apresentado no âmbito desta Tese consolida a investigação no campo da reparação de perfurações de furca com biocerâmicas e reforça os dados sobre o potencial do Biodentine em aplicações em medicina dentária.

Palavras-chave: Biocerâmicas; Biodentine; Modelo animal - cão; Endodontia; Reparação de perfuração de furca*; in vivo*.

Table of Contents

Index of Tables

Index of Figures

Abbreviations and Acronyms List

CBCT - Cone beam computed tomography

CEM - Calcium-enriched mixture

CHX - Chlorhexidine gluconate

FP - Furcation perforation

IRM - Intermediate restorative material

IQR - Interquartile range

ISO - International organization for standardization

MicroCT - Micro-computed tomography

MTA - Mineral trioxide aggregate

NaOCl - Sodium hypochlorite

prMTA - ProRoot™ MTA (Dentsply Endodontics, Tulsa, OK, USA)

SDR - Smart Dentin Replacement

List of Publications / Submitted Manuscripts

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Chapter I - General Introduction

General Introduction

In today's society, great importance is given to the maintenance of dental pieces as an integral part of a chewing, phonation and aesthetic apparatus. Clinicians should remember the many benefits that well performed endodontics can bring to their patients with the maintenance of the natural tooth with all the well-known advantages. New techniques and materials have been developed, which allow the majority of dentists to achieve favorable results in endodontic treatments.

In the case of a dental injury, where the damaged portion needs replacement or repair, the anatomical characteristics of the lesion, the repair technique, and the physical, chemical and biological properties of the material to be used have equivalent importance in the treatment success. The development of biomaterials that promote the repair of damaged living tissues aiming at the rapid restoration of their physiological functions has been the object of intense and growing investigation around the world.

FURCATION PERFORATIONS

Furcation perforations (FP) are serious pathological conditions in clinical practice which affect the vital prognosis of the tooth and are the second most frequent cause of endodontic treatment failure following obturation.[291] FP can be defined as anomalous communications between the root canal system and the external dental surface in the interradicular region of multirooted teeth, connecting the pulp cavity with the surrounding periodontal tissues. Perforations in this region have been considered one of the major complications leading to failure of endodontic treatment.[330]

These perforations can occur iatrogenically, namely during endodontic treatment, or by internal or external resorption or large carious lesions. Regardless of the cause, a furcation perforation can be a hazardous complication, triggering an inflammatory reaction of the periodontium which may induce irreversible destruction of the periodontal ligament with loss of support, threatening the viability of the tooth.[101,105,291] Timely detection and intervention decrease the likelihood of infection, improving prognosis and often preventing extraction of the affected tooth.[105,124]

The size of the defect and its location in relation to the crestal bone, the length of the root trunk, the existence of periodontal communication, the time lapse between the occurrence of the perforation and the repair, the characteristics of the repair material, the accessibility of the lesion for repair, and other factors such as the technical skill of the clinician and the patient's oral hygiene can affect the long term prognosis.[139,245]

The involved teeth will have a poor prognosis if FP are not adequately and promptly treated. Two main techniques have been proposed for the repair of such defects: intracoronally or through an external surgical access.[58] Since surgical procedures in this context may lead to pocket formation, nonsurgical methods are preferred.[58,267]

Current advances in endodontics and biomaterials made the recovery of tooth structure and function feasible even in the most complicated cases.

REPAIR MATERIALS

An ideal perforation repair material should be nontoxic, noncarcinogenic, nongenotoxic, biocompatible and bioactive, promoting tissue regeneration in the area of the perforation. It is also generally accepted that it should be insoluble in tissue fluids, dimensionally stable, non-resorbable, radiopaque, easy to handle, reasonably priced and bacteriostatic. Furthermore, it should possess good sealing ability and be unaffected by environmental conditions such as the presence of moisture.[232,233,245,269,330]

A plethora of materials have historically been used for perforation repair such as amalgam, gutta-percha, composite resins, glass ionomers, zinc oxide eugenol based cements including Intermediate Restorative Material (IRM™) or Super-EBA™.[341] Although not devoid of drawbacks, mineral trioxide aggregate (MTA) is the current material of choice for FP repair and has demonstrated good potential for clinical success.[235]

Unfortunately, none of these materials fulfill all of the ideal requirements.[147]

Several promising materials have emerged in recent years for the repair of perforations, prompting exhaustive research of their performance, safety and biocompatibility.

Bioceramics

Within the past decade significant changes occurred in endodontic material science, which is continuing to evolve. A new line of research is the increased use of bioceramic technology in endodontics.

The appearance of new bioactive materials with favorable properties to conservatively repair complex clinical situations where there is invasion of the pulp and/or periodontium with dentine loss, such as FP, has stimulated intensive experimental and clinical investigation of the new bioceramics.

Bioceramics are ceramic materials specifically designed for application in medicine and dentistry, which are used to restore normal activity of diseased or damaged parts of the body.[37] Nowadays, they are used in many medical applications, such as dentistry (namely in dental restorations, repair of periodontal disease, maxillofacial reconstruction, prosthodontic devices or orthodontic brackets), orthopedics (joint replacements), cardiology (prosthetic heart valves), neurosurgery (cranioplasty repair), otolaryngology (middle ear implants) and others.

These ceramic materials include alumina and zirconia, bioactive glass, glass ceramics, coatings and composites, hydroxyapatite and resorbable calcium phosphates, and radiotherapy glasses.[37,47]

Bioceramics can be classified into three groups:[37]

- Bioinert ceramics - which result in little or no physiological reaction in the human body, such as alumina or zirconia used for prosthetic devices, and tend to exhibit inherently low levels of reactivity. These are attached by compact morphological fixation.
- Bioactive ceramics - which react in a positive way with local cells, i.e. directly attach by chemical bonds and have a substantially higher level of reactivity such as bioactive glass ceramics.
- Resorbable bioceramics - which are porous or nonporous structures that are slowly and gradually replaced by bone, such as tricalcium phosphate. These show even higher levels of reactivity.

A bioactive material reacts with body fluids promoting the repair processes of the tissue. An important requirement is the formation of a biologically active bone-like apatite layer on its surface in a biological environment. This concept is closely associated with biointeractivity, that is the ability to exchange information within a biological system.[108]

A new group of bioactive bioceramics has been emerging, which can be classified as calcium-silicate cements.

Calcium-silicate cements are hydrophilic materials which tolerate moisture (hydraulic materials) and are capable of polymerizing and hardening (setting), namely in the presence of biological fluids such as blood, plasma, saliva or dentinal fluid. Their ion-leaching properties enable the release of calcium and hydroxyl ions into the surrounding fluids, with alkalinizing activity and creating the conditions for apatite formation.[107,315] Specifically, calcium-silicate particles hydrate and decalcify after mixing with water, with the formation of a calcium silicate hydrate gel and calcium hydroxide.[107,315,317]

Apatite formation is a characteristic shared by calcium silicate–containing biomaterials.[115,344] MTA is a bioactive material which is mainly composed of calcium and silicate.[63,95] Several studies have shown that it can promote hard tissue formation.[36,133,156,159,237,306,338]

1 Mineral Trioxide Aggregate

MTA was developed at Loma Linda University for use as a root-end filling material in surgical endodontic treatment.[63] Since its introduction in the 1990's numerous studies have shown its use in several clinical settings. It was first described in literature in 1993 and was approved for endodontic use by the U.S. Food and Drug Administration in 1998, being first manufactured commercially as ProRoot™ MTA (Dentsply Endodontics, Tulsa, OK, USA) (prMTA).[63,310] Unless stated otherwise, the term ProRoot MTA or its abbreviation prMTA stand for ProRoot™ MTA (Dentsply Endodontics, Tulsa, OK, USA) throughout this Thesis.

Up to 2002, only one MTA version, comprising gray-colored powder, was available. In that year white mineral trioxide aggregate was presented as White ProRoot™ MTA (Dentsply Endodontics, Tulsa, OK, USA) to address esthetic concerns.[310]

MTA has been widely studied and is cited in more than one thousand articles.

MTA materials are a mixture of a refined Portland cement (composed of dicalcium silicate, tricalcium silicate, tricalcium aluminate, gypsum, and tetracalcium aluminoferrite) and bismuth oxide. It has been shown to contain trace amounts of silicon dioxide, calcium oxide, magnesium oxide, potassium sulfate, and sodium sulfate.[63,85,272]

MTA is prepared by mixing the powder with sterile water in a 3:1 powder/liquid ratio.[314] A moist cotton pellet is recommended to be temporarily placed in direct contact with the material, until a follow-up appointment.[262] The mean setting time reported for MTA is 165±5 minutes.[319]

Upon hydration, MTA materials become a colloidal gel that then hardens.[85,319] The setting reaction, a hydration reaction of tricalcium silicate and dicalcium silicate, is purportedly promoted by moisture from the surrounding tissues.[178] Hydrated MTA products have an initial pH of 10.2, which rises to 12.5 three hours after mixing.[63,319] Dicalcium silicate is reported to be responsible for the development of the strength of MTA.[85]

On the basis of available information in literature, MTA appears to be a bioactive material that has the capacity to create an ideal environment for healing.[233]

MTA induces dental tissue regeneration in contact with pulp[100] or periradicular tissue.[101,132,226,339,346] The ability to form hydroxyapatite when exposed to simulated body tissue fluid supports its biocompatibility.[27,272] In general, MTA presents better sealing ability than conventional endodontic repair materials tested by various methods.[262] MTA has been shown not to be affected by tissue fluid or blood contamination,[316] to have low cytotoxicity[109] and antibacterial effects[13] and to be capable of promoting cementogenesis when used as root-end fillings.[318]

When placed in direct contact with human tissues, MTA is reported to:

- Form calcium hydroxide that releases calcium ions, inducing cell attachment and proliferation;[54,56,60,102,204,230,272,304]

- Produce an antibacterial environment by its alkaline pH;[92,103,307]

- Modulate cytokine production;[2,122,135,159,160]

- Promote differentiation and migration of hard tissue–producing cells;[170,311]

- Form hydroxyapatite or carbonated apatite on the MTA surface and provide a biologic seal.[56,261,272]

From the above, MTA has been a revolutionary material in endodontics.[310] These features made MTA the gold standard for perforation repair because of its predictable periodontal ligament regeneration.[101,132,134]

MTA has several clinical applications such as management of internal root resorption, root-end filling, pulp capping, pulpotomy for primary teeth, apical barrier formation for teeth with necrotic pulps and open apexes, perforation repair, apexification and regenerative procedures.[69,75,187,217,221,233,265,269,310]

Despite the many well-known favorable properties, MTA has several disadvantages that justify the need for improvement.[334]

The main drawbacks of MTA include discoloration potential, presence of toxic elements in its composition, poor handling characteristics, long setting time, high price, absence of a known solvent and the difficulty of its removal after cure.[50,55,222,234,240,288,300,335,80,88,104,182,195,206,207,216]

These disadvantages attest to the necessity to create new more ideal restorative materials.[334]

2 New calcium silicate-based cements

Several new calcium silicate-based cements have been launched commercially to overcome the limitations of MTA.[86] Table I.1 describes the composition of the available formulations.

Table I.1: Calcium silicate based materials

Material	Powder composition	Liquid	Manufacturer
Biodentine	Tricalcium silicate; dicalcium silicate; zirconium dioxide; calcium carbonate	Water / Calcium chloride	Septodont, France
ProRoot MTA	Tricalcium silicate; dicalcium silicate; tricalcium aluminate; tetracalcium aluminoferrite; calcium sulfate dihydrate; bismuth oxide	Purified water	Dentsply Tulsa, USA
BioAggregate	Tricalcium silicate; dicalcium silicate; hydroxyapatite; tantalum oxide; calcium silicate oxide; calcium phosphate silicate	Distilled water	Innovative BioCeramix, Vancouver,Canada
Endosequence/ IRoot BP Plus	Tricalcium silicate; dicalcium silicate; zirconium oxide; tantalum pentoxide; calcium sulfate	Premixed (presence of water)	Innovative BioCeramix, Vancouver,Canada
IRoot FS	Tricalcium silicate; dicalcium silicate; calcium phosphate; tantalum oxide; anhydrous calcium sulphate	Presence of water	Innovative BioCeramix, Vancouver,Canada
TheraCal LC	Portland cement; polyethylene glycol dimethacrylate; barium zirconate; barium sulphate	Light cure	Bisco, Inc. Schaumburg, USA
Calcium Enriched Mixture (CEM)	Calcium silicate; calcium phosphate; aluminum oxide; calcium carbonate; calcium sulfate; calcium oxide	Calcium chloride	Bionique Dent, Iran
MM-MTA	Tricalcium silicate; dicalcium silicate; tricalcium aluminate; bismuth oxide; calcium sulfate dehydrate; calcium carbonate	Capsules with powder and liquid	Micro-Mega, France
MTA Plus	Tricalcium silicate; dicalcium silicate; bismuth oxide; sílica; calcium sulfate	Unspecified gel	Avalon Biomed, Bradenton, USA
Neo MTA Plus	Tricalcium silicate; dicalcium silicate; tantalum oxide; sílica; calcium sulfate	Unspecified gel	Avalon Biomed, Bradenton, USA

(Table I.1 continued)

Material	*Powder composition*	*Liquid*	*Manufacturer*
Retro MTA	Calcium zirconia complex; silicon dioxide; aluminum oxide; calcium carbonate	Purified water	BioMTA, Korea
Ortho MTA	Tricalcium silicate; dicalcium silicate; tricalcium aluminate; Tetracalcium aluminoferrite; bismuth oxide; calcium cxide	Purified water	BioMTA, Korea
MTA Angelus	Tricalcium silicate; dicalcium silicate; tricalcium aluminate; tetracalcium aluminoferrite; bismuth oxide	Purified water	Angelus, Londrina, Brazil
MTA HP	Tricalcium silicate; dicalcium silicate; tricalcium aluminate; calcium carbonate; calcium oxide; calcium tungstate	Water and a plasticizing agent	Angelus, Londrina, Brazil
Portland cement	Tricalcium silicate; dicalcium silicate; tricalcium aluminate; tetracalcium aluminoferrite; calcium sulfate dihydrate	Purified water	Elwan Cement Company, Egypt
Tech Biosealer	Tricalcium silicate; dicalcium silicate; bismuth oxide; calcium carbonate; calcium sulfate	Phosphate buffered saline	Isasan, Italy
Endocem MTA	Calcium oxide; silicate oxide; aluminum oxide; magnesium oxide; aluminum oxide; bismuth oxide	Deionized water	Maruchi, Wonju, Korea
Endocem Zr	Calcium oxide; silicate oxide; aluminum oxide; magnesium oxide; aluminum oxide; zirconium oxide	Deionized water	Maruchi, Wonju, Korea
Supra MTA	Tricalcium silicate; mineral oxides	Purified water	Sarp Dental, Lda, Turkey

The composition of each material was written based on the MSDS (Material Safety Data Sheets) provided by the manufacturers.

Although not devoid of drawbacks, the newer products, especially Biodentine, could be promising alternatives to MTA; however, further research is required. Although many have similar basic components and biological effects, they differ from each other in their setting times and physical properties, which may significantly alter their characteristics and performance.[86]

2.1 Biodentine

Biodentine™ (Septodont, Saint-Maur-des-Fosses, France) has recently emerged as a promising material for several endodontic applications. Unless stated otherwise, the term Biodentine stands for Biodentine™ (Septodont, Saint-Maur-des-Fosses, France) throughout this Thesis.

Biodentine was approved for dental use in the USA by FDA in 2009 and is commercially available in Europe since 2010. It is a bioactive cement, in which the powder is mainly composed of tricalcium silicate, with addition of zirconium dioxide and calcium carbonate, and the liquid has calcium chloride that acts as a setting accelerator. Having tricalcium silicate cement as the main component makes it comparable to MTA materials.

Biodentine is currently being used as a dentine substitute under composite resins and shows favorable clinical outcomes.[253] It is a versatile material that has proven to be an advantageous endodontic reparative material in the endodontic armamentarium for surgical procedures such as root-end filling, root perforation repair, or apexification. Biodentine has also been used for vital pulp therapy including pulp capping, pulpotomies, and revascularization procedures.

Even though Biodentine did not reveal favorable results in washout tests in one study,[117] various other tests published in literature revealed promising results, such as ease of handling, fast setting time (12 minutes), increased compressive and push-out bond strength, decreased porosity, high density, color stability, immediate formation of calcium hydroxide, high release and depth of incorporation of calcium ions, low cytotoxicity, induction of cell proliferation and biomineralization and gingival fibroblast viability preservation. Tables I.2 to I.15 summarize the findings of the main studies in literature regarding Biodentine's structural, physical, mechanical and biological properties.

2.1.1 Composition, Setting and Structural Properties

Studies addressing composition, setting and general structural properties of Biodentine under different conditions are summarized in Table I.2.

Table I.2: Studies addressing composition, setting and structural properties of Biodentine

Study	Main findings
Camilleri 2012[62]	Minimal leaching in Hank's balanced salt solution, but higher level of acid-extractable arsenic than the limit set by ISO 9917-1
Camilleri 2013[67]	Composition: tricalcium silicate (main component), calcium carbonate (filler material), zirconium oxide (radiopacifier), dicalcium silicate (traces), calcium oxide (traces), iron oxide (traces) and the liquid is an aqueous solution of a hydrosoluble polymer (water reducing agent) with calcium chloride (decreases the setting time) Faster setting promoted by calcium carbonate additive acting as a nucleation site
Camilleri 2013[64]	Set Biodentine consists of 5μm round particles embedded in a calcium silicate hydrate matrix
Grech 2013[118]	Alkalin cement pH similar to that of BioAggregate™ and IRM™
Grech 2013[117]	Final setting time: 45 minutes High washout with more material lost with each consecutive drop Deposition of substance on the material when immersed in Hank's balanced salt solution, similar to BioAggregate™
Butt 2014[59]	Initial setting time 6.5 ± 1.7 minutes
Jang 2014[137]	Shorter setting time than MTA and BioAggregate™
Natale 2014[219]	Higher ion release than Dycal™ in pH 7.0
Camilleri 2014[65]	Formation of calcium hydroxide which was present in the material matrix and also on the material surface after hydration for 28 days
Setbon 2014[277]	Important differences in composition compared to MTA Angelus™, MM-MTA™, MTA-Caps™ and ProRoot MTA, which directly influence their setting kinetics
Kaup 2015[149]	Lower setting time than ProRoot MTA
Agrafioti 2016[7]	Microstructure changed by exposure to citric acid, showing a relatively smooth surface with more spheroidal crystals
Lucas 2017[183]	Shorter initial (16.2±1.48 minutes) and final setting time (35.4±5.55 minutes) than White MTA Angelus™ and zinc oxide eugenol-based cement Production of an alkaline environment (approximately pH 10)

2.1.2 Handling

One of the acclaimed advantages of Biodentine is to overcome some of MTA drawbacks such as its inconvenient handling. Handling properties have been addressed in studies concerning Biodentine (Table I.3).

Table I.3: Studies addressing handling properties of Biodentine

Study	Main findings
Koubi 2012[166]	Operating time longer than resin modified glass ionomer
Butt 2014[59]	Better handling consistency than MTA Angelus™

2.1.3 Microhardness

Since the structural design of a given material affects its microhardness, Biodentine's microhardness has been studied under different conditions (Table I.4).

Table I.4: Studies addressing microhardness of Biodentine

Study	Main findings
Camilleri 2013[64]	Microhardness not affected by etching Higher surface microhardness than unetched conventional glass ionomer or resin modified glass ionomer, but similar microhardness to the etched materials
Grech 2013[117]	Higher microhardness than BioAggregate™ and IRM™
Caronna 2014[74]	Hardness not affected by setting in moist or dry conditions
Kaup 2015[149]	Higher microhardness than ProRoot MTA
Antonijević 2016[24]	Better micromechanical properties (microhardness, average unloading slope and indentation distance increase) than Portland cement™ and experimental calcium silicate cement Improved micromechanical surface performance after immersion in simulated body fluid Reduced microhardness by acid-etching treatment
Majeed 2017[188]	Higher microhardness than BioAggregate™

2.1.4 Compressive Strength

Given its clinical uses, it is vital that a cement used in dentistry has sufficient compressive strength to resist external impacts in order to be strong enough to withstand masticatory forces. Therefore, compressive strength is considered as one of the main physical characteristics of hydraulic cements. Studies testing this property in Biodentine show good results (Table I.5).

Table I.5: Studies addressing Biodentine's compressive strength

Study	Main findings
Sawyer 2012[273]	Biodentine and MTA Plus reduce the ability of dentine to resist deformation (strength) and to absorb energy without fracturing (toughness)
Grech 2013[117]	Enhanced compressive strength due to the low water/cement ratio
Kayahan 2013[150]	Compressive strength not affected by acid etching procedures
Butt 2014[59]	Higher compressive strength than MTA Angelus™, close to that reported for human dentine
Jang 2014[137]	Higher compressive strength than BioAggregate™
Natale 2014[219]	Superior flexural strength, flexural modulus and compressive strength than MTA Angelus™ and Dycal™
Alsubait 2016[19]	Compressive strength not affected by acid-etching, however higher compressive strengths were achieved by acid etching at 7 days than by etching at 24 hours
Elnaghy 2016[94]	Resistance to root fracture after 12 months similar to White MTA Angelus™
Ranjkesh 2016[258]	Inferior early diametral tensile strength than Protooth™ Diametral tensile strength not affected by humid condition during 180 days
Govindaraju 2017[116]	Higher compressive strength than White ProRoot MTA, NeoMTA Plus™ and MTA Angelus™ Compressive strength reduced by EDTA but not affected by sodium hypochlorite
Lucas 2017[183]	Higher compressive strength and higher dentine bond strength than White MTA
Subramanyam 2017[302]	Compressive strength not adversely affected by contamination with oral tissue fluids like blood and saliva, similar to ProRoot MTA

2.1.5 Push-out Bond Strength

An ideal endodontic biomaterial should adhere to the cavity walls and resist dislodging forces to help maintain the integrity of the root filling–dentine interface either under static conditions or during function and operative procedures. Hence, a sufficient amount of bond strength with dentinal walls is needed to prevent dislodgement from the repair site. Mechanical tests that measure the dislodgement resistance of dental materials are commonly used not only in endodontics but also in other fields of dentistry. The push-out bond test is a reliable method for measuring the dentinal bond strength of endodontic cements and the test's loading simulates clinical stresses. Table I.6 summarizes studies addressing Biodentine's push-out bond strength.

Table I.6: Studies addressing Biodentine's push-out bond strength

Study	Main findings
Poplai 2012[244]	Bond strength impaired at low pH values (4.4)
Aggarwal 2013[4]	Push-out bond strength not affected by blood contamination Higher push-out bond strength with increase in setting time from 24 hours to 7 days
El-Ma'aita 2013[91]	Smear layer removal worsens push-out bond strength between calcium silicate cements and dentine
Guneser 2013[120]	Push-out bond strength not influenced by endodontic irrigants, such as sodium hypochlorite, chlorhexidine, and saline Higher push-out bond strength than MTA
Üstün 2015[326]	Dislocation resistance not affected by blood contamination of furcation perforations Lower bond strength than ProRoot MTA and RetroMTA™
Akcay 2016[10]	Higher bond strength than MTA Bond strength as root-end filling material negatively affected by the presence of blood Suitable for apical surgery
Centenaro 2016[77]	Push-out bond strength not affected by the prior use of calcium hydroxide, similar to MTA Angelus™ Higher push-out bond strength than MTA Angelus™ regardless of the use of calcium hydroxide
Küçükkaya Eren 2016[168]	Enhanced bond strength by ultrasonic activation Higher bond strength than MTA Angelus™
Nagas 2016[212]	Higher bond strength to root canal dentine than ProRoot MTA Increased dislodgment resistance with prior intracanal placement of calcium hydroxide in distilled water
Silva 2016[283]	Higher push-out bond strength than MTA HP™ and White MTA Angelus™

Ulusoy 2016[324]	Higher resistance to dislodgement than BioAggregate™ Dislodgement resistance from root dentine influenced by remaining dentine thickness (thinner remaining dentine associated with lower dislodgement resistance), which determines dentinal tubular density
Alsubait 2017[20]	Higher bond strength than White ProRoot MTA, EndoSequence™ and NeoMTA Plus™ Decreased push-out bond strength after exposure to 2.5% sodium hypochlorite during the early setting phase
Ashofteh Yadzi 2017[28]	Higher push-out bond strength than White ProRoot MTA, EndoSequence™ and CEM in different exposure times Suitable for situations encountering higher dislocation forces in a short time after cement application
do Carmo 2017[72]	Precipitation of crystals and increased dislodgment resistance from root-end cavities after contact with PBS for 14 days
Majeed 2017[188]	Higher bond strength than BioAggregate™ Higher displacement force than ProRoot MTA Suitable material for root repair procedures and retrograde fillings
Nagas 2017[213]	Higher dislocation resistance than ProRoot MTA as a perforation repair material No adverse effect of Er,Cr:YSGG laser activation of irrigation aqueous solutions on push-out dentine bond strength

2.1.6 Shear Bond Strength

Given that Biodentine can be used as a dentine substitute under permanent restorations, several authors studied its bond strength with different bonding systems. Results are summarized in Table I.7.

Table I.7: Studies addressing Biodentine's shear bond strength

Study	Main findings
Odabas 2013[229]	Bond strength to resine composite not affected by type of adhesive system (etch-and-rinse, two-step self-etch, or one-step self-etch) Highest bond strength value for two-step self-etch adhesive at a 24-hour period
Al-Ashou 2014[11]	High shear bond strength with resin composite, glass ionomer restorative filling material, self-adhesive resin cement and poly-acid modified resin composite
Cantekin 2014[70]	Higher shear bond strength than ProRoot MTA when used with the methacrylate-based composite Low bond strength to silorane-based composite and to glass ionomer cement, similar to ProRoot MTA
Hashem 2014[130]	Weak restorative material in its early setting phase Adequate setting/maturation after 2 weeks

Raju 2014[254]	Less shear bond strength than glass ionomer cement in both primary and permanent teeth Good marginal integrity
Altunsoy 2015[21]	Lower shear bond strength to a self-adhering flowable composite than CEM
Kaup 2015[148]	Higher adhesion to dentine surface than ProRoot MTA
Abdelmegid 2016[1]	Lower shear bond strength to caries-affected dentine of primary teeth than Smart Dentin Replacement (SDR™), Multicore Flow™ and Fuji II LC™
Alkhudhairy 2016[18]	Worse shear bond strength than SDR™
Deepa 2016[87]	Weak material in its early setting phase. Lower bond strength when immediately bonded to composite resin than TheraCal LC™ or resin-modified glass ionomer cement
Meraji 2017[202]	Surface modified by etching, unlike TheraCal LC™ and Fuji IX™ Weaker bond to composite resin than TheraCal LC™ and Fuji IX™, with complete failure of bonding shown after demolding and thermocycling

2.1.7 Sealing Ability/Marginal Adaptation

An ideal root-end filling material should adhere to the prepared cavity walls forming a tight seal in the root canal system. The quality of apical seal achieved by root-end filling materials has been assessed by various means such as the degree of leakage measured by dye, bacterial, glucose or fluid filtration techniques. Many studies assessed the sealing ability and marginal adaptation of Biodentine under various conditions (Table I.8).

Table I.8: Studies addressing sealing ability/marginal adaptation of Biodentine

Study	Main findings
Kokate 2012[162]	Less leakage than MTA and glass ionomer cement
Raskin 2012[259]	Adequate marginal seal at the interface of enamel, dentine and dentine-bonding agents
Koubi 2012[166]	Microleakage similar to that of resin-modified glass ionomer cement
Camilleri 2013[64]	Leakage at the dentine to material interface either with or without etching
Koubi 2013[165]	Good marginal adaptation after restoration of posterior teeth
Butt 2014[59]	Less leakage than white MTA Angelus™ at 4 and 24hours, but similar at 1, 2, 4, 8, and 12 weeks Valid and stable apical seal
Jeevani 2014[138]	Worse sealing ability than Endosequence™

Raju 2014[254]	Good marginal integrity Less microleakage at the junctional interface between material and dentine both in primary and permanent teeth than glass ionomer cement
Ravichandra 2014[260]	Better marginal adaptation than glass ionomer cement and ProRoot MTA
Soundappan 2014[298]	Similar marginal adaptation at 1mm level to ProRoot MTA and IRM™, but lower than ProRoot MTA at 2mm level, when used as retrograde filling material Inferior overall marginal adaptation compared to ProRoot MTA and IRM™
Aggarwal 2015[5]	Better marginal adaptation than MTA Plus™, similar to glass ionomer cement, when used as a dentine substitute under composite restorations in “open sandwich” technique Can be used as dentine substitute materials in “open sandwich” class II restorations
Bani 2015[44]	Apical sealing ability, similar to ProRoot MTA at any apical plug thickness Reduction of the apical plug thickness significantly increases apical microleakage 3 and 4mm thickness of apical plugs revealed a good sealing ability
Bolhari 2015[51]	Marginal adaptation not affected by blood contamination, similar to ProRoot MTA, CEM and BioAggregate™
Gupta 2015[121]	More microleakage when manually manipulated than with machine trituration
Mandava 2015[190]	Higher microleakage than White ProRoot MTA
Naik 2015[215]	Less microleakage than ProRoot MTA, irrespective of the presence of smear layer Sealing ability improved by the removal of smear layer
Nanjappa 2015[218]	Lower microleakage than MTA and Chitra-calcium phosphate cement Promising material for use as a root-end filling material
Sinkar 2015[293]	Better sealing ability than RetroMTA™
Suri 2015[303]	Sealing ability enhanced by the addition of 2% chlorhexidine to the liquid
Agrafioti 2016[7]	Ability to prevent fluid movement over time enhanced in acidic environment
Alkhudhairy 2016[18]	Worse microleakage than SDR™
Katge 2016[147]	Sealing ability in furcation repair similar to MTA Plus™
Ramazani 2016[255]	Similar bacterial leakage to ProRoot MTA Promising results as perforation repair materials; suitable for repair of furcation perforation of primary molars
Abraham 2017[3]	Superior cavity adaptation at dentine/liner interface than ProRoot MTA when used as a composite liner

Küçükkaya Eren 2017[169]	Sealing ability and marginal adaptation negatively affected by laser application
Ramezanali 2017[256]	Similar coronal microleakage to CEM cement and MTA Angelus™ Efficient coronal seal when used as an intra-orifice barrier in endodontically treated teeth
Tanomaru-Filho 2017[308]	Higher filling ability than MTA Angelus™ and zinc oxide eugenol Higher lateral cavity filling than zinc oxide eugenol
Tsesis 2017[322]	Depth of bacterial penetration into the dentinal tubules similar to ProRoot MTA and IRM™ Less viability of the colonized bacteria than ProRoot MTA

2.1.8 Radiopacity

Radiopacity is an important property of retrograde or repair materials, as they are generally applied in low thicknesses and need to be distinguished from the surrounding tissues. Table I.9 summarizes findings of studies addressing Biodentine's radiopacity.

Table I.9: Studies addressing radiopacity of Biodentine

Study	Main findings
Grech 2013[117]	Lower radiopacity (4.1 and 3.3mmAl respectively after 1 and 28 days) than BioAggregate™ and IRM™, but higher than 3mmAl (established as the minimum radiopacity value for endodontic cements by ISO 6876 specifications)
Tanalp 2013[305]	Higher radiopacity than dentine Lower radiopacity than MM-MTA™ and MTA Angelus™ Lower mean radiopacity (2.8 ± 0.48mmAl) than required by ISO 6876 specifications
Ceci 2015[76]	Lower values of radiopacity than MTA Angelus™ and ProRoot MTA
Kaup 2015[149]	Lower radiopacity than ProRoot MTA; not in accordance with ISO 6876 specifications
Lucas 2017[183]	Inferior radiopacity (2.79±0.27mmAl) than required by ISO 6876 specifications

2.1.9 Porosity

Hermetic sealing is crucial when bioceramics are used in cases such as perforation repair, vital pulp treatments or retrograde fillings. Since the degree of porosity is a critical factor determining the amount of leakage and has an impact upon other factors including adsorption, permeability, strength and density, it has been addressed in studies involving Biodentine's characterization (Table I.10).

Table I.10: Studies addressing porosity of Biodentine

Study	Main findings
Camilleri 2013[68]	Denser and less porous than MTA Angelus™
de Souza 2013[299]	Similar porosity to IRoot BP Plus™, Ceramicrete™, and ProRoot MTA
Camilleri 2014[61]	Lower porosity than BioAggregate™, prototype radiopacified tricalcium silicate cement and IRM™ Cracks in the material and gaps at the root dentine to Biodentine interface associated with dry storage

2.1.10 Colour Stability

Esthetics play an important role in dentistry and discoloration of a single tooth can have a significant impact on the patient's quality of life. Many materials used in endodontic procedures can lead to tooth discoloration with an unesthetic outcome. Therefore, an optimal material for use in dentistry should fulfil both functional and esthetic criteria, especially in the anterior region. Extrinsic discoloration of silicate filling materials and dental tissues can be measured visually and with specific instruments. Table I.11 summarizes studies focusing on colour stability of Biodentine under various settings.

Table I.11: Studies addressing colour stability with Biodentine

Study	Main findings
Vallés 2013[328]	Colour stability over time independent of oxygen and light irradiation Suitable for use under light-cured restorative materials in esthetically sensitive areas
Beatty 2015[46]	Higher bovine tooth discoloration after 8 weeks than ProRoot MTA
Keskin 2015[151]	Clinically perceptible discoloration when immersed in sodium hypochlorite (NaOCl) and chlorhexidine gluconate (CHX), however lower than White ProRoot MTA Absence of clinically perceptible discoloration caused by distilled water in material Higher discoloration when immersed in CHX than in NaOCl
Kohli 2015[161]	Absence of perceptible colour change in the tooth structure when left in the pulp chamber for extended periods of time (6 months), similar to EndoSequence™ and AH Plus™ sealer, and unlike triple antibiotic paste, White ProRoot MTA and gray MTA

Vallés 2015[329]	Color stability, with better results than White ProRoot MTA, which showed increasing discoloration over time
Marconyak 2016[192]	Less discoloration than White ProRoot MTA, MTA Angelus™, and ProRoot MTA, similar to EndoSequence™
Ramos 2016[257]	Delayed (1 year) tooth discoloration, less evident than for White ProRoot MTA Less decrease in luminance than White ProRoot MTA
Shokouhinejad 2016[281]	Increased discoloration associated with blood contamination Less tooth discoloration than Ortho-MTA™ in the absence of blood
Yoldas 2016[340]	Lower discoloration potential than BioAggregate™ and MTA Angelus™ in the presence of blood

2.1.11 Solubility

Some degree of porosity is characteristic of dental cements prepared by mixing the powder and liquid due to incorporation of microscopic air bubbles during the mixing operation. Classically, lack of solubility has been mentioned as one of the ideal characteristics of root-end filling materials because endodontic and restorative materials should provide a long-term seal and avoid leakage from the oral cavity and/or the periapical tissue. However, it has also been stated that cements which form calcium hydroxide or calcium oxide during setting, such as MTA or Biodentine, should present a certain degree of solubility to improve the mineralization process in contact with vital tissue.[149] Studies concerning Biodentine's solubility are summarized in Table I.12.

Table I.12: Studies addressing solubility of Biodentine

Study	Main findings
Grech 2013[117]	Low fluid uptake and absorption in Hank's balanced salt solution, similar to IRM™ Negative solubility values (does not loose particulate matter to result in dimensional instability)
Ceci 2015[76]	Lower solubility than IRM™, similar to MTA Angelus™ and ProRoot MTA; fulfills the requirements of ISO 6876
Kaup 2015[149]	More soluble than ProRoot MTA, but fulfills ISO 6876 requirements Increased solubility may be an advantage in regard to bioactivity
Singh 2015[292]	Higher solubility than IRM™, Fuji IX™ and ProRoot MTA Suggests that solubility may be a contributing factor towards clinical healing

2.1.12 Biocompatibility

Biocompatibility of a dental material is a major factor that should be taken into consideration specifically when it is used in pulp capping, perforation repair or as a retrograde filling. During the aforementioned procedures, the material is in direct contact with the connective tissue and has the potential to affect the viability of periradicular and pulpal cells. Therefore, it is essential that toxic materials are avoided and materials promoting repair or that are biologically neutral are preferred during procedures in which the material is directly in contact with the surrounding tissue. Studies addressing Biodentine's biocompatibility generally attest its lack of cytotoxicity and tissue acceptability (Table I.13).

Table I.13: Studies addressing biocompatibility of Biodentine

Study	Main findings
Laurent 2008[175]	Non-cytotoxic and non-genotoxic for pulp fibroblasts at any concentration Specific functions of pulp fibroblasts not modified when Biodentine is used as a direct pulp-capping agent or as a lining material
Shayegan 2012[280]	Highly biocompatible after *in vivo* (pig) direct pulp capping, similar to White ProRoot MTA No signs of moderate or severe pulp inflammation response Complete calcified bridge with normal pulp tissue 90 days after pulp capping Thick calcification under the pulpotomy site
Marijana 2013[194]	Biocompatible after *in vivo* (Vietnamese pigs) pulp capping Pulp reaction similar to ProRoot MTA Preservation of functional and morphological integrity of the pulp Induction of dentine bridge formation
Pérard 2013[239]	Similar biocompatibility to ProRoot MTA in cell cultures May slightly modify the proliferation of cells from odontoblastic or undifferentiated pulp cell lines Weak cytotoxic effects of both Biodentine and ProRoot MTA on internal cells
Attik 2014[32]	Biocompatible High cellular viability of osteoblast-like cells, similar to White ProRoot MTA
Corral Nuñez 2014[82]	Similar cytotoxicity and induction of a similar pattern of cytokine expression to ProRoot MTA
Jang 2014[137]	Biocompatible Absence of cytotoxic effects on human periodontal ligament fibroblasts Somewhat higher cytotoxicity than MTA
Jung 2014[142]	No cytotoxicity and good biocompatibility in contact with the human osteoblasts and periodontal ligament cells

Khedmat 2014[153]	Biocompatible, similar to ProRoot MTA Increasing storage time improved viability of monocytes Less cytotoxic than CEM and Biosealer™ after 48 hours of incubation
Lee 2014[177]	Lower cell viability than ProRoot MTA and BioAggregate™ at higher concentrations, but similar at lower concentrations
Mori 2014[208]	Biocompatible Nonsignificant or mild presence of inflammatory reaction 14 days after subcutaneous implantation in rat
Poggio 2014[242]	Lower cytotoxicity than calcium hydroxide-based materials
Rossi 2014[264]	Biocompatible Formation of mineralized tissue bridge after pulpotomy with similar morphology and integrity to White ProRoot MTA
Bortoluzzi 2015[54]	Time and concentration dependent cytotoxic effects
Ceci 2015[76]	Biocompatible Promotion of cell vitality of murine odontoblasts
Chang 2015[295]	Biocompatible Favorable cell viability, similar to Ortho-MTA™ and MTA Angelus™ and superior to IRM™ Formation of mineralized nodules and expression of odontoblastic marker genes, similar to Ortho-MTA™, MTA Angelus™ and IRM™ Lower levels of proinflammatory mediators than IRM™
Margunato 2015[193]	Biocompatible Absence of cytotoxic effect on human bone marrow stem cells after 14 days in culture Stimulation of osteogenic differentiation of human bone marrow stem cells, similar to MM-MTA™, however lower than ProRoot MTA
Poggio 2015[243]	Lower cytotoxicity than calcium hydroxide-based materials, similar to ProRoot MTA, in murine odontoblasts Suitable for pulp-capping treatment
Simsek 2015[289]	Higher biocompatibility than MM-MTA™ and BioAggregate™ one week after subcutaneous implantation in rat Similar biocompatibility to MM-MTA™ and BioAggregate™ after 45 days Quicker inflammation decline
Akbulut 2016[9]	Biocompatible Supported periodontal ligament fibroblasts adhesion and spreading
Escobar-Garcia 2016[96]	Biocompatible Not cytotoxic when evaluated in cultured fibroblasts of periodontal ligament in incubation periods of up to 5 days
da Fonseca 2016[99]	Biocompatible Regression in inflammatory reaction at 60 days after dorsal subcutaneous implantation in rat Gradual reduction in the immunoexpression of IL-6, a proinflammatory cytokine, from 7 to 60 days Immunoexpression of IL-6 at 60 days similar to White MTA Angelus™

Kim 2016[157]	Biocompatible Induction of favorable effects on the reparative process during vital pulp therapy Hard tissue formation after pulp capping in rat
Küçükkaya 2016[167]	Similar cytotoxicity profile to White MTA Angelus™ and CEM
Michel 2016[203]	Low levels of cytotoxicity which do not seem to increase over time Allowed for primary human gingival fibroblasts and human osteoblast cells proliferation
Nikfarjam 2016[223]	Biocompatible No influence on cell morphology, proliferation or cell integrity
Silva 2016[282]	Low cytotoxicity in a 3D cell culture model associated with an *in situ* root-end filling model, similar to White MTA Angelus™ Overproduction of IL-1α, similar to White MTA Angelus™
Saberi 2016[266]	Optimal biocompatibility when exposed to stem cells from the apical papilla Less cytotoxicity than CEM and octacalcium phosphate on human gingival fibroblasts, similar to ProRoot MTA Decreased cytotoxicity over time
Simsek 2016[290]	Nontoxic trace elements in brain, kidney and liver samples of rats after subcutaneous implantation
Arias-Moliz 2017[25]	Similar biocompatibility to TheraCal LC™ 3 days after cell culture exposure, although initially worse
Köseoğlu 2017[164]	Biocompatibility and cytotoxicity better than MTA Angelus™ and similar to Endocem™ and MM-MTA™ in mouse fibroblast cell culture
Rodrigues 2017[263]	Biocompatible Not cytotoxic at the higher dilution (1:8) to transgenic cultures of human osteoblast cells overexpressing BMP-2, similar to MTA Angelus™
Silva 2017[285]	No bone resorption, fewer inflammatory cells, and greater RUNX2 immunostaining intensity than gutta-percha (positive control), and similar to White ProRoot MTA (considered negative control), after furcation perforation repair in dogs

2.1.13 Bioactivity

A bioactive material reacts chemically with body fluids in a manner compatible with the repair processes of the tissue. Hence, a suitable dentine replacement material will initiate formation of secondary dentine once placed over the pulp, maintaining pulp vitality. The bioactivity of Biodentine has been extensively studied (Table I.14).

Table I.14: Studies addressing bioactivity of Biodentine

Study	Main findings
Han 2011[125]	Higher amount of calcium ions released and depth of incorporation into human root canal dentine than White ProRoot MTA
Atmeh 2012[31]	Intertubular diffusion of carbonate into dentine, leading to ‘‘mineral infiltration zone’’ formation Highly dynamic and interactive dentine-Biodentine interface
Laurent 2012[174]	Induction of cell proliferation and biomineralization in a human entire tooth culture model Increased TGF-β1 (growth factor which signals reparative dentinogenesis with role in angiogenesis, recruitment of progenitor cells, cell differentiation and mineralization) secretion from pulp cells Induction of an early form of reparative dentine synthesis
Leiendecker 2012[179]	Adverse effect on the integrity of the dentine collagen matrix
Shayegan 2012[280]	Bioactive properties Promotes hard tissue regeneration *in vivo* Marginal integrity due to the formation of hydroxyapatite crystals at the surface, enhancing sealing potential
Tran 2012[320]	Reparative dentine synthesis shown by expression of dentine sialoprotein and osteopontin after application to mechanically exposed rat pulps Dentine bridge formation, well localized at the injury site, with percentage porosity comparable to White ProRoot MTA and better than calcium hydroxide
Zanini 2012[342]	Bioactive on immortalized murine pulp cells Induction of cell proliferation and biomineralization Suitable pulp capping material
Camilleri 2013[68]	Bioactivity indicated by the deposition of hydroxyapatite on Biodentine when exposed to simulated body fluid
Chalas 2013[78]	Active dental material Formation of an interfacial layer at the Biodentine/dentine border
Gjorgievska 2013[111]	Favorable results as dentine substitute - Biodentine crystals firmly attached to the underlying dentine surface
Han 2013[126]	Higher amount of calcium ions released than EndoSequence™ BC sealer
Nowicka 2013[227]	Induction of a dentine bridge after pulp capping in caries-free intact human molars Complete dentinal bridge formation and absence of inflammatory response
Camilleri 2014[66]	Composition optimized, and the environmental conditions did not affect material microstructure or hydration Formation of calcium hydroxide and calcium ion leaching, beneficial to the dental pulp

Jung 2014[142]	Well-tolerated endodontic material with stimulatory bioactive properties Good proliferation and cell attachment Organized spreading and parallel alignment of the human periodontal ligament cells Higher cell density in osteoblast and human periodontal ligament cell culture than amalgam and composite resin, similar to ProRoot MTA
Lee 2014[177]	Osteogenic differentiation of mesenchymal cells into osteoblasts
Luo 2014[185]	Bioactive Favorably affected healing when placed directly in contact with the pulp by enhancing the proliferation, migration, and adhesion of human dental pulp stem cells
Luo 2014[184]	Bioactive Induction of odontoblast differentiation of human dental pulp stem cells, regulated via mitogen-activated protein kinase and calcium-/calmodulin-dependent protein kinase II pathways
Atmeh 2015[30]	Greater mineralization in totally demineralized dentine than glass ionomer cement
Bortoluzzi 2015[54]	Enhanced osteogenic differentiation of human dental pulp stem cells after exposure to Biodentine without its cytotoxic components Extracellular mineralization better than zinc oxide-eugenol-based cement Favorable tissue response anticipated with the use as a blood clot-protecting material for pulpal revascularization
Jung 2015[141]	Odontoblastic differentiation and mineralization nodule formation by activating the mitogen-activated protein kinase pathway, similar to MTA Useful for regenerative endodontic procedures
Kim 2015[158]	Bioactive Formation of interfacial layer on the root canal dentine
Tziafa 2015[323]	Tertiary dentine with occasional intermediate formation of osteodentin observed after restoration of deep dentinal cavities of miniature swine teeth with or without the application of a calcium hydroxide-containing pulp protective base Higher thickness of the tertiary dentine zone in the absence of the protective base
Agrafioti 2016[6]	Higher induction of cell proliferation than ProRoot MTA Dental pulp stem cells viability and adherence enhanced by setting in the presence of citric acid
Costa 2016[83]	Proliferation and differentiation of human mesenchymal stem cells and human umbilical vein endothelial cells Slightly lower overall osteogenic and angiogenic outcome than ProRoot MTA and MTA Plus™

El Karim 2016[144]	Reduced Tumor Necrosis Factor Alpha–induced TRPA1 expression (an ion channel that is considered the gatekeeper of pain and inflammation) in odontoblast-like cells Potential for modulating nociceptive receptors in odontoblasts, possibly with implications in postoperative pain relief
Mullaguri 2016[210]	Higher TGF-β1 release and integrity of fibrin structure than glass ionomer cement, and IRM, when layered over platelet-rich fibrin
Peters 2016[241]	Enhanced angiogenesis Increased cell viability 1 day after exposure of stem cells from the apical papilla
Widbiller 2016[337]	Cell proliferation and bioactive effect on three-dimensionally cultured dental pulp stem cells by induction of mineralization-associated gene expression, similar to ProRoot MTA
Aksoy 2017[171]	Increased calcium release during the first 7 days followed by a linear decrease Higher hydroxide rates than MTA, TheraCal LC™ and Calcimol™ Stimulation of hard tissue formation and ion-releasing ability Suitable for indirect pulp capping
Arias-Moliz 2017[25]	Higher amounts of calcium leached than TheraCal LC™ Uptake of phosphorus from solution 1 day after immersion in Hank's balanced salt solution
Corral Nuñez 2017[81]	Bioactivity improved by incorporation of bioactive glass nanoparticles, accelerating the formation of a crystalline apatite layer on its surface after immersion in simulated body fluid and greatly enhances the formation of a mineral-rich interfacial layer when in contact with dentine
Gandolfi 2017[106]	Osteoinductive properties with dynamic biomineralization processes 30 days after *in vivo* repair of surgical bone defects in rabbit tibiae Formation of new trabecular bone with marrow spaces and sparse traces of residual material Absence of destructive inflammatory processes or of necrotic processes at the interface with the pre-existing bone Angiogenic activity with neovascularization and formation of capillaries Osteoid matrix deposition by activated osteoblasts Formation of direct bond with the mineralized bone matrix
Giraud 2017[110]	Use as control confirmed the bioactivity and capacity to induce the specific functions of pulp cells necessary for the regenerative process
Gomes-Cornelio 2017[114]	Bioactive Dose-dependent responses in human osteoblastic cell viability Enhanced cell proliferation
Li 2017[181]	Higher speed and intensity of re-mineralization than TheraCal LC™ Overall dentine re-mineralization efficacy appeared insufficient, as the deepest demineralized dentine zone was never re-mineralized, similar to TheraCal LC™ and ProRoot MTA
Rodrigues 2017[263]	Bioactive Stimulatory effect on the formation of mineralized nodules Suitable material to improve osteoblastic cell mineralization

2.1.14 Antimicrobial Activity

Microorganisms play an important role in the development and progression of pulpal and periapical disease and in the failure of endodontic treatment. Therefore, it is imperative to eliminate microorganisms and infected tissue and achieve an effective seal to prevent recontamination. A perforation repair material that has antimicrobial properties might theoretically eliminate residual microorganisms located around it. Biodentine's antimicrobial properties, both antibacterial and antifungal, have been studied (Table I.15).

Table I.15: Studies addressing antimicrobial properties of Biodentine

Study	Main findings
Kharabe 2014[152]	Antimicrobial activity against aerobes and facultative anaerobes
Bhavana 2015[49]	Higher antimicrobial activity than ProRoot MTA and glass ionomer cement Antifungal (Candida) activity
Poggio 2014[242]	Antibacterial activity
Ceci 2015[76]	Larger inhibition zone for *S. sanguis* than MTA Angelus™ and ProRoot MTA Lower inhibition zone for *S. salivarius* than MTA Angelus™ and ProRoot MTA No inhibition zone for *S. mutans*
Hiremath 2015[131]	Antibacterial property and antifungal activity
Koruyucu 2015[163]	Antibacterial activity, similar to MTA Angelus™
Özyürek 2016[231]	Antimicrobial activity against *E. coli*, *S. aureus*, *P. aeruginosa*, and *E. faecium* Higher antimicrobial activity against *E. coli* and *S. aureus* than MTA Angelus™ Lower antimicrobial activity against *P. aeruginosa* and *E. faecium* than MTA Angelus™
Arias-Moliz 2017[25]	Absence of antimicrobial activity

Randomized Clinical Trials

Table I.16 summarizes the randomized clinical trials which used Biodentine as a test material.

Table I.16: Randomized controlled clinical trials using Biodentine

Clinical application	n	BD group (n)	Comparator materials (n)	Type of teeth	Follow-up	Author, date
Performance and safety in the restoration of posterior teeth	212	96	Z100 (116)	1st premolar to 3rd molar	1 yr	Koubi, 2013[165]
Indirect pulp capping materials in patients with reversible pulpitis	na	na	Glass ionomer cement Fuji IX™ (na)	85% were molars	1 yr	Hashem, 2015[129]
Pulpotomy in primary teeth	75	25	ProRoot MTA (25); Propolis™ (25)	Primary molars	9 mt	Kusum, 2015[172]
	60	20	MTA Angelus™ (20); Diode Laser (20)	Primary molars	6 mt	Niranjani, 2015[225]
	78	39	MTA (39)	Primary molars	1 yr	Cuadros-Fernández, 2016[84]
	112	56	Formocresol (56)	Primary molars	6 mt	El Meligy, 2016[200]
	30	15	Calcium Hydroxide (15)	Primary molars	1 yr	Grewal, 2016[119]
	88	44	ProRoot MTA (44)	Primary molars	12 mt	Togaru, 2016[312]
	58	19	White ProRoot MTA (22); Tempophore™ (17)	Primary molars	18 mt	Rajasekharan, 2017[252]
Tomographic evaluations of reparative dentine bridges formed after direct pulp capping	44	11	White ProRoot MTA (11); Calcium Hydroxide (11); Single Bond Universal™ (11)	Intact 3rd molars scheduled for extraction	6 w	Nowicka, 2015[228]
Direct pulp-capping agent in young permanent molars	42	21	MTA Angelus™ (21)	Young permanent molars	12 mt	Katge, 2017[146]

n: number of teeth; BD: Biodentine; yr: year(s); na: information not available; MTA: mineral trioxide aggregate; mt: months, w:weeks.

From the above properties of Biodentine, it is perceptible that the endodontic indications of this novel material are similar to MTA, but are reported to offer several advantages including better consistency than MTA, suited to clinical use; improved handling; and quicker setting time, which eliminates the two step obturation needed with MTA and decreases the risk of bacterial contamination.

Because of its favorable reported properties, Biodentine could be an adequate alternative to MTA[86] to be used in a variety of indications in the field of endodontics, dental traumatology, restorative dentistry and paediatric dentistry.[253]

OBJECTIVES

This Thesis globally aims to give insight into furcation perforation repair options and can be divided into two complementary arms:

To evaluate the role of Biodentine as a FP repair material;

To improve the methodology of experimental studies of FP and approach for canine endodontics.

Specific objectives

A) To identify FP experimental animal models validated in literature, to determine whether there is evidence that one is superior to others and to provide a comprehensive synthesis to aid and homogenize future preclinical investigation in this field.

B) To systematically summarize what is known about Biodentine's results when used in FP repair, compared with currently used materials.

C) To develop and describe a new line of clamps made especially for dogs in order to improve dental dam isolation efficiency without damaging the furcation of the tooth and review step-by-step the procedure and materials used for isolating the operative field in dogs' endodontic treatments.

D) To compare the *in vivo* histological responses and radiographic and micro-computed tomographic outcomes after FP repair with Biodentine or ProRoot MTA in dogs' teeth.

E) To summarize the clinical applications of Biodentine reported in literature and describe a personal case series in this setting, including the first case of FP repair with Biodentine.

Chapter II - Animal Models Used in Furcation Perforation Studies: a Systematic Review and Comprehensive Synthesis of Model Characteristics

Part of this chapter has been based on the following article:

Cardoso M, Catré D, Noites R, Paulo M, Viegas C. Animal Models Used In Furcation Perforation Studies - A Systematic Review And Comprehensive Synthesis Of Model Characteristics. Australian Endodontic Journal. *In Press* DOI: 10.1111/aej.12221

Animal Models of Furcation Perforation - Systematic Review of Model Characteristics and a Comprehensive Synthesis

INTRODUCTION

Furcation perforations are serious pathological conditions in clinical practice which affect the vital prognosis of the tooth and are the second most frequent cause of endodontic treatment failure following obturation.[291] FP can be defined as anomalous communications between the root canal system and the external dental surface in the interradicular region of multirooted teeth, connecting the pulp cavity with periodontal support tissues.

These perforations can occur iatrogenically, namely during endodontic treatment, or by internal or external resorption. Regardless of the cause, a FP can be a hazardous complication, triggering an inflammatory reaction of the periodontium which may induce irreversible destruction of the periodontal ligament with loss of support, threatening the viability of the tooth.[101,105,291]

Timely detection and intervention decrease the likelihood of infection, improving prognosis and often preventing extraction of the affected tooth.[105,124]

Current advances in endodontics and biomaterials made the recovery of tooth structure and function feasible even in the most complicated cases. Several promising materials have emerged in recent years for the repair of perforations, prompting exhaustive research of their performance, safety and biocompatibility. Clarification of the best approach to FP is incomplete, mostly based on rare and inconsistent clinical cases, requiring the development of *in vivo* and *in vitro* models that simulate the clinical features of this pathology.

The aim of this systematic review was to identify FP experimental animal models validated in literature, to determine whether there is evidence that one is superior to others and to provide a comprehensive synthesis to aid and homogenize future preclinical investigation in this field.

MATERIALS AND METHODS

MEDLINE/PubMed, SciELO and Cochrane Library were used to find studies indexed from inception to June 2017, using (furcal OR furcation) and (perforation OR perforations) as search Keywords and Boolean operators. Potentially relevant articles were identified by title and summary and further studies were included by backward tracking and using PUBMED related articles function.

All titles, abstracts and full texts of potentially relevant studies were assessed for eligibility based on inclusion and exclusion criteria.

Inclusion criteria: the survey was limited to animal studies and included *in vivo* experimental models of furcation perforation with abstract or article available online.

Exclusion criteria: *in vitro* models, human studies or case reports and publications referring to perforations outside the furcation region were excluded. Articles published in languages other than English and without the major information set for this review available in the abstract were also excluded.

Two reviewers independently reviewed and selected studies for inclusion. Data were gathered concerning model characteristics, chosen groups, sample numbers, type of outcomes, journal and main area studied. Details of all studies were synthesized and individually entered into a SPSS Software Version 19.0 for Windows (SPSS Inc., Chicago, IL, USA) database. Median and lower and upper limits were used for descriptive statistics.

RESULTS

Our search found 174 articles in Pubmed, 9 articles in SciELO (none new) and 6 articles (none new) in Cochrane, totalling, after the exclusion of the repeated ones, 174 articles, of which 24 met the inclusion criteria for this study. One further article was included by backward tracking. Thus, 25 articles were included in the study (Figure II.1), 4 conducted in rodent models,[173,245,284,286] 3 in non-human primates[43,128,250] and 18 in dogs.[12,17,285,287,309,330,333,339,341,346,40,101,176,209,226,251,267,269]

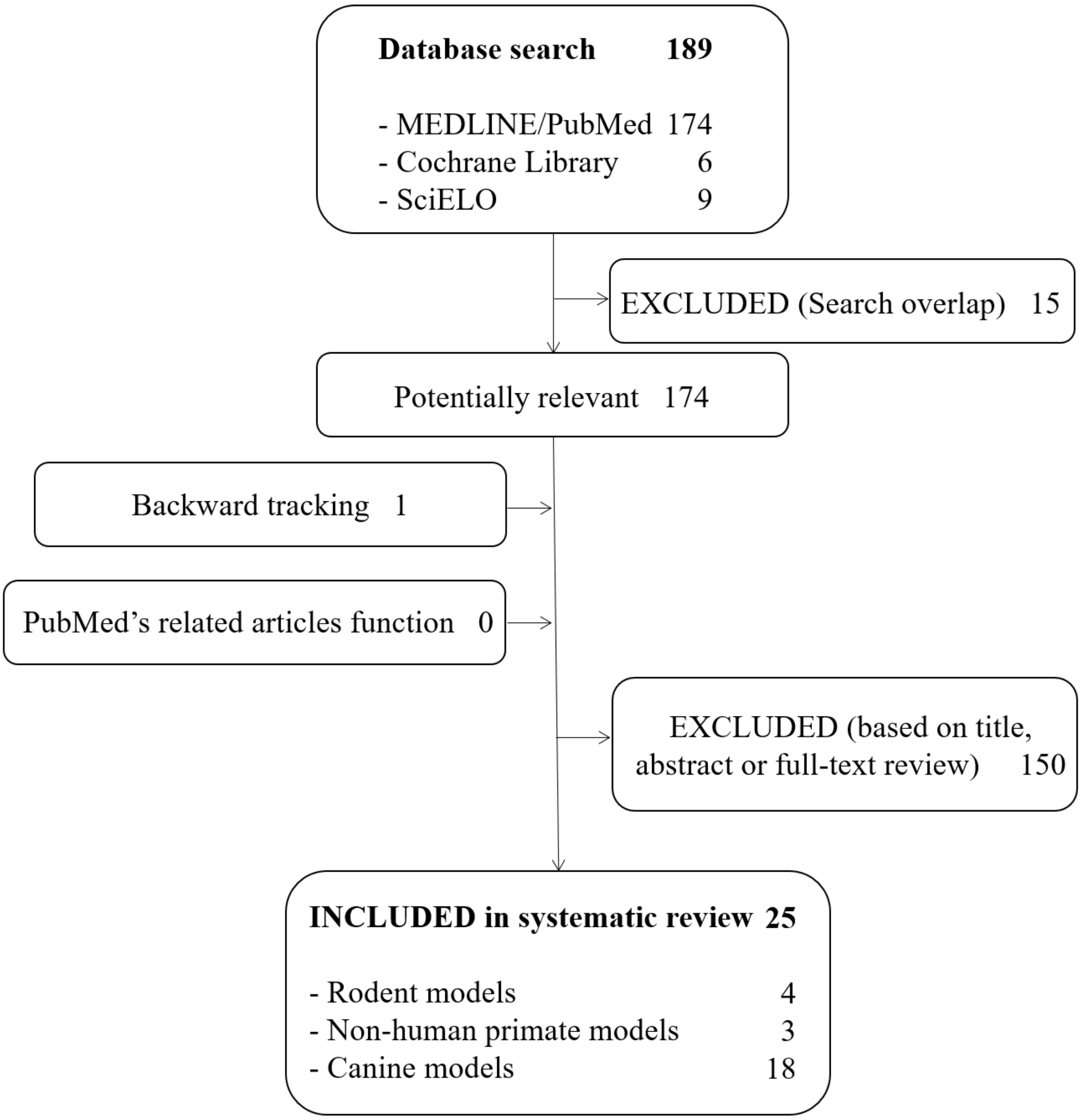

Figure II.1: Systematic literature search flow chart.

Characteristics of the 25 studies included in this review are summarized in Table II.1.

Table II.1: Characteristics of included studies

1st author. Year	Species/Breed	Teeth (n)	Animals (n)	Teeth/ group (n)	Studied material(s)	Type of teeth	Control teeth (n)	Type of controls	Perforation diameter (mm)	Study endpoints (days)
Rodent models										
Lara 2015[173]	Balb/c mice	30	15	5	MTA	Maxillary molar (1st)	5	Pos.	NA	7, 14 and 21
da Silva 2011[284]	Holtzman rats (*Rattus norvegicus albinus*)	120	60	5	Endo-CPM-Sealer, MTA, ZOE	Maxillary molar (1st)	20	Neg.	0.25	7, 15, 30, and 60
Silva 2009[286]	Wistar rat	18	21	6	None	Molar (1st)	3	Neg.	0.5	14, 21 and 28
Prescott 2008[245]	Mouse (NU/NU Mice Strain)	20 †	20	4	DPSCs, collagen scaffold, DMP1	Human (sc implantation)	5	Control material	1	42
Non-human primate models										
Rafter 2002[250]	Baboons (*Papio anubis*)	80	4	16	HAPSET, Hydroxyapatite	Premolars and molars	16x2	Pos. and neg.	1.4	7, 30, 90 and 210
Hartwell 1993[128]	Rhesus monkey	18	3	15	Decalcified freeze-dried bone	Molars	3	Pos.	NA	180
Balla 1991[43]	Rhesus monkey	120	6	10	TCP, Hydroxylapatite, Amalgam, Life	Premolars and molars	10	Pos.	NA	60, 120 and 240
Canine models										
Silva 2017[285]	Beagle	30	3	10 or 14	Biodentine, MTA	Premolars (2nd, 3rd, 4th)	6	Control material	1,2	120
Bakhtiar 2017[40]	Mixed breed	32	5	6	MTA, SC+TCP, SC+TDM	Premolars (2nd, 3rd, 4th)	1x2	Pos. and neg.	2	90
Tawfik 2016[309]	Mongrel	192	12	8	MTA, platelet rich plasma, platelet rich fibrin	Premolars (2nd, 3rd, 4th) and molars (1st)	8x2	Pos. and neg.	1	7, 30 and 90
Silva Neto 2012[287]	Mongrel	80	10	20	White, Type II and Type V Portland cements	Premolars	NA	NA	1.8	120
Zairi 2012[341]	Beagle	66	6	6; 10 or 14	Growth factors (OP-1, TGFβ1, bFGF, IGF-I), MTA, ZOE	Premolars and molars	8	Control material	1.4	21 and 56

Table II.1 (continued)

1st author. Year	*Species/Breed*	*Teeth (n)*	*Animals (n)*	*Teeth/ group (n)*	*Studied material(s)*	*Type of teeth*	*Control teeth (n)*	*Type of controls*	*Perforation diameter (mm)*	*Study endpoints (d)*
Vanni 2011[330]	Mongrel	48	6	12	MTA, AH Plus, Vitremer, Gutta-percha	Premolars (2nd, 3rd)	12	Control material	NA	90
Samiee 2010[269]	NA	34	4	15	CEM, MTA	Premolars	2x2	Pos. and neg.	1	90
Al-Daafas 2007[12]	Beagle	72	9	15	MTA, Amalgam, Calcium sulphate	Mandibular premolars (3rd, 4th) and maxillary premolars (2nd, 3rd)	6x2	Pos. and neg.	1.4	120
Vladimirov 2007[333]	NA	24	4	12	ProRoot MTA, Titan cement	Mandibular premolars (2nd, 3rd, 4th)	NA	NA	NA	30
Noetzel 2006[226]	Mixed breed	24	6	12	TCP, MTA	Mandibular premolars (2nd, 3rd)	NA	Control material	1.2	84
Rahimi 2005[251]	German-shepherd	30	5	6	Root MTA, Pro Root MTA	Mandibular premolars (2nd, 3rd, 4th)	3x3	Pos. and neg.	NA	30 and 90
Yildirim 2005[339]	Mongrel	90	9	36	MTA, Super EBA	Mandibular premolars and molars	18	Pos.	1.4	30, 90 and 180
Zhu 2003[346]	Beagle	42	3	7	MTA, Dycal, GIC	Premolars and molars	NA	NA	NA	120
Salman 1999[267]	Labrador	36	6	15	Atrisorb under GIC	Mandibular premolars (2nd, 3rd, 4th)	6	Neg.	1	90
Alhadainy 1998[17]	NA	72	9	8	Hydroxylapatite, Calcium sulfate (under GIC)	Premolars (4th) and molars (1st)	12	Neg.	NA	30, 90 and 180
Leder 1997[176]	Beagle	8	1	5	ePTFE membranes	Molars (1st,2nd)	3	Pos.	1.4	NA
Ford 1995[101]	Beagle	28	7	7	Amalgam, MTA	Mandibular premolars	NA	NA	1.4	120
Morinaga 1989[209]	NA	25	5	5	Cyanoacrylate cement	Premolars and molars	NA	NA	NA	7, 14, 28, 56, and 84

MTA: mineral trioxide aggregate; Pos.: positive control; NA: information not available; ZOE: zinc oxide-eugenol cement; Neg.: negative control; DPSCs: dental pulp stem cells; DMP1: dentine matrix protein 1; † human third molar teeth; sc: subcutaneous; HAPSET: 65% non-resorbable hydroxyapatite and 35% plaster of Paris; TCP: tricalcium phosphate; TDM: treated dentine matrix; OP-1: osteogenic protein-1; TGFβ1: transforming growth factorβ1; bFGF: basic fibroblast growth factor; IGF-I: insulin growth factor-I; d: days; CEM: calcium enriched mixture cement; Super EBA: super ethoxybenzoic acid; GIC: glass ionomer cement; e-PTFE: expanded polytetrafluoroethylene.

Statistical analysis showed the following medians (limits): 6 animals (1 to 60) and 34 teeth (8 to 192) per study; 10 teeth (4 to 20) per test group and, when performed, 6 teeth (1 to 20) per control group. Of the 20 studies reporting the use of negative or positive control groups, 6 included both (a negative group with unperforated teeth and a positive group with perforated but untreated teeth) and the remaining included only one type of control.

Almost all studied outcomes focused on the use of materials for FP repair, focusing primarily on the reaction of the surrounding tissues to the presence of the material. The only exception was a study aiming to validate a new experimental model of furcation perforation.[286] Eight authors[12,17,40,245,250,267,309,341] also tested the use of adjuvant materials and/or substances, such as matrices and growth factors, in FP repair.

Histology was used for evaluation in all studies except two.[173,330] Six authors[17,128,176,284,286,309] also used imaging techniques, most often radiography.

Mineral trioxide aggregate, currently regarded as the gold standard in FP repair, was used as a comparison in all the studies included in this review published in the last fifteen years except in one.[173]

Regarding the scope of the journals, 8 articles were published in the field of endodontics, 12 in other dentistry journals and the remaining in multidisciplinary health or biomaterial related journals.

Studies in Rodents

Of the 4 studies undertaken in rodents, one aimed to validate a new experimental model of FP in Wistar rats[286] and another used the immunodeficient mouse (NU/NU Mice Strain) as a model for simulated furcation perforation by subcutaneous implantation of dentine slices from perforated human molars, transplanted with 3 components obtained by tissue engineering.[245]

The remaining two studied tissue response to endodontic materials used to repair FP in rodent teeth, either by histological and histomorphometric evaluation in Holtzman rat molars (*Rattus norvegicus albinus*)[284] or by real time polymerase chain reaction for determination of cytokine expression levels in Balb/c mice.[173]

Studies in non-human primates

Of the 3 studies performed in non-human primates, all published more than 10 years ago, two used Rhesus monkeys[43,128] and one used Baboons (*Papio anubis*).[250] All 3 aimed to investigate tissue response to FP repair of mandibular and maxillary molars and, in 2 studies,[43,250] also premolars. From all the articles included in this review, these last two studies, having used 20 teeth per animal, were those which used each specimen more efficiently, decreasing the number of animals needed, with ethical and economic advantages.

Studies in dogs

Of the 18 studies performed in dogs, 6 used Beagles,[12,101,176,285,341,346] 1 used Labradors,[267] 1 used German-shepherds[251] and 10 used mongrel dogs or do not mention the breed.[17,40,209,226,269,287,309,330,333,339] Dogs were either obtained from breeders or kennels, after inspection by a veterinarian, or from certified laboratories where animals were bred specifically for experimental use.

Only five studies used more than 8 dogs, five studies used 6 to 7 dogs, five studies included 4 to 5 dogs and the remaining used 1 and 3 dogs. The maximum number of dogs included was 12. A median of 8 teeth per dog was used (limits: 4 to 14). Only one author used exclusively molar teeth[176] while the remaining used molars and premolars or, in 11 cases, only premolars. Most authors used both mandibular and maxillary teeth, although 6 only used mandibular teeth. Some articles do not specify the teeth used, but only the type, such as "mandibular premolars". When specified in methodology, the second and third premolars were the most often used teeth.

Six authors[17,209,251,309,339,341] included more than one time point for evaluation; for each of these studies the longest time point was considered for descriptive statistics. Study endpoints ranged from 1 month (1 study) to 6 months (2 studies). Most authors performed euthanasia after 3 or 4 months (respectively 8 and 5 studies). Median time for evaluation was 3 months.

Perforation diameter ranged from 1 to 2mm (mainly 1.4mm) in twelve studies, while the remaining studies were excluded from the analysis of this parameter due to absence of obtainable data.

DISCUSSION

Furcation perforations are among the complications of endodontic treatment with poorest prognosis.[42,124,271] In the absence of appropriate and timely intervention, inflammation caused by bacterial contamination may lead to irreversible destruction of the periodontal ligament and even tooth loss.[101,105,124,205] Therefore, the immediate sealing of the defect with a non-irritating material to prevent microbial penetration increases the possibility of repair.[101,276,336] Non-surgical treatment is considered the best option, since surgical repair can be impaired by difficult or even impossible accessibility and can lead to formation of chronic pockets by excessive removal of alveolar bone.[269,321] However, non-surgical treatment is not devoid of complications, such as material extrusion into the periodontal space.[291]

Thus, to achieve the best result, new repair materials must be biocompatible, show good sealing ability, stimulate new bone mineralization and extrusion should be preventable.[269,291,330] Since no repair material fulfils all these requirements, many researchers have tried to determine the best and safest alternative from within the currently available options, thus justifying the number of studies focusing on repair materials. On the other hand, many authors aimed to develop and test techniques to overcome common issues, such as the use of carrier matrices which prevent material extrusion.

Since it is unethical to intentionally create furcation perforations in humans for research purposes, FP models are important to study consequences in a simulated homogeneous setting, allowing comparison of performance results of different materials and techniques. Experimental models, both *in vitro* and *in vivo*, simulate the structure and environment of perforated teeth. However, only *in vivo* studies can replicate the surrounding tissues and clinical and physiological conditions (such as mastication, thermal variations, saliva exposure and flow).[286,330]

In vitro models attempt to replicate all factors to mimic FP situations without the need for animal sacrifice, with logistical, ethical and economic advantages. Although *in vitro* studies in this area have *ex vivo* characteristics, these do not mirror periradicular tissue behaviour, hampering the thorough evaluation of biocompatibility and, therefore, making *in vivo* studies essential. The use of animal models in oral disease investigation is widespread, with well-known anatomy, physiology and histology in this area.[15]

Ideally, *in vivo* models to evaluate FP repair should be developed in a previously validated species for endodontic studies, readily available and easy to maintain. Furthermore, specifically, teeth from the chosen model should be comparable to human anatomy and have well differentiated roots, making surgery and subsequent analytical, radiographic or histological evaluation easier.

This review systematized the experimental animal models available for the study of furcation perforations and identified rodent, non-human primates and canine models. The wide variety of animals found shows the need for standardization of models to allow comparability and reproducibility of findings. Advantages and disadvantages of different animal models of furcation perforation are summarized in Table II.2.

Table II.2: Advantages and disadvantages of different animal models of furcation perforation

Type	Advantages	Disadvantages
Rodent	Low cost Easily manageable Well-known anatomy, physiology and pathology Can be used in subcutaneous model for biomaterial safety profile	Different dental anatomy and oral microbiota Smaller mouth and teeth size, difficult to work Less usable teeth per specimen Subcutaneous model does not allow full study
Non-human primates	Anatomical, immunological and microbiological similarities to the human oral cavity	Expensive to acquire and maintain Handling difficulty/aggressiveness High susceptibility to infections and systemic disease
Canine	Large dental anatomy, easy to work Docile behaviour (some breeds such as Beagles) Well-known model for furcation perforation – easier to compare among studies	Expensive to acquire Relationship between the furcation and the bone edge not directly comparable to human teeth

The use of rodents is generally appealing due to their smaller size, lower cost, easier accessibility, maintenance and handling and detailed knowledge available regarding anatomy, physiology and pathology. Nevertheless, it is limited for FP studies by the differences in dental anatomy, mouth and teeth size and oral microbiota,[15] possibly explaining its scarce use found in this study contrasting with the widespread use in other fields. Subcutaneous implantation in rats, placing perforated human teeth in close proximity to connective tissue and blood vessels, is a potentially useful model to overcome some of these difficulties with the advantage of the ease of accomplishment. However, it is limited to the analysis of histopathological events in the soft tissues.[245]

Regarding non-human primates, anatomical, immunological and microbiological similarities to the human oral cavity are recognized advantages. However, significant limitations such as high costs, ethical considerations, handling difficulty, aggressiveness and high susceptibility to infections and systemic disease[15] led to a decrease in use in experimental settings in general including in FP as is shown.

The present review established that *in vivo* FP studies more often use canine models, predominantly Beagles. This breed is favoured in research given its well documented physiological responses, relatively small size and docile behaviour. Canine morphology and physiology make it a useful model for dental and medical investigation, with application in varied fields such as toxicology, surgery or implantology.[15] Although more expensive and harder to maintain than rodents, dogs' larger dental anatomy, easier to work and closer to human, makes this species a more suitable model for FP studies.[205,341] Premolars are preferred given their two distinct roots. Mandibular teeth have the advantage of allowing easier access, with more similar conditions to the ones found in human mandibular molars. However, the concomitant choice of maxillary second and third molars allows the use of more teeth per animal, decreasing the number of animals needed, with ethical and economic advantages.[226,267,339] Dogs' premolars and molars are large, offer good accessibility and visibility and allow the creation of FP without tooth hemisection.[12,205,341] On the other hand, the relationship between the furcation and the bone edge in dog teeth is not directly comparable to human teeth. Posterior dog teeth are narrower buccolingually than mesiodistally, have long pointed cusps, do not have root trunk and the furcation is closer to the cementoenamel junction, with root bifurcation in mandibular premolars often as close as 1 to 2mm, while in humans it is deeper in the alveolar bone and less associated with epithelialization and connective tissue formation.[205,267,341] Therefore, any technique that shows favorable results in dog models will probably have an even better performance in humans.[12,226,267,269,341]

Perforation diameter ranged from 1 to 2mm in canine models, depending on the drill, corresponding to the sizes commonly used in endodontic treatment of human teeth.

Rules for acquiring dogs for animal studies vary among countries, with 3 possible sources: specialized laboratories for breeding of animals for research, kennels or shelters, or individual breeders with specific licenses. In some European countries, including Portugal, the purchase to specialized laboratories is required by Council Directive 86/609/EEC of November 24th 1986 and by its revised version 2010/63/EU of September 22nd 2010. In the United States of America, dogs can be acquired from breeders or kennels licensed by the Department of Agriculture. In other countries, namely Brazil, experimental studies can be performed in dogs obtained from kennels, after inspection by a veterinarian for confirmation of the required conditions for the investigation. In general, research is best guaranteed if subjects are homogeneous, bred in ideal conditions, healthy and have known origin. Animal training since pups for research also makes the relationship with humans easier and safer and reduces stress from laboratory exposure. Nonetheless, this is a more expensive method, increasing the costs associated with research, limiting sample size or even hindering the study.

Despite being limited by some missing data especially due to inconsistent reports in the included studies, the present study has strengths since it is based on a comprehensive review of three major databases, with very broad time limits, and is the first, to our knowledge, to collect, critically evaluate and organize information regarding FP models.

In conclusion, although there is no model without disadvantages that can be stated as ideal and clearly higher than others for furcation perforation studies, dogs appear to present the most suitable characteristics, with due caution when extrapolating results to human settings, given the known differences. We anticipate that this review will have an immediate impact on preclinical research by critically reporting the main models used for furcation perforation and the comprehensive synthesis will provide valuable information in standardizing the study design for future preclinical investigations of FP.

Chapter III - Biodentine Use in Furcation Perforation Repair – Systematic Review

Part of this chapter has been based on the following article:

Cardoso M, Paulo M, Viegas C. Biodentine in furcation perforation repair. Submitted to Brazilian Dental Journal.

Biodentine Use in Furcation Perforation Repair – Systematic Review

INTRODUCTION

Furcation perforations are serious pathological conditions in clinical practice which affect the vital prognosis of the tooth and are the second most frequent cause of endodontic treatment failure following obturation.[291] FP can be defined as mechanical or pathologic communications between the root canal system and the external dental surface in the interradicular region of multirooted teeth, connecting the pulp cavity with periodontal support tissues.

These perforations can occur iatrogenically, namely during endodontic treatment, or by internal or external resorption.

Current advances in endodontics and biomaterials made the recovery of tooth structure and function feasible even in the most complicated cases.[33] Timely detection and intervention decrease the likelihood of infection, improving prognosis and often preventing extraction of the affected tooth.[105,124] Additionally, several promising materials and new techniques have emerged in recent years for the repair of perforations, prompting exhaustive research of their performance, safety and bioactivity.

Mineral trioxide aggregate is a biocompatible and bioactive material which was first introduced in dental practice in 1993. MTA powder is mainly a mixture of tricalcium silicate, tricalcium aluminate, tricalcium oxide and silicate oxide. Bismuth oxide has been added as a radiopacifier. The first formulation of MTA was gray followed by a white version and thereon by other similar materials, mostly composed of a tricalcium silicate-based cement and radiopacifier, developed to overcome the shortcomings of the original MTA. Despite drawbacks, such as poor handling characteristics, initial looseness and slow setting time,[4] the currently marketed ProRoot MTA has been considered the gold standard material for FP repair.[327]

Biodentine has recently emerged as a promising material for several endodontic applications. It was approved for dental use in the USA by FDA in 2009 and is commercially available in Europe since 2010. Biodentine is a bioactive cement, in which the powder is mainly composed of tricalcium silicate, with addition of zirconium dioxide and calcium carbonate, and the liquid has calcium chloride that reduces the setting time. Having tricalcium silicate cement as the main component makes it comparable to mineral trioxide aggregate materials.

Biodentine is currently being used as a dentine substitute under composite resins and shows favorable clinical outcomes.[253] It is a versatile material that has proven to be an advantageous endodontic reparative material in the endodontic armamentarium for surgical procedures such as root-end filling, root perforation repair, or apexification. Biodentine has also been used for vital pulp therapy including pulp capping, pulpotomies, and revascularization procedures. However, studies addressing its use as a FP repair material are scarce and have not yet been analyzed together. Its high success in various clinical procedures has been attributed to many of the “ideal” material qualities for use in endodontics. Given its bioactivity, Biodentine can be considered an attractive material for clinical indications that require regeneration, such as FP repair.

This work aims to systematically summarize what is known about Biodentine’s results when used in FP repair, compared with currently used materials.

METHODS

A MEDLINE/PubMed search was conducted, from January 1st 2009 (year of Biodentine’s approval for dental use by the FDA) to May 31st 2017, to identify citations of furcation perforation repair using Biodentine. In Pubmed search function, Key words / MeSH terms (Biodentine OR MTA OR mineral trioxide aggregate OR bioceramic OR material) AND (Furcation perforation OR Furcal perforation OR furcation repair OR furcal repair) found in title, abstract or full text were used for selecting relevant papers. Further studies were searched by backward tracking and using PUBMED related articles function. All titles, abstracts and full texts of hypothetically relevant studies were independently reviewed for eligibility by two authors based on inclusion and exclusion criteria. The inclusion criteria were studies on FP repair using Biodentine. Exclusion criteria were articles regarding FP diagnosis or repair technique, FP repair with other materials, Biodentine use in settings other than FP, and non-English language studies.

RESULTS

Our search found 73 published articles, from which nine met the inclusion criteria of this systematic review.[4,90,138,147,213,255,270,293,326] One further study[285] was included in the review since, even though not yet published, it was already available ahead of print and was the only *in vivo* investigation addressing this issue. Thus, ten articles were included in this systematic review, reporting one *in vivo* and nine *in vitro* studies. Table III.1 lists the included studies and their basic characteristics and conclusions. No human studies or case reports were found.

Table III.1: Included studies and their basic characteristics and conclusions

Study	Test	Test groups other than Biodentine (BD)	Relevant findings on BD performance *versus* other groups
Aggarwal 2013	Push-out bond strength	ProRoot MTA and MTA Plus (c or nonc; 24h or 7d)	BD24h stronger than ProRoot MTA24h (P*) BD7d stronger than MTA Plus7d (P*) BD7d stronger than BD24h (P*) BD not affected by contamination
Jeevani 2014	Dye-extraction	MM MTA and EndoS	BD worse than EndoS (P*) MM-MTA (P*)
Ustun 2015	Push-out bond strength	ProRoot MTA, RetroMTA and Supra MTA (c or nonc)	BD ~ ProRoot MTA ~ RetroMTA ~ Supra MTA (NS)
El-Khodary 2015	Fluid filtration	MTA-A, PC, TechB (24h, 1m, 6m, 1-y)	BD ~ MTA-A ~ PC ~ TechB, at all time points after 24h (NS)
Sinkar 2015	Dye-penetration	ProRoot MTA and RetroMTA	BD better than RetroMTA (P*) BD ~ ProRoot MTA (NS)
Ramazani 2016	Bacterial leakage	ProRoot MTA and CEM	BD~ CEM~ ProRoot MTA (NS)
Samuel 2016	Dye-leakage	MTA-A	BD better than MTA-A (P*)
Katge 2016	Dye-extraction	MTA Plus	BD ~ MTA Plus (NS)
Nagas 2017	Push-out bond strength	ProRoot MTA	BD stronger than ProRoot MTA (P*) BD not affected by laser-assisted irrigation
Silva 2017	Histopathologic and indirect immunofluorescence analysis	ProRoot MTA	BD ~ MTA for new mineralized tissue formation, bone resorption, inflammatory cell infiltrate and RUNX2 immunostaining intensity (NS) BD worse than MTA for frequency of complete sealing and thickness and area of newly formed mineralized tissue (P*)

BD: Biodentine; MTA: mineral trioxide aggregate; c: contamination; nonc: non-contamination; 24h: 24 hours; 7d: 7 days; P*: statistically significant; EndoS: Endosequence; ~: similar; NS: non-significant; MTA-A: MTA Angelus™; PC: Portland Cement; TechB: Tech Biosealer; 1m: 1 month; 6m: 6 months; 1-y: 1 year; CEM: Calcium-enriched mixture cement.

Aggarwal *et al.* in 2013[4] used a push-out bond strength test in an *in vitro* FP model to evaluate the sealing properties of Biodentine compared to ProRoot MTA and MTA Plus™. In total, 120 extracted human molars were used for this study. The test material groups were submitted to the push-out bond test under blood contamination or noncontaminated environment at 24 hours and 7 days. Push-out bond strengths significantly increased with time for all the tested materials. Contrarily to the MTA Plus group, the bond strengths of both Biodentine and ProRoot MTA were not affected by blood contamination. Additionally, Biodentine was found to have significantly higher push-out bond strength than ProRoot MTA in uncontaminated samples at 24 hours and than MTA Plus at 7 days. The authors concluded that caution should be taken while condensing restorative materials over FP defects.

Jeevani *et al.* in 2014[138] compared the sealing ability of Biodentine *versus* MM-MTA™ and EndoSequence™, when used to repair FP, employing a dye-extraction method. In this study, 40 human extracted permanent molars with experimentally induced FP were divided into 4 groups: Biodentine, MM-MTA, Endosequence and a positive control. In the ultraviolet spectrophotometric analysis of dye-absorbance the comparison of the three tested materials showed that Biodentine had significantly higher dye-absorbance compared to MM-MTA and Endosequence. The authors concluded that Endosequence presented better sealing ability than Biodentine or MM-MTA.

Üstün *et al.* in 2015[326] used a push-out bond strength test in an *in vitro* FP model to evaluate the effect of blood contamination on sealing properties of Biodentine, ProRoot MTA, RetroMTA™ and Supra MTA™. In total, 96 extracted molars were used for this study. Four test material groups (Biodentine, ProRoot MTA, Retro MTA and Supra MTA) were submitted to the test under blood contamination or a noncontaminated environment. Although push-out bond strengths did not show significant differences among the tested materials, a trend towards lower push-out bond strengths of both Biodentine and Supra MTA was found compared to ProRoot or Retro MTA groups ($P > 0.05$). In addition, blood contamination did not affect the materials' bond strength. Microscopical examination revealed adhesive failure as the most common pattern of failure in all groups. The authors concluded that, regarding dislodgment resistance, ProRoot and Retro MTA seem to be more suitable to treat FP.

El-Khodary *et al.* in 2015[90] used a fluid filtration technique in furcation perforations to assess the sealing ability of Biodentine, MTA Angelus™, Portland cement™ and Tech Biosealer™. In this study, 50 human extracted primary molars were used, divided into six groups (four test materials, positive and negative controls). No significant differences were found in fluid penetration among the test material groups at 24 hours, 1 month, 6 months and 1-year after the repair. In addition, sealing ability improved with time after 24 hours. The authors concluded that all the tested materials have a similar sealing ability at the analyzed time points.

Sinkar *et al.* in 2015[293] used a dye-leakage method in furcation perforations to assess the sealing ability of Biodentine, ProRoot MTA and RetroMTA™. In this study, 35 extracted human molars were used, divided into four groups (Biodentine, ProRoot MTA, Retro MTA, and positive control). Biodentine group showed the least dye absorbance, significantly lower than Retro MTA group, however, without significant difference compared to ProRoot MTA. The authors concluded that, although all materials showed sealing capability, Biodentine was the best material for FP repair.

Ramazani and Sadeghi in 2016[255] compared the microleakage of Biodentine and calcium-enriched mixture cement (CEM) with ProRoot MTA, as FP repair materials, using a bacterial leakage model. In this study, 61 human extracted primary molars were used, divided into five groups (Biodentine, ProRoot MTA, CEM, positive and negative controls). There were no significant differences in bacterial leakage among the test material groups. The authors concluded that Biodentine and CEM can be recommended as alternatives to ProRoot MTA for FP repair.

Samuel *et al.* in 2016[270] compared the sealing ability of Biodentine and MTA Angelus™ when used to repair FP, employing a dye-leakage test. In this study, 40 human extracted primary molars with experimentally induced FP were divided into 2 groups: MTA Angelus and Biodentine. The authors found significantly less microleakage with Biodentine compared to MTA Angelus and thus concluded that it may be a good alternative.

Katge *et al.* in 2016[147] compared the sealing ability of Biodentine and MTA Plus™ in furcation perforations, using a dye-extraction test. In this study, 90 human extracted permanent molars were divided into 4 groups: Biodentine, MTA Plus, positive and negative controls. No significant microleakage differences were found between Biodentine and MTA Plus. The authors concluded that both materials can be used to repair FP.

Nagas *et al.* in 2017[213] studied the effect of erbium, chromium: yttrium–scandium–gallium–garnet laser-activated irrigation of sodium hypochlorite on the push-out bond strength of FP repaired with Biodentine and ProRoot MTA. In this study, 100 extracted human mandibular molars were used, divided into two groups (Biodentine, ProRoot MTA) and then further assigned into five subgroups depending on the irrigation protocol (needle irrigation or laser-activated irrigation of distilled water or 5.25% sodium hypochlorite, and control with no irrigation). Biodentine yielded greater dislodgement resistance and laser-assisted irrigation had no effect on the push-out dentine bond strength of either repair materials independently of the irrigation solutions.

Silva *et al.*[285] in 2017 evaluated the *in vivo* response of periradicular tissues 120 days after sealing of furcation perforations with Biodentine, ProRoot MTA and gutta-percha in dogs (total of 30 teeth repaired in 3 dogs). Histopathologic and indirect immunofluorescence analysis were performed. The authors found that prMTA and Biodentine induced the formation of significantly more new mineralized tissue than gutta-percha. prMTA presented highest frequency of complete sealing and greater thickness and area of newly formed mineralized tissue. Bone resorption was similar between Biodentine and prMTA and Biodentine showed fewer inflammatory cells, and greater RUNX2 immunostaining intensity than gutta-percha and similar to prMTA. Thus, the authors concluded that Biodentine could be considered as an adequate FP repair material.

DISCUSSION

Biodentine performance in FP repair is scarcely studied and available results are scattered and disorganized, prompting our purpose to examine and analyze them together. To our knowledge this is the first study to systematically address this issue.

Most recently developed MTA-like materials, including Biodentine, newest MTA formulations and new bioceramics have not yet been proven as an advantageous alternative to ProRoot MTA for the repair of FP in clinical practice. Regarding their use in clinical settings, while numerous literature reports attest the efficacy and safety of ProRoot MTA[33,38,196] as well as the long-term healing success rates in historical cohorts,[201] only two studies reporting the clinical use of CEM as repairing material in human cases of furcation perforation[26,89] could be found and none for other materials, including Biodentine. Nine out of ten studies found by our search were done *in vitro*, which cannot simulate *in vivo* conditions. Moreover, various different methodologies were used for accessing the material's sealing ability. On the other hand, all studies but one used controls and all performed statistical analysis. Taken together, the comparative results of sealing ability of Biodentine and different formulations of MTA or other materials such as Endosequence™, Tech biosealer™, CEM or Portland cement™ using *in vitro* push-out bond strength, fluid filtration, bacterial or dye leakage tests (Table III.1), most commonly showed similar or superior sealing ability results for Biodentine. Specifically, Biodentine was found to be significantly better than ProRoot MTA in two studies,[4,213] MTA Angelus™ in one study,[270] MTA Plus™ in one study[4] and RetroMTA™ in one study.[293] However, Biodentine was found to have worse sealing ability than Endosequence™ or MM-MTA™ in one study.[138]

Furthermore, Biodentine was found to induce favorable periradicular tissues response when used in FP repair in the only *in vivo* study available, even though with inferior frequency of complete sealing and thickness and area of newly formed mineralized tissue than ProRoot MTA.[285]

In conclusion, taken together, these results support that Biodentine is a good FP repair material with overall better or equivalent performance when compared with currently used materials. However, research on Biodentine for repair of FP remains scarce with only one *in vivo* and nine *in vitro* studies addressing this specific topic and larger clinical studies are still lacking.

Chapter IV - Dental Dam Application for Endodontics in Dogs – a Novel Clamp Kit

Part of this chapter has been based on the following article:

Cardoso M, Catré D, Noites R, Paulo M, Viegas C. Dental Dam Application in Endodontics in Dogs - New Clamps Kit. Journal of Veterinary Dentistry. *In Press*

Dental Dam Application for Endodontics in Dogs – a Novel Clamp Kit

INTRODUCTION

The rubber dam has been used in dental care for decades and its use is well-described. It was introduced in dental practice by Dr. Sanford Barnum in 1864.[23]

In humans, its use is mandatory in modern endodontic practice,[97] but in veterinary practice this procedure is rare.[93]

The use of a dental rubber dam in humans provides better control of cross-infection and improves treatment efficiency. It provides an aseptic field isolating the tooth from oral and salivary contamination, improves the access and visibility to the operating field by retraction of soft tissues and protects from possible aspiration or swallowing of instruments, drugs, irrigating solutions and tooth/material debris.[112,274]

The importance of oral microorganisms in the pathogenesis of apical periodontitis is well established.[143] Successful treatment depends on effective contamination control measures to eliminate infection and prevent re-infection of the root canal system. This can be more predictably achieved by isolating the operating field, as is shown by direct evidence demonstrating that using a dental dam improves the outcome of endodontic treatment.[8]

The purpose of this work is to describe a new line of clamps made especially for dogs in order to improve dental dam isolation efficiency without damaging the furcation of the tooth and review step-by-step the procedure and materials used for isolating the operative field in dogs' endodontic treatments.

MATERIALS AND METHODS

Initially the authors created a new design of clamps, better adapted to the dog's specific tooth anatomy, with different sizes and conformations (Figure IV.1A). This design was then sent to a surgical materials factory (Manuel Nunes Antão, Lda) to be assembled.

The absolute isolation may be accomplished before or after opening of the access cavity. Placing the isolation before opening prevents contamination, but can also contribute to complications, including excess removal of tooth structure which may lead to perforation. Therefore, in cases where the clinical and radiographic examination indicates difficulties in opening the access cavity, particularly in the absence of experience in the field of endodontics by the clinician performing the treatment, it is advisable to place the isolation after the access to the pulp chamber has been prepared. Adequate prophylaxis of the tooth to be treated must be done before placing the isolation, in order to eliminate bacterial biofilm.

The necessary materials are a clamp, rubber dam sheet, rubber dam punch, rubber dam frame, and clamp forceps (Figure IV.1).

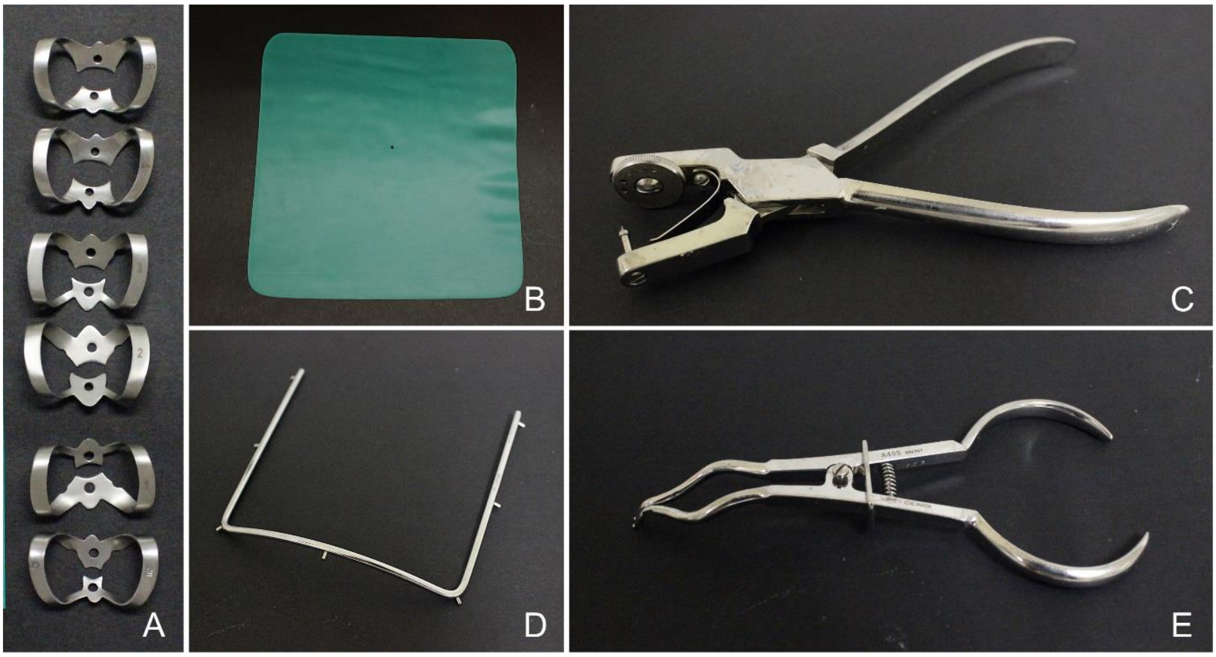

Figure IV.1: Materials. (**A**) Clamps; (**B**) rubber dam sheet; (**C**) rubber dam punch; (**D**) rubber dam frame; (**E**) clamp forceps.

Clamp

There are a variety of shapes and sizes of clamps, designed for human use, intending to suit different teeth and situations. The fit of the rubber dam essentially depends on the choice of the appropriate clamp and of its correct positioning. Clamps shown in Figure IV.1A were designed by the authors to better adapt to dog's teeth.

Rubber dam sheet

Rubber dam sheets (Figure IV.1B) usually measure around 6x6 inches (152x152mm) and can be made of natural latex or can be latex free for allergic patients. Depending on the manufacturer, there are different thicknesses (which vary between 0.15 and 0.35mm) available. Medium thickness is recommended for veterinary use, because it is easier to manipulate and the risk of splitting is reduced.

Rubber dam punch

A rubber dam punch (Figure IV.1C) is used to create a hole in the rubber dam sheet through which the tooth will pass. Different hole sizes can be created, depending on the tooth to be treated.

Rubber dam frame

A rubber dam frame (Figure IV.1D) is necessary to maintain tension in the dam sheet so that the lips and cheeks may be retracted well. Some frames, including the Young frame, are made of very thin metal; others, including the Nygaard-Ostby, are plastic.

Clamp forceps

Clamp forceps (Figure IV.1E) are used to carry and remove the clamp from the tooth. This instrument is necessary to open the clamp and position it around the tooth. There are several designs with the same function. For each clinician, experience will reveal the most comfortable for clinical practice.

Techniques for placement of the rubber dam

There are different techniques for rubber dam placement for isolation (Figures IV.2-4). The choice of the technique depends primarily on the type of tooth to be treated, its position in the dental arch and the preference of the clinician.

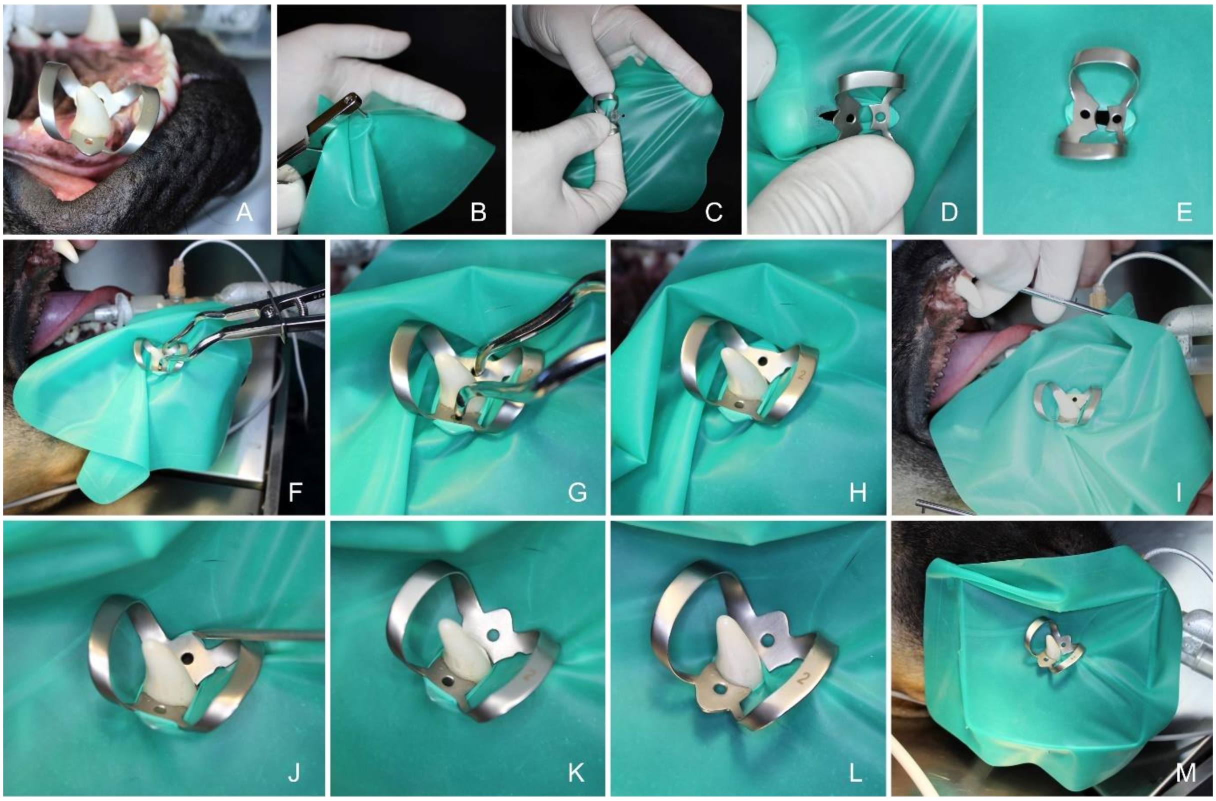

Figure IV.2: Rubber dam application: rubber dam sheet + clamp followed by the rubber dam frame. (**A**) Choice of clamp that best fits the tooth; (**B**) perforation of the rubber dam with the rubber dam punch; (**C**) placement of the clamp in the previously created hole; (**D**) clamp with rubber dam sheet placed in one fin; (**E**) clamp with rubber dam sheet placed in both fins; (**F** and **G**) application of clamp with rubber dam sheet in the tooth with the aid of clamp forceps; (**H**) clamp and rubber dam sheet placed around the tooth; (**I**) application of the rubber dam frame stretching the sheet; (**J**) removal of rubber dam sheet from the clamp fins; (**K**) sheet removed from one clamp fin; (**L**) sheet removed from both clamp fins; (**M**) rubber dam assembly applied to a dog right mandibular canine tooth.

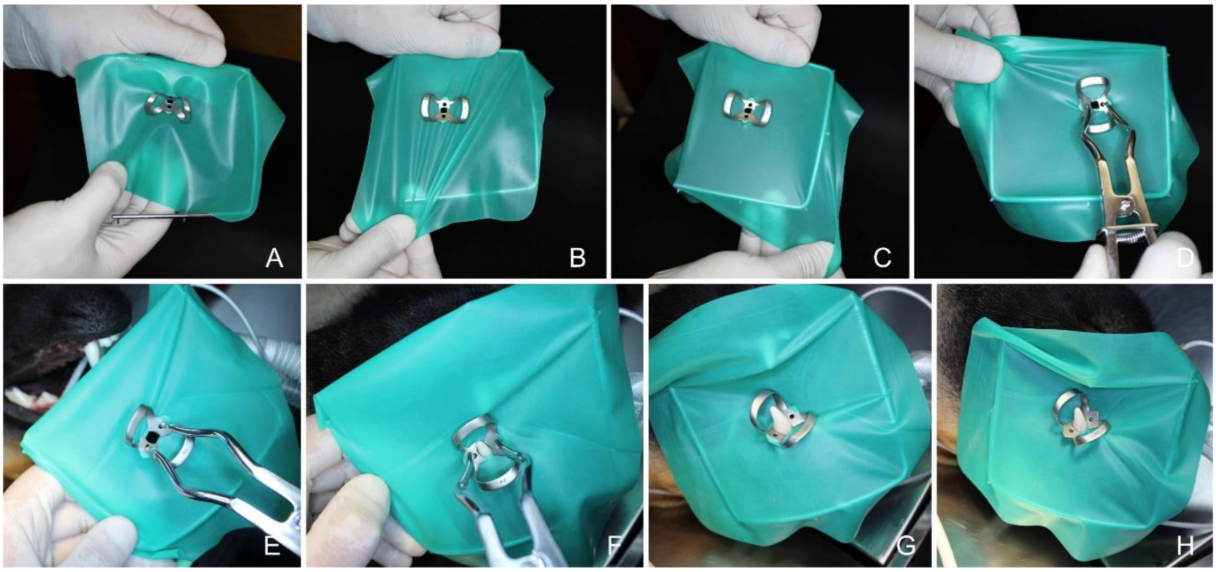

Figure IV.3: Rubber dam application: rubber dam sheet + clamp + rubber dam frame. (**A, B** and **C**) Application of the rubber dam frame stretching the sheet; (**D, E** and **F**) placement of the rubber dam sheet, frame and clamp assembly around the tooth with the aid of a clamp forceps; (**G**) rubber dam apparatus applied with the sheet over the clamp fins; (**H**) rubber dam assembly applied to a dog mandibular canine tooth with the sheet removed from the clamp fins and adapted to the tooth collar.

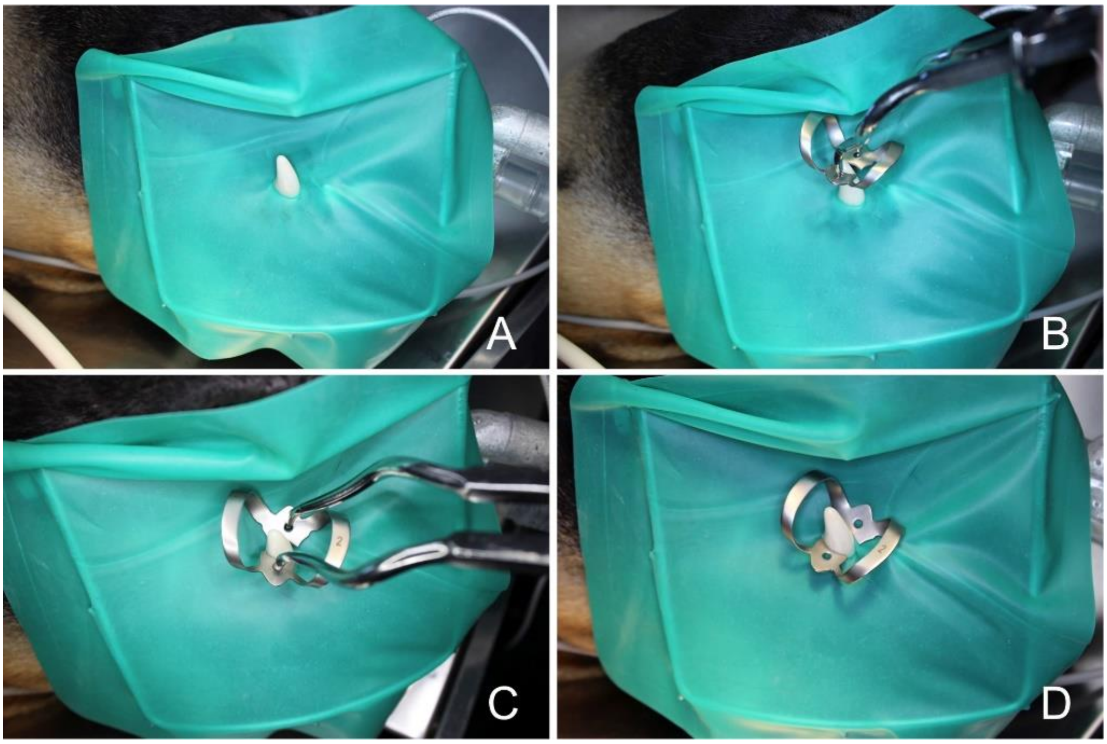

Figure IV.4: Rubber dam application: rubber dam sheet and frame first followed by the clamp. (**A**) Perforated rubber dam sheet and frame applied together around the tooth; (**B** and **C**) placement of the clamp with the aid of a clamp forceps; (**D**) rubber dam assembly adapted to the tooth collar applied to a dog mandibular canine tooth.

1- Rubber dam sheet + clamp followed by the rubber dam frame

Choose the clamp that best fits the tooth to be treated. The clinician must test the clamp so that it is properly adapted to the cementoenamel junction of the tooth crown without traumatizing the gums (Figure IV.2A).

Perforate the rubber dam sheet with the rubber dam punch (Figure IV.2B).

Place the chosen clamp in the previously created hole, fixing it by its fins (Figures IV.2C-E).

Attach this apparatus to the tooth (Figures IV.2F-H) and then apply the rubber dam frame (Figures IV.2I). Afterwards, adapt the sheet to the tooth by removing it from the clamp fins (Figures IV.2J-M), ensuring its proper fit to the tooth collar.

2- Rubber dam sheet + clamp + rubber dam frame

As described before, perforate the rubber dam with the rubber dam punch (Figure IV.2B) and, after selecting the appropriate clamp (Figure IV.2A), place it in the previously created hole in the rubber dam sheet (Figures IV.2C-E).

Apply the rubber dam frame stretching the sheet (Figures IV.3A-C).

With the aid of clamp forceps, the rubber clamp assembly is attached and carried to the mouth (Figures IV.3D-G). Adapt the sheet to the tooth removing it from the clamp fins, which must be properly fitted to the tooth collar (Figures IV.2J-M and IV.3H).

The main difference from the previous technique is the timing of the frame placement, which here is performed before the placement of the apparatus in the mouth.

3 - Rubber dam sheet and frame first followed by the clamp

In this technique one uses clamps with or without fins.

First, the perforated sheet and the frame are applied together in the tooth (Figure IV.4A) and afterwards the clamp is placed (Figures IV.4B-D), ensuring the sheet is properly fit to the tooth collar.

DISCUSSION

Although rubber dams are widely used in endodontic treatment in humans, with well-known advantages, their use in veterinary dentistry is very uncommon. The new line of adapted clamps presented in this article has been successfully used by the authors (Figure IV.5).

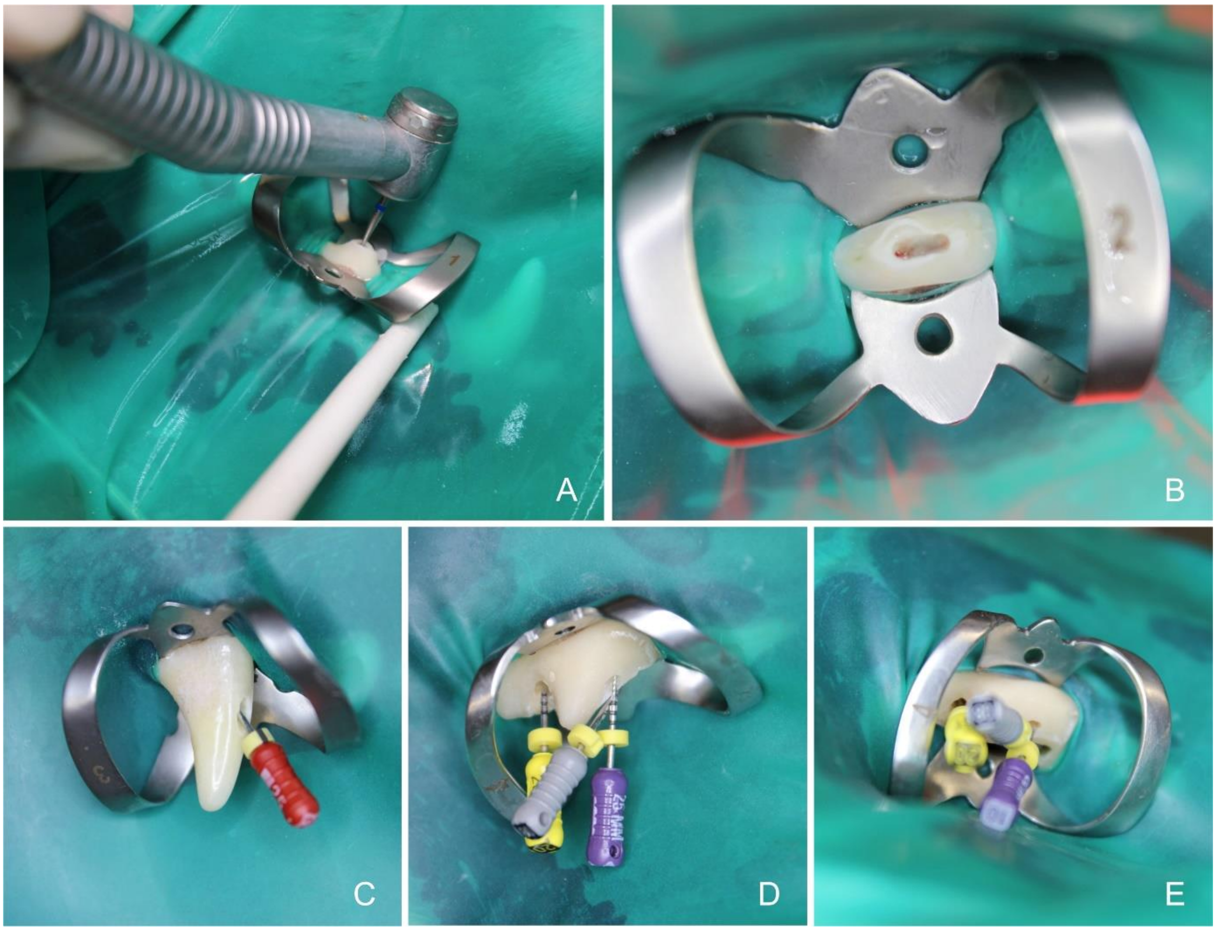

Figure IV.5: Rubber dam applied to different types of teeth. (**A**) Access cavity preparation in a mandibular second premolar; (**B**) access cavity with the root canals entrance in a mandibular third premolar; (**C**) access cavity in a maxillary canine with a #25 file in the root canal (**D** and **E**) access cavity in a maxillary fourth premolar with a #20 file in the distal canal, a #10 file in the mesiobuccal canal and a #08 file in the mesiopalatal canal.

To the best of our knowledge there is only one other report in the literature of the use of the dental dam in endodontic procedures in dogs.[180] In it, LeVan *et al.* used clamps made for human teeth which have a different anatomy from the dog. In order to improve isolation of the operating field, we developed a specific line of clamps for dogs. Butterfly clamps provide better access to the tooth. Usually dog premolars are long in the medial distal direction but short in the buccal lingual direction. The first mandibular molar is a big tooth with unique anatomy. The clamps with this new adapted design allow for a better adaptation to the tooth, without injuring the gum or the furcation, and increase retention, which grants a more effective isolation. Taking into account results in humans, this may offer a better prognosis for maintaining the tooth.

Although current perception of dentistry is evolving due to the efforts of specialized clinicians, many still believe that fractured teeth do not need to be treated, or intervention should consist of extraction only. Common causes of endodontic disease in dogs are fractures of maxillary fourth premolar, first mandibular molar and canine teeth, which are considered strategic teeth given their relevance to the daily activities of prehension, defense and mastication.[297] Endodontic treatment can be useful in returning a diseased tooth to a normal, pain-free function, thus avoiding more invasive procedures, such as tooth extraction.

Veterinarians are now being asked to preserve tooth function and structure rather than to extract injured or diseased teeth.[199] A variety of instruments are available that improve and make endodontic treatment easier.

In humans, the use of a rubber dam for endodontic treatment has many clinical implications on treatment outcome, which are believed to also be valid in veterinary dentistry.[180] Root canal irrigants play an integral role in canal preparation procedures and are needed to eliminate microorganisms, dissolve organic debris and lubricate root canal instruments.[97] Sodium hypochlorite is considered the irrigant of choice because of its broad antimicrobial spectrum and unique capacity to dissolve necrotic tissue,[343] but it is an irritant. Irrigation with sodium hypochlorite should be accompanied by tooth isolation with a well fitting rubber dam.[180]

Barriers for the use of a rubber dam include lack of experience, underestimation of its benefits and lack of motivation. Another reason is that the amount of time required to place a rubber dam is often overestimated. In the experience of regular rubber dam users, placing a rubber dam in routine circumstances does not require more than one to two minutes of additional time, which will be compensated by efficiency during the treatment.[8,98,186]

Given the proven benefits in similar situations in humans, the authors expect that the widespread use of a rubber dam in endodontic treatment, particularly with clamps specifically designed for dogs’ anatomy, will greatly improve clinical outcomes.

Chapter V - Biodentine for Furcation Perforation Repair: *In Vivo* Study with Histological, Radiographic and Micro-computed Tomographic Assessment

Part of this chapter has been based on the following article:

Cardoso M, Pires MA, Lourenço L, Correlo V, Reis R, Catré D, Paulo M, Viegas C. Biodentine for Furcation Perforation Repair: *In Vivo* Study with Histological, Radiographic and Micro-computed Tomographic Assessment. Submitted to Australian Endodontic Journal.

Biodentine for Furcation Perforation Repair: *In Vivo* Study with Histological, Radiographic and Micro-computed Tomographic Assessment

INTRODUCTION

Furcation perforations are anomalous communications between the root canal system and the external dental surface in the interradicular region of multirooted teeth, connecting the pulp cavity with periodontal tissues.[101,291]

Current advances in endodontics and biomaterials made the recovery of tooth structure and function possible even in the most complicated cases. Several promising materials have emerged for the repair of perforations, prompting exhaustive research, however, none was considered as the ideal.[101,132,147,226,267] Despite the breakthroughs in techniques and resources, FP management remains challenging[90,226] and the best approach is still unclear. Unintentional extrusion of the repair material, inadequate sealing and lack of biocompatibility are among the described difficulties.[12,127] Available options are mostly based on inconsistent clinical reports, raising the need for *in vitro* and *in vivo* studies under controlled conditions that simulate the clinical features of this pathology.

Two main techniques have been advocated for the repair of such defects.[58] Since surgical procedures for FP repair may induce pocket formation, nonsurgical methods, especially in inaccessible areas, are favored.[58,267] Ideally, perforations should be immediately repaired with a biocompatible material to interrupt the communication between the perforation site and the gingival sulcus to achieve a more favorable prognosis.[58,132]

Mineral trioxide aggregate is a bioactive material which was introduced in dental practice in 1993. MTA powder is mainly a mixture of tricalcium silicate, tricalcium aluminate, tricalcium oxide and silicate oxide, with bismuth oxide added as a radio-opacifier agent. Because of their biocompatibility and bioactivity, MTA formulations, namely ProRoot MTA, have become the most common choice for FP repair.

Biodentine is a bioactive cement, in which the main component of the powder is tricalcium silicate, with addition of zirconium dioxide and calcium carbonate; the liquid has calcium chloride as a setting accelerator. Biodentine has been reported to provide good biocompatibility, bioactivity,[174,175] high compressive strength and a short setting time of 12 minutes.[189,253] Since its approval for dental use by the FDA in 2009, Biodentine was widely studied for several applications, however its use in FP has been scarcely addressed, mostly by *in vitro* studies.[4,90,138,147,213,255,270,285,293,326] Despite the promising results in this field, further studies are necessary with larger *in vivo* samples, stronger methodologies and complementary assessment, including imaging.

The aim of this *in vivo* study was to compare the histological responses and radiographic and micro-computed tomographic (microCT) outcomes after FP repair with Biodentine or ProRoot MTA in dogs' teeth. In this context, the null hypothesis was that there would be no significant differences in the histological or imaging findings of Biodentine or prMTA.

MATERIALS AND METHODS

All animal procedures were approved by the institutional Ethical Committee and conformed with the ethical guidelines and regulations of the national Directorate-General for Food and Veterinary (Process number 0421/000/000/2014), and with the ones laid down by the European Union Directive 2010/63/EU for animal experiments. All measures were undertaken to minimize pain and animal discomfort.

The second and third maxillary premolars and the second, third, and fourth mandibular permanent premolars of 5 male beagle dogs aged 18 months and weighing 17 kg on average were selected for the study, providing 50 teeth.

The teeth were divided into two experimental groups, prMTA (FP repaired with ProRoot MTA) and BDT (FP repaired with Biodentine), with 20 teeth each, and two control groups, PCont (positive control, with FP without repair) and NCont (negative control, without perforation), with 5 teeth each. Each animal had 4 teeth repaired with Biodentine, 4 with prMTA, 1 positive control and 1 negative control, randomly assigned within the teeth included in the study. Table V.1 summarizes the experimental groups and the 50 teeth distribution.

Table V.1: Distribution of the 50 teeth included in the study by the experimental groups

Experimental Groups	Procedures	n per dog*	Total n per group
Test Groups			
BDT	FP repaired with Biodentine	4 teeth**	20
prMTA	FP repaired with ProRoot MTA	4 teeth**	20
Control Groups			
PCont	Positive control with FP without repair	1 tooth**	5
NCont	Negative control without perforation	1 tooth**	5

n: number; *number of teeth in each of the 5 dogs used in this study; BDT: Biodentine group; FP: furcation perforation; **randomly assigned within the teeth included in the study for each dog; prMTA: ProRoot MTA group; PCont: positive control group; NCont: negative control group.

All procedures were carried out under general anesthesia. Each animal was pretreated with 0.2 mg/Kg morphine, 0.005 mg/Kg acepromazine and 0.004 mg/Kg dexmedetomidine, administered intramuscularly, and general anesthesia was induced with intravenous 0.5 mg/Kg ketamine, 0.2 mg/Kg diazepam and 2 mg/Kg propofol. The animals were intubated with a cuffed endotracheal tube, and anesthesia was maintained with isoflurane and a constant rate infusion of 10 µg/Kg/minute of ketamine. Adequate prophylactic antibiotic and analgesia were provided.

Preoperative standardized periapical radiographs were performed by using film-holding devices. After prophylaxis and root scaling, all teeth were isolated with a rubber dam and the operative field was disinfected with 5% iodine.

In the test and PCont groups, access cavities were prepared in occlusal surfaces using a round diamond bur (ISO 012) in a high-speed handpiece under copious water spray. After complete removal of the pulp chamber roof, coronal pulp tissue was extirpated using a #10 curette up to the canal entrance level. A 1.2mm diameter perforation was created in the center of the pulp chamber floor, using a sterile round bur (ISO 012) at low speed until hemorrhage was noted (Figure V.1). Hemostasis was achieved with abundant saline irrigation and sterile cotton pellets.

Figure V.1: Access cavity with furcation perforation (arrow) in a third mandibular right premolar.

Both cements (Biodentine and prMTA) were prepared according to the manufacturers' protocol, placed into the perforation defects and over the remaining radicular pulp tissue of the respective test group's teeth and gently compacted with a plugger. After the initial setting of the materials, the access cavity was sealed with light-cured glass ionomer cement (Riva Light Cure LC™/Southern Dental Industries SDI*)* and the teeth were radiographed again.

The PCont perforation was left open. The NCont group teeth received no intervention, to evaluate the potential effects of external variables that could have developed during the study period.

One hundred and twenty days after the procedures, new periapical radiographs were performed and the animals were euthanized using an overdose of sodium pentobarbital.

Each experimental tooth with the surrounding tissues was cut in a block section and placed in 10% buffered formalin.

Fixed block sections were scanned using a high-resolution micro-CT system (vivaCT 80™, ScancoMedical) with the root oriented vertically. The x-ray transmission was set at 90 degrees rotation, with the x-ray source set at 70 kVp/114 μA,8W. The scanning time for each sample was approximately 35 minutes.

The specimens were demineralized using Morse's solution (50% formic acid and 20% sodium citrate) for three months. After complete decalcification, the specimens were dehydrated, embedded in paraffin and serially sectioned (3μm thickness). All sections passing through the FP site were stained with hematoxylin-eosin. Samples of these sections were stained with Gram staining for histomicrobiological analysis.

Radiographic assessment

The immediate postoperative radiographs and those after 4 months were analyzed to determine the development or increase of radiolucency in the furcation region bordering the repaired perforations.

This radiographic evaluation was performed blindly by two independent experienced dentists. The cases where evaluation did not match were discussed to reach a consensus.

MicroCT

In the test groups, the volume of material that extruded to the periodontal tissues area was quantified for each tooth using microCT images.

Histological assessment

The specimens were blindly evaluated by two investigators under an optical microscope. The cases where evaluation did not match were discussed to reach a consensus.

The connective tissue reactions and the periodontal specific reparative tissue response were evaluated under microscopy according to established criteria for inflammatory cell infiltration, hard tissue resorption, hard tissue repair and cementum repair in the furcation area (Table V.2).

Table V.2: Evaluation criteria for histological assessment parameters

Inflammatory cell infiltration*
Grade 1 - No detectable cells
Grade 2 - Mild (few scattered inflammatory cells)
Grade 3 - Moderate (focal accumulation of inflammatory cells)
Grade 4 - Severe (dense infiltration of inflammatory cells)
Hard tissue response**
Hard tissue resorption*:* presence of tissue resorption in both bone and cementum adjacent to the amputated area
Yes
No
Hard tissue repair*:* presence of tissue repair in both bone and cementum adjacent to the amputated area
Yes
No
Cement repair at furcation area
Grade 1 - Totally repaired
Grade 2 - Repair up to half of furcation
Grade 3 - Repair up to a quarter of furcation
Grade 4 - No repair

*Performed according to Ørstavik and Mjör as described by Noetzel *et al.*[226]
**Performed according to *Zairi et al.*[341]

Statistics

Statistical analysis was performed using SPSS™ (version 22, SPSS, Inc., Chicago, Il).

Normality of distribution of continuous variables was determined using Kolmogorov-Smirnov and Shapiro-Wilk tests.

Comparisons between multiple groups (inflammation scores and cementum repair at the furcation area) were performed with Kruskal-Wallis test and, where differences were identified, pairwise comparisons were performed using Mann-Whitney U test with correction by Holm's sequential Bonferroni method. Chi-square testing with Fisher's Exact test was used for categorical data (radiographic and hard tissue responses). For analysis of continuous variables (microCT results), independent-samples t-test was used. All differences were considered significant at $P \leq 0.05$.

Values are expressed as means±standard deviation, median and interquartile range (IQR) or number (percentage), as appropriate.

RESULTS

1. Clinical and macroscopic observations

The animals remained healthy throughout the study period. No signs of infection or of local intolerance were observed.

2. Mortality

No deaths occurred. As expected, all the animals awoke uneventfully and reached the defined end-point (4th postoperative month).

3. Radiographic assessment:

Six prMTA specimens (30%) presented development or increase of radiolucency in the furcation area, in contrast with only two cases (10%) in BDT group.

Both test materials showed similar radiographic evolution 4 months after surgery (P>0,05) (Figure V.2).

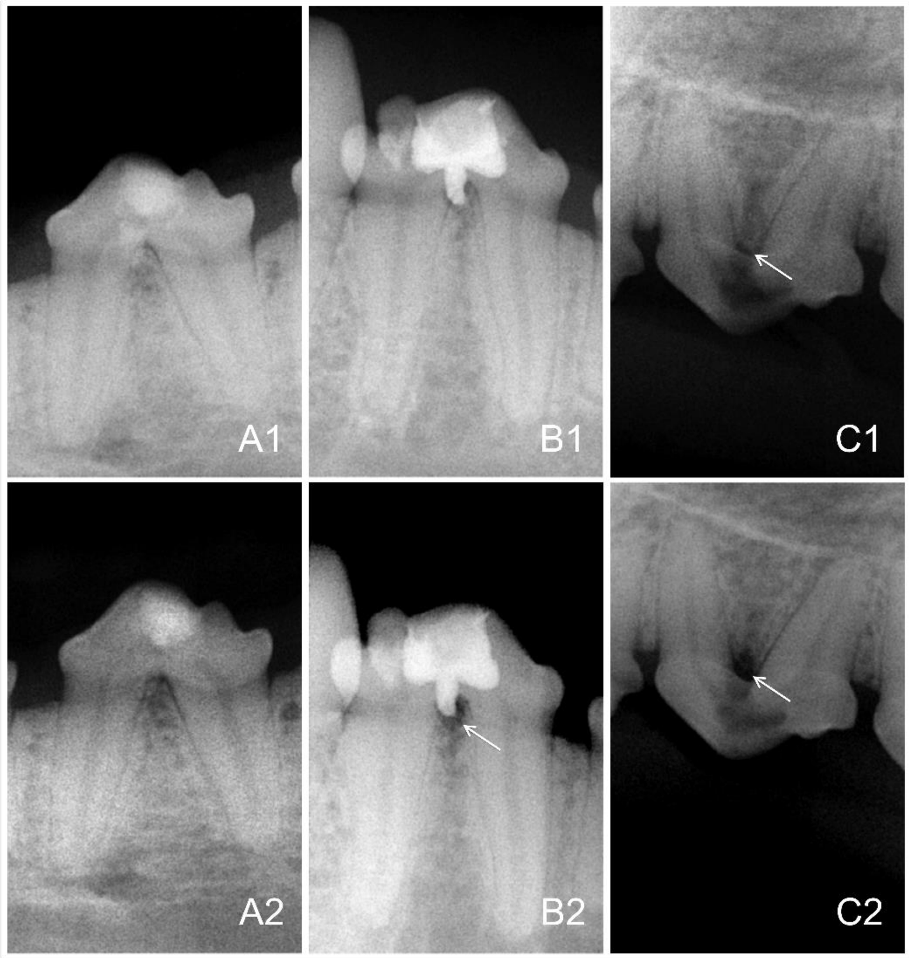

Figure V.2: Radiographic images. (**A**) Radiographs of a third mandibular left premolar restored with Biodentine: (A1) immediate postoperative, (A2) 120 days after furcation perforation repair. (**B**) Radiographs of a fourth mandibular right premolar restored with prMTA: (B1) immediate postoperative, (B2) 120 days after furcation perforation repair with development of radiolucency (arrow). (**C**) Radiographs of second left maxillary premolar, positive control: (C1) immediate postoperative with radiolucency (arrow), (C2) 120 days after furcation perforation with increase of radiolucency (arrow).

4. MicroCT

Total volume of extruded material (Figure V.3A) was significantly lower in BDT group than in prMTA group (BDT: 1.42±0.80mm^3; prMTA: 2.27±1.67mm^3; P=0.049).

In both test material groups, microCT showed continuity between the extruded repair material and the surrounding bone (Figure V.3B and C).

Along with the study's included outcomes, further evaluation of microCT images allowed the identification of new mineralized tissue bridges over the remaining radicular pulp tissue in specimens of both test groups (Figure V.3D-F).

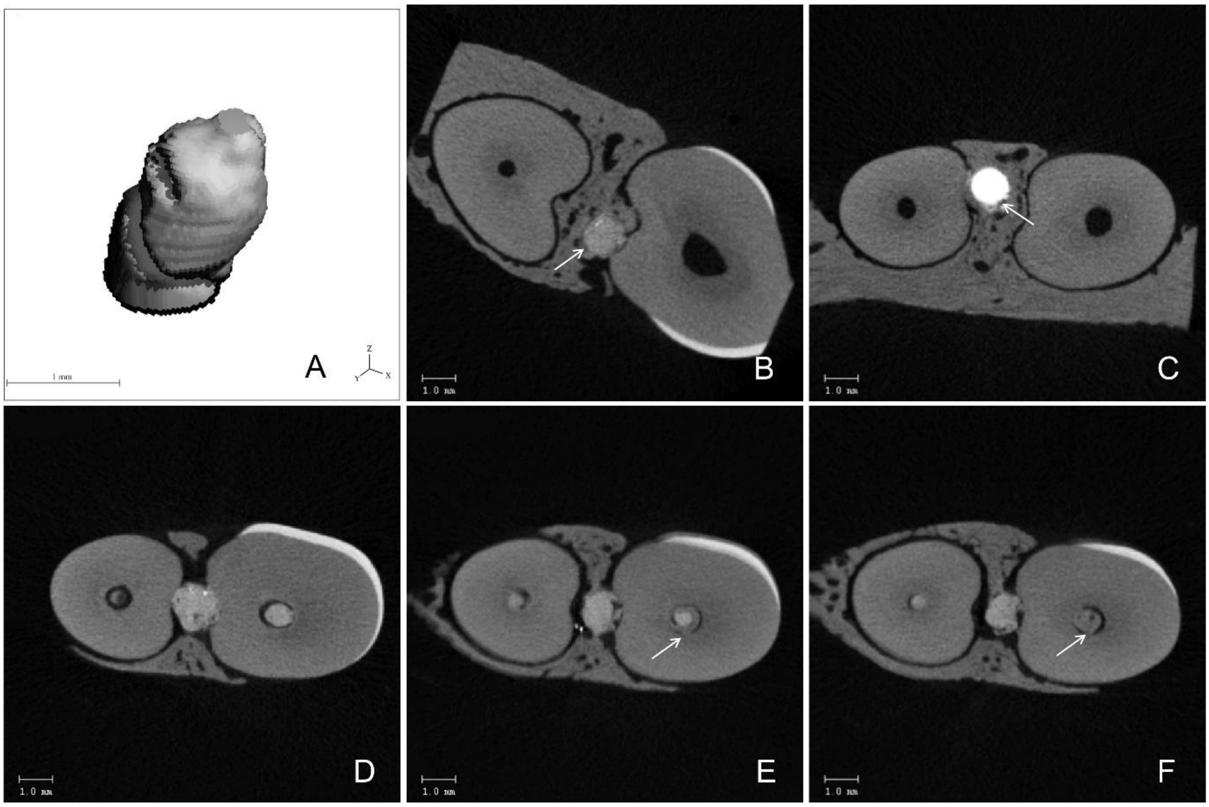

Figure V.3: Micro-CT volume reconstruction and axial sections. (**A**) Micro-CT 3D model reconstruction representative of extruded material volume in a fourth right mandibular premolar. (**B**) Micro-CT axial section of a fourth left mandibular premolar restored with Biodentine (arrow) in continuity with adjacent bone. (**C**) Micro-CT axial section of a third left mandibular premolar restored with prMTA (arrow) in continuity with adjacent bone. (**D, E, and F**) Micro-CT axial sections of a third right mandibular premolar restored with Biodentine showing dentine bridge (arrow) formation from coronal to apical (from D to F).

5. Histological assessment

From the 50 teeth, 3 specimens from prMTA group and 2 specimens from BDT group, were excluded from the histological assessment due to technical problems during the histological processing.

Table V.3 summarizes the results of the variables of histological assessment and Figure V.4 shows illustrative histological images.

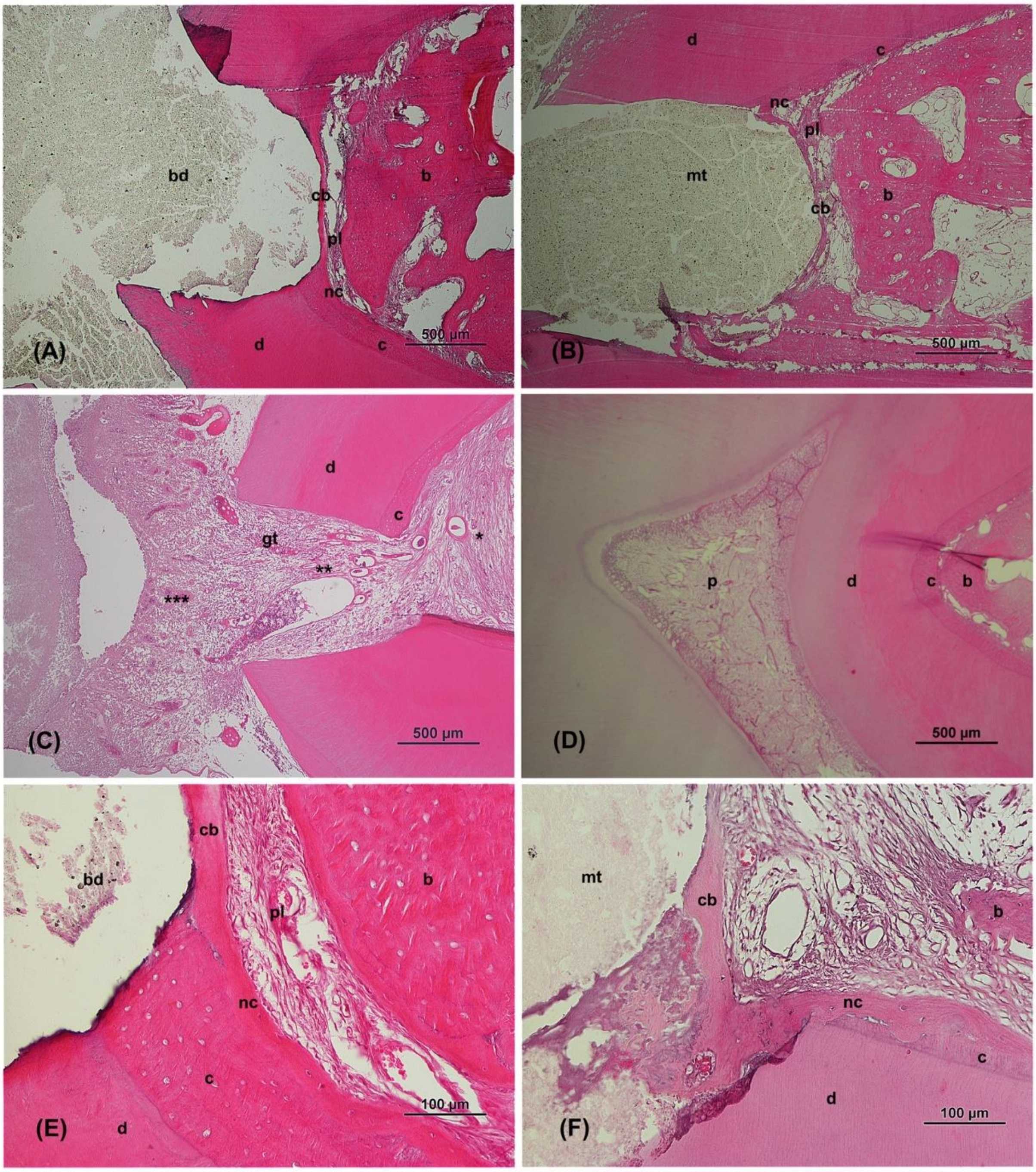

Figure V.4: Histological images 120 days after furcation perforation repair. (**A**) Third left maxillary premolar restored with Biodentine (bd) and (**B**) third left mandibular premolar restored with prMTA (mt), both showing cementum bridge (cb) formation, presence of new cementum (nc) over the previous cementum (c) and the periodontal ligament (pl) attached to the alveolar bone (b). (**C**) Second left maxillary premolar with unrepaired perforation (PCont group) presenting granulation tissue (gt) in the furcation (*), in the perforation (**) and in the pulp chamber (***). (**D**) Second left maxillary premolar without perforation (NCont group) with vital pulp (p), dentine (d), cementum (c), periodontal ligament (pl) and bone (b). (**E**) Third left maxillary premolar restored with Biodentine (bd) in higher magnification showing cementum bridge (cb) formation and presence of new cementum (nc) over the previous cementum (c) and periodontal ligament (pl) attached to the alveolar bone (b). (**F**) Third left maxillary premolar restored with prMTA (mt) in higher magnification showing hard tissue remodeling with signs of cementum (c) resorption and formation of new cementum (nc) and the cementum bridge (cb). Conventional light microscopy; Hematoxylin-eosin; (A, B, C and D) x4, (E and F) x20.

Table V.3: Histological assessment results

Histology	BDT (n=18)	prMTA (n=17)	PCont (n=5)	NCont (n=5)
Inflammation **Scores 1/2/3/4 (n)**	16/2/0/0	11/3/3/0	0/0/1/4	5/0/0/0
Hard Tissue Resorption **Yes/No (n)**	1/17	4/13	5/0	0/5
Hard Tissue Repair **Yes/No (n)**	18/0	17/0	0/5	NA
Cement repair at furcation area **Scores 1/2/3/4 (n)**	9/8/1/0*	4/7/6/0*	0/0/0/5	NA

BDT: Biodentine group; n: number; prMTA: ProRoot MTA group; PCont: positive control group; NCont: negative control group; NA: not applicable. *Significant difference between test materials (*P*<0.001).

As for microCT, further histological observation also identified new mineralized tissue bridges over the remaining radicular pulp tissue in specimens of both test groups (Figure V.5).

There was no evidence of bacterial presence in test groups.

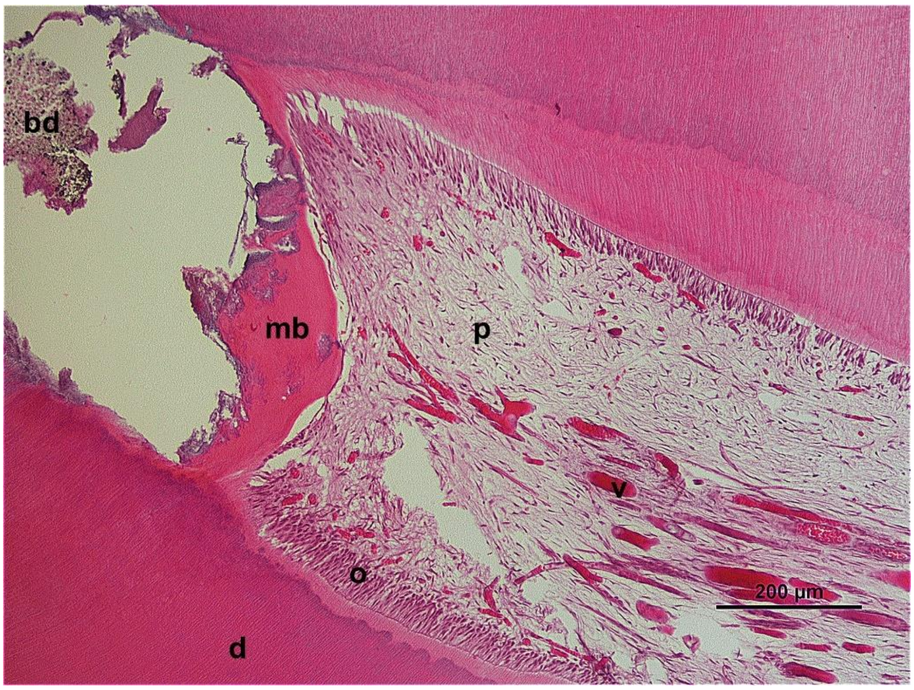

Figure V.5: Second left mandibular premolar restored with Biodentine (bd) showing a mineralized bridge (mb) over vital pulp (p) with blood vessels (v) and odontoblasts (o) attached to the dentine (d) in the root canal lateral wall. Conventional light microscopy; Hematoxylin-eosin; x10.

5.1 Inflammation scores

Concerning the grade of inflammation 4 months after FP repair, both test groups presented favorable results with only two cases of few scattered inflammatory cells in BDT group and six cases in prMTA group. There was no significant difference in inflammation scores between repair with Biodentine or prMTA (P>0.05), although BDT group showed a trend towards more favorable results, with median (IQR) score 1 (0) for BDT group and 1 (1) for prMTA group. Median score for the PCont group was 4 (1) and all NCont samples were graded 1. Both test groups showed significant difference when compared to the positive control (BDT *vs.* PCont: P<0.001; prMTA *vs.* PCont: P=0.001) and no statistically significant difference to the negative control (both P>0.05).

5.2 Hard tissue resorption

After 4 months, resorption was present in all the specimens of the PCont group, in none of the NCont group, in four prMTA group specimens and in only one case in the BDT group, however without significant difference (P>0,05) between test-material groups.

5.3 Hard tissue repair

At the fourth postoperative month, all specimens of the prMTA and BDT groups showed signs of hard tissue repair.

5.4 Cementum repair at furcation area

Four months after FP repair, all the test groups' specimens presented cementum formation (Figure V.4 A, B, E, F). BDT group showed significantly better scores than prMTA group [Median scores (IQR); BDT: 1.5 (1) *vs.* prMTA: 2 (2), P=0,03]. The two groups presented significantly higher repair than the PCont group (both P<0.001). As expected, all PCont samples were graded 4, with no cementum repair.

DISCUSSION

This report presents an *in vivo* qualitative and quantitative analysis of histological and imaging findings after FP repair with Biodentine, using an established large animal model. In this study, Biodentine's *in vivo* behavior was at least comparable to the gold standard, confirming and eventually surpassing our null hypothesis. Regarding prMTA and BDT groups, the tested variables showed equivalent inflammatory and radiographic responses, together with similar hard tissue resorption and repair; on the other hand, BDT group showed significantly lower volume of extruded material and higher cementum repair.

Different materials have been used for perforation repair and their performance has been assessed through several methodologies, however, so far, none was considered as the ideal.[101,132,147,226,267] In cases where there is direct contact with the surrounding connective tissue, biocompatibility is of primary significance.

In this study, prMTA was used as a comparison material, because, despite drawbacks such as poor handling characteristics and slow setting time,[4,235] it has been considered a gold standard in FP repair,[235] due to the favorable sealing abilities and high biocompatibility.[101,132,226,235]

Even though Biodentine did not reveal favorable results in washout tests in one study,[117] various other tests published in literature revealed promising results, such as increased compressive and push-out bond strength,[4,189] decreased porosity, high density,[68,191] colour stability,[257,329,340] induction of cell proliferation and biomineralization,[68,114,157,174,193,238,342,345] immediate formation of calcium hydroxide, high release and depth of incorporation of calcium ions,[106,264] low cytotoxicity,[175] gingival fibroblast viability preservation,[345] ease of handling and faster setting time (12 minutes).[117,253,264] Hence, Biodentine could be an efficient alternative to mineral trioxide aggregate formulations.[253]

The favorable results regarding inflammation scores and hard tissue analysis support biocompatibility and bioactivity of both materials in FP repair, which is in consonance with previous general studies regarding Biodentine.[174,175,189,253] Also, even though it was not part of study's outcomes, the observation of new mineralized tissue bridges over the remaining radicular pulp in the test groups is consistent with other findings in literature, namely an *in vivo* study by Rossi *et al.*,[264] who used Biodentine and prMTA after pulpotomy in dogs and described similar results, demonstrating tissue compatibility. Very recently, Silva *et al.*[285] compared the periradicular tissue response of Biodentine, ProRoot MTA, and gutta-percha in FP repair, by histopathologic and indirect immunofluorescence analyses, using a total of 30 teeth[285] in 3 dogs and concluded that Biodentine could be considered as an adequate FP repair material. Even though the sample size is small, as well as uneven among materials, and the choice of study groups is dubious with subsequent possibly biased interpretations, the presented results complement our study using different methodologies.

Concerning radiographic assessment results, when the furcation perforations were performed with a bur until hemorrhage was found, in some cases a small defect was created in the bone, viewable on the postoperative radiographs. The radiological examination evaluated the appearance or increase of previously present radiolucency at the fourth postoperative month; the absence of significant difference between the test groups was consistent with the histological results.

Micro-CT is a non-destructive technique that does not require specimen demineralization and therefore can provide additional information.[14] In this study, micro-CT was used to quantify the volume of extruded material to the periodontal tissues. Extrusion of filling material into the periodontal space may hinder periodontal reattachment[205] and adversely affect tissue repair,[132] because there is no absorption of the material by the tissues.[287] This is even more relevant for FP which, given their anatomical specificities, act as bottomless pits.[347] The use of internal biocompatible matrices has been advocated to help control the extrusion of the filling materials as well as increase their sealing ability, however with equivocal results.[12,17,250] The greater amount of extruded material found for prMTA group is consistent with its lengthier setting time, which may contribute to the unintended compaction of the unset material into the furcation defect.

The higher grade of cementum repair found in BDT group may be explained by lesser amount of extruded material than in prMTA group. Also, the difference in the chemical reaction's velocity during the setting of materials is considered to be associated with better repair conditions. Although both materials produce the same chemical compounds,[68] Biodentine allows a greater release of ions during the initial setting, which reduces over time,[67] subsequently improving conditions.[253] Moreover, hydration of the calcium oxide contained in MTA may prompt an exothermic reaction,[68] possibly inducing less favorable conditions.[264] It may be hypothesized that if the study had a longer evaluation period, the overall results for prMTA and BDT groups could be even more similar.

Regarding the hard tissue repair found in all test group specimens, although the perforations were done using slow speed, temperature may have increased when inserting the bur into the alveolar bone, generating low thermal trauma,[12,101,267] with subsequent resorption and deposition of new bone and cementum, as hypothesized by Al-Daafas *et al.*[12]

According to the results of this study, both prMTA and Biodentine yield acceptable results in FP repair in dogs' teeth. Dogs were selected for this study because of their well-documented physiological responses and dental anatomy, with a suitable furcation that provides good accessibility and visibility. However, the furcation of the posterior teeth is often as close as 1 to 2mm from the cementoenamel junction.[12] Therefore, epithelialization of a furcation perforation in dogs is considered more likely than in human teeth, where the furcation is deeper in the alveolus. Thus, our favorable results obtained in a dog model of FP may be associated with even better responses in humans.[12] Nevertheless, extrapolation of our data to a clinical setting must be cautious, as with every animal study.

It may be concluded that Biodentine presented tissue compatibility and allowed for mineralized tissue formation after FP repair in dogs' teeth, with similar morphology and integrity but greater cementum formation than prMTA. The excellent outcomes of the present study, complementing other favorable results obtained by different research methods, are highly suggestive that Biodentine is a promising biomaterial to be used for the repair of furcation perforations.

Chapter VI - Clinical Applications of Biodentine

Part of this chapter has been based on the following articles:

Cardoso MA, Noites RB, Martins MA, Paulo MP. Nonsurgical endodontic retreatment of fused teeth with transposition: a case report. Restorative Dentistry and Endodontics. 2016.41:148-153.

Cardoso M, Paulo M, Viegas C. Biodentine in furcation perforation repair. Submitted to Brazilian Dental Journal.

Clinical Applications of Biodentine

INTRODUCTION

Biodentine's uniqueness relies on its biocompatibility, bioactivity and versatility of applications.

In comparison with other calcium silicate based materials, Biodentine possesses better biological and physico-chemical properties such as easier handling, faster setting time, biocompatibility, stability, increased compressive strength, increased density, decreased porosity, tight sealing properties, and reparative dentine synthesis.

Biodentine has a wide range of clinical applications. Up until July 2017, 38 case reports could be found in MEDLINE/Pubmed under the keyword "Biodentine" (Table VI.1). The cases reported the use of Biodentine in different applications such as: 1 of indirect pulp capping, 4 of apexogenesis, 4 of retrograde filling / apical surgery, 1 of vertical root fracture repair, 6 of apexification, 4 of palatogingival groove repair, 2 of pulpotomy in a mature permanent tooth, 2 of internal root resorption, 5 of external resorption, 6 of regenerative treatment in necrotic immature teeth, 1 of full canal obturation in immature permanent tooth, 1 of dentine substitute base in a compromised tooth, 1 of *dens invaginatus* and none specifically in furcation perforation repair.

CASE REPORTS – PERSONAL EXPERIENCE

In this part of the Thesis a selection of 11 case reports of the author's personal experience is presented. Biodentine was used as material of choice for different applications such as: apexification, apexogenesis, pulpotomy, indirect pulp capping, pulp capping, deciduous pulpectomy, repair of root perforation, repair of internal or external resorptions, palato-gingival groove and apical surgery. With this description we aim to emphasize, with our personal experience in a case series, the versatility of applications of Biodentine and describe its use for the first time specifically in furcation perforation repair.

Table VI.1: Case reports in literature using Biodentine

Clinical apllication	Teeth (n)	Type of teeth	Follow-up	Author, Date
Indirect pulp capping	1	Right mandibular first molar	4 mt	Rada, 2013[249]
Apexogenesis	6	Right mandibular second premolar	6 mt	Villat 2013[332]
		Right maxillary central incisor	1 yr	Bhat 2014[48]
		Left maxillary central incisor	3 yr	Martens 2015[197]
		Right maxillary central incisor	4 yr	Martens 2015[197]
		Left maxillary lateral incisor	2 yr	Martens 2015[197]
		Right mandibular first molar	18 mt	Ashraf 2017[29]
Retrograde filling / apical surgery	7	Right maxillary central incisor	1 w	Bachoo 2013[34]
		Left mandibular second premolar*	0	Bachoo 2013[34]
		Right maxillary central incisor	18 mt	Pawar 2013[236]
		Right maxillary lateral incisor	18 mt	Pawar 2013[236]
		Left maxillary second premolar (x2)	2 yr	Caron 2014[73]
		Right mandibular central incisor	1 yr	Jain 2015[136]
Vertical root fracture	1	Right maxillary central incisor**	2 yr	Hadrossek 2014[123]
Apexification	6	Left maxillary lateral incisor	18 mt	Khetarpal 2014[154]
		Left maxillary central incisor	1 yr	Nayak 2014[220]
		Left maxillary central incisor	1 mt	Bajwa 2015[39]
		Right maxillary central incisor	6 mt	Sharma 2015[279]
		Right maxillary central incisor	1 yr	Niranjan 2016[224]
		Left maxillary central incisor	18 mt	Vidal 2016[331]
Palatogingival groove	5	Left maxillary lateral incisor	2 yr	Johns 2014[140]
		Left maxillary lateral incisor	6 mt	Naik 2014[214]
		Right maxillary lateral incisor	1 yr	Sharma 2015[278]
		Right maxillary central incisor	1 yr	Nadig 2016[211]

(Table VI.1 continued)

Clinical apllication	***Teeth (n)***	***Type of teeth***	***Follow-up***	***Author, Date***
Pulpotomy in a mature permanent tooth	5	Right maxillary central incisor (x3)	18 mt	Borkar 2015[52]
		Right maxillary lateral incisor	18 mt	Borkar 2015[52]
		Right mandibular first molar	18 mt	Soni 2016[296]
Internal root resorption	2	Left mandibular first molar	43 mt	Borkar 2015[53]
		Left maxillary first premolar	10 mt	Umashetty, 2015[325]
External resorption	5	Right mandibular second premolar	4 mt	Salzano, 2015[268]
		Maxillary central incisors (x2)	11 mt	Baranwal, 2016[45]
		Maxillary central incisors (x2)	3 yr	Karypidou, 2016[145]
		Left maxillary central incisor	1 yr	Ambu, 2017[22]
		Right maxillary central incisor**	18 mt	Pruthi, 2015[246]
Regenerative treatment in necrotic immature teeth	12	Left mandibular second premolar	1 yr	Khoshkhounejad, 2015[155]
		Right mandibular second premolar	2 yr	Aldakak, 2016[16]
		Left mandibular second molar	1 yr	Subash, 2016[301]
		Mandibular first molars (x3)	18 mt	Topçuoglu, 2016[313]
		Maxillary central incisors (x4)	18 mt	Bakhtiar, 2017[41]
		Right maxillary lateral incisor	18 mt	Bakhtiar, 2017[41]
		Right maxillary central incisor	2 yr	Chaniotis, 2017[79]
Full canal obturation in immature permanent teeth	3	Maxillary central incisors (x3)	2 yr	Martens, 2016[198]
Dentine substitute base in a compromised tooth	1	Right mandibular first molar	20 mt	Soares, 2016[294]
Dens invaginatus	1	Right maxillary lateral incisor	30 mt	Goel, 2017[113]

n: number; mt: months; yr: year(s); x3: 3 teeth; w: week; x2: 2 teeth; *with lateral perforation; **after intentional extraction; x4: 4 teeth.

Case report 1: Furcation perforation

A 21-year-old male patient presented with pain in the left mandibula and recurrent abscesses in this region. The patient's medical history was noncontributory. The clinical examination revealed a carious lesion on the left mandibular third molar. The tooth was sensitive to percussion. Periapical radiography showed the carious lesion, an already existing furcation perforation and a radiolucency between the two roots (Figure VI.1A). Based on the clinical and radiographic information, symptomatic apical periodontitis was diagnosed. The treatment plan involved a root canal treatment with repair of the furcation perforation.

Figure VI.1: Periapical radiographs of case report #1. (**A**) Preoperative radiograph showing furcation perforation (arrow); (**B**) furcation reparation with Biodentine (arrow); (**C**) postoperative radiograph; (**D**) two-year follow-up.

Following informed consent provided by the patient, the tooth was anesthetized and isolated with a rubber dam. After opening the access cavity (Figure VI.2A and B) and performing irrigation with 2.5% sodium hypochlorite (NaOCl), the three root canals (mesiobuccal, mesiolingual and distal) were shaped with a ProTaper™ Universal F1 file (Dentsply Maillefer, Switzerland). The canals were dried with sterile paper points and three gutta-percha points F1 were introduced in the canals (Figure VI.2C). The furcation perforation was repaired with Biodentine (Figure VI.2D), and, after setting, the gutta-percha points were removed and calcium hydroxide paste (Calcicur™ - VOCO, Germany) was applied, and the access cavity was temporarily sealed with IRM™. Even though the radiopacity of Biodentine is similar to dentine, a radiograph showed the repair of the furcation perforation (Figure VI.1B).

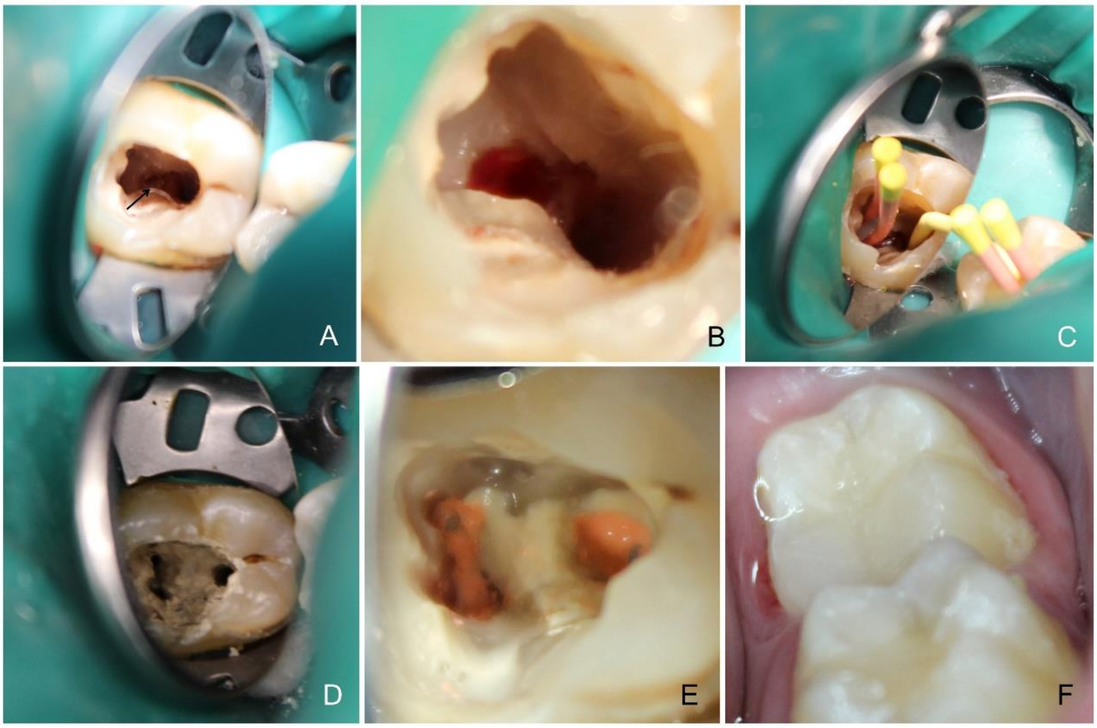

Figure VI.2: Operative photographs of case report #1. (**A**) Furcation perforation (arrow); (**B**) detail of furcation perforation; (**C**) gutta-percha points placed before Biodentine application; (**D**) Biodentine application; (**E**) Biodentine after completely setting and canal obturation with gutta-percha; (**F**) direct composite restoration.

After one month, the patient returned and the calcium hydroxide paste was removed. Irrigation was performed with 2.5% NaOCl. The mesiobuccal and mesiolingual canals were shaped with a finishing ProTaper™ Universal F3 file and the distal canal with a #80 K file. Subsequently passive ultrasonic irrigation was performed, and the canals were dried with sterile paper points. AH Plus™ sealer (Dentsply, Germany) was applied with a lentulo and the excess was removed with paper points. The root canal filling was done with a thermoplasticized gutta-percha system, using GuttaCore™ (Dentsply, Germany) (Figure VI.2E). A postoperative radiograph (Figure VI.1C) confirmed that the root canal system filling was complete. The crown was restored permanently with direct composite (Figure VI.2F).

At the two-year follow-up, there was no symptomatology and less radiolucency was observed around the apical and furcation region (Figure VI.1D) in comparison with the preoperative radiograph.

Case report 2: Apexification

A 36-year-old male patient presented at our clinic reporting pain in the maxillary right central incisor. Radiographic examination showed that the tooth had a previous endodontic treatment with an obturation with radiolucent areas, an incomplete root formation with an open apex and an associated periapical lesion (Figure VI.3A).

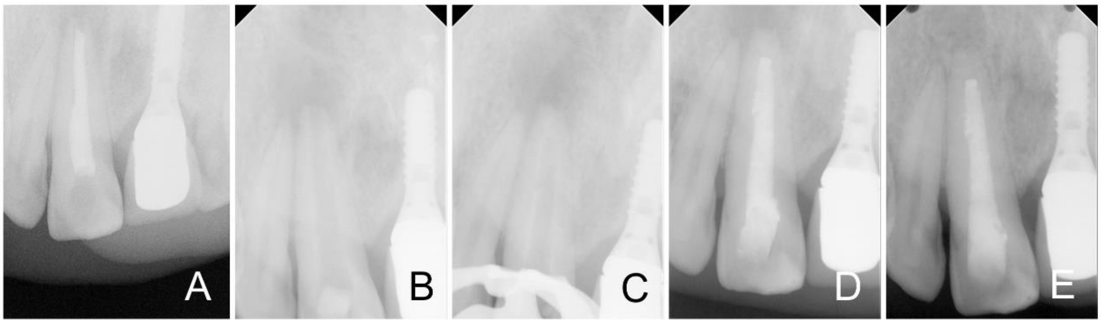

Figure VI.3: Periapical radiographs of case report #2. (**A**) Preoperative radiograph; (**B**) after gutta-percha removal; (**C**) Biodentine filling the apical 3mm; (**D**) postoperative radiograph; (**E**) three-year follow-up.

Nonsurgical endodontic retreatment with apexification with Biodentine was proposed. After the patient provided informed consent, the tooth was anesthetized and isolated with rubber dam. After opening the access cavity, gutta-percha was removed using ProTaper™ Universal retreatment files D1, D2 and D3 (Figure VI.3B). Biomechanical preparation was carried out with #80K file with irrigation with 2.5% sodium hypochlorite. After drying the canal with paper points the apical 3mm were obturated with Biodentine using a plugger performing vertical condensation (Figure VI.3C). AH Plus™ cement was applied in the remaining canal. After removing the cement excess with paper points, the obturation was performed with the injection of warm gutta-percha with Calamus™ (Dentsply Maillefer, Switzerland) (Figure VI.3D). The access cavity was restored with a composite.

After a three-year follow-up, there was no symptomatology and the periapical lesion diminished (Figure VI.3E).

Case report 3: Apexification/internal resorption

A 27-year-old male patient was reported with the maxillary right incisor presenting a periapical lesion, an internal resorption, a pulp chamber calcification, apical resorption and open apex. Radiographic examination confirmed the previous description (Figure VI.4A).

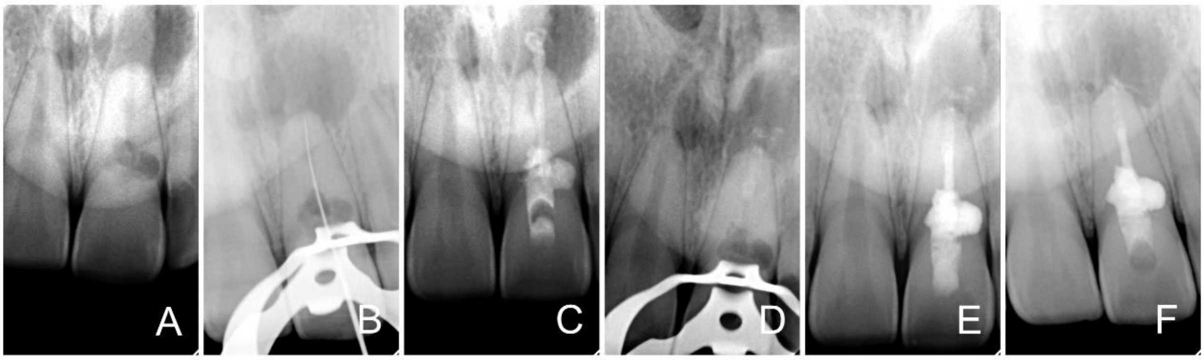

Figure VI.4: Periapical radiographs of case report #3. (**A**) Preoperative radiograph; (**B**) working length determination; (**C**) calcium hydroxide application; (**D**) Biodentine apical plug; (**E**) postoperative radiograph; (**F**) one-year follow-up.

Endodontic treatment with apexification with Biodentine was proposed. After the patient provided informed consent, the tooth was anesthetized and isolated with rubber dam. After opening the access cavity the working length (22mm) (Figure VI.4B) was determined using an electronic apex locator. The bottom of the resorption was demonstrated to be at 16mm, confirmed by a radiograph.

Biomechanical preparation was performed with a #70K file using 2.5% sodium hypochlorite irrigation. The resorption cavity was cleaned with a precurved #25K file with the aid of ultrasounds. Calcium hydroxide paste Calcicur™ was applied and the access cavity was temporarily sealed with DuoTEMP™ (Coltène/Whaledent AG, Switzerland) (Figure VI.4C). After rubber dam isolation and access cavity opening, irrigation with 2.5% sodium hypochlorite powered by ultrasounds was performed. After drying the canal with paper points, the apical 4mm were obturated with Biodentine using a plugger (Figure VI.4D). AH Plus™ cement was applied with a lentulo in the remaining canal. After removing the cement excess with paper points, the obturation of the remaining canal and of the root resorption was performed with the injection of warm gutta-percha with the BeeFill™ 2-in-1 (VDW, Germany) (Figure VI.4E). The access cavity was restored with a composite. After a 1-year follow-up, there was no symptomatology and the periapical lesion diminished (Figure VI.4F).

Case report 4: External resorption

A 48-year-old female patient presented for a routine appointment. Radiographic examination showed an external root resorption on the maxillary left lateral incisor, which was restored with composite and a fiber post (Figure VI.5A).

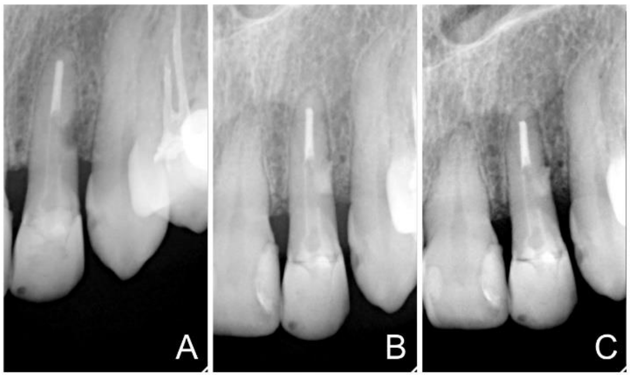

Figure VI.5: Periapical radiographs of case report #4. (**A**) Preoperative radiograph; (**B**) postoperative radiograph; (**C**) six-month follow-up.

A fistula could be observed in the buccal aspect (Figure VI.6A). Periodontal probing revealed normal values of 1mm on the palatal aspects, 2mm on buccal and mesial aspects (Figure VI.6B) and 9mm on the distal aspect (Figure VI.6C). A Cone Beam Computed Tomography (CBCT) was done which confirmed the presence and the localization of the external resorption (Figure VI.7).

After the patient provided informed consent, surgery with repair of the external resorption was performed. After anesthesia, a triangular flap was done, with the horizontal incision between 21 and 24 teeth and the vertical incision was executed in mesial (Figure VI.6D). After removing granulation tissue (Figure VI.6E), the resorption cavity was restored with Biodentine (Figure VI.5B and 6F) and the suture was performed with Surgycril™ PGA 5/0. At the time of the removal of the suture the fistula had disappeared (Figure VI.6G).

After a 6-month follow-up, there was no symptomatology, the resorption was restored and periodontal probing was stabilized, predicting a good evolution (Figures VI.5C and 6H).

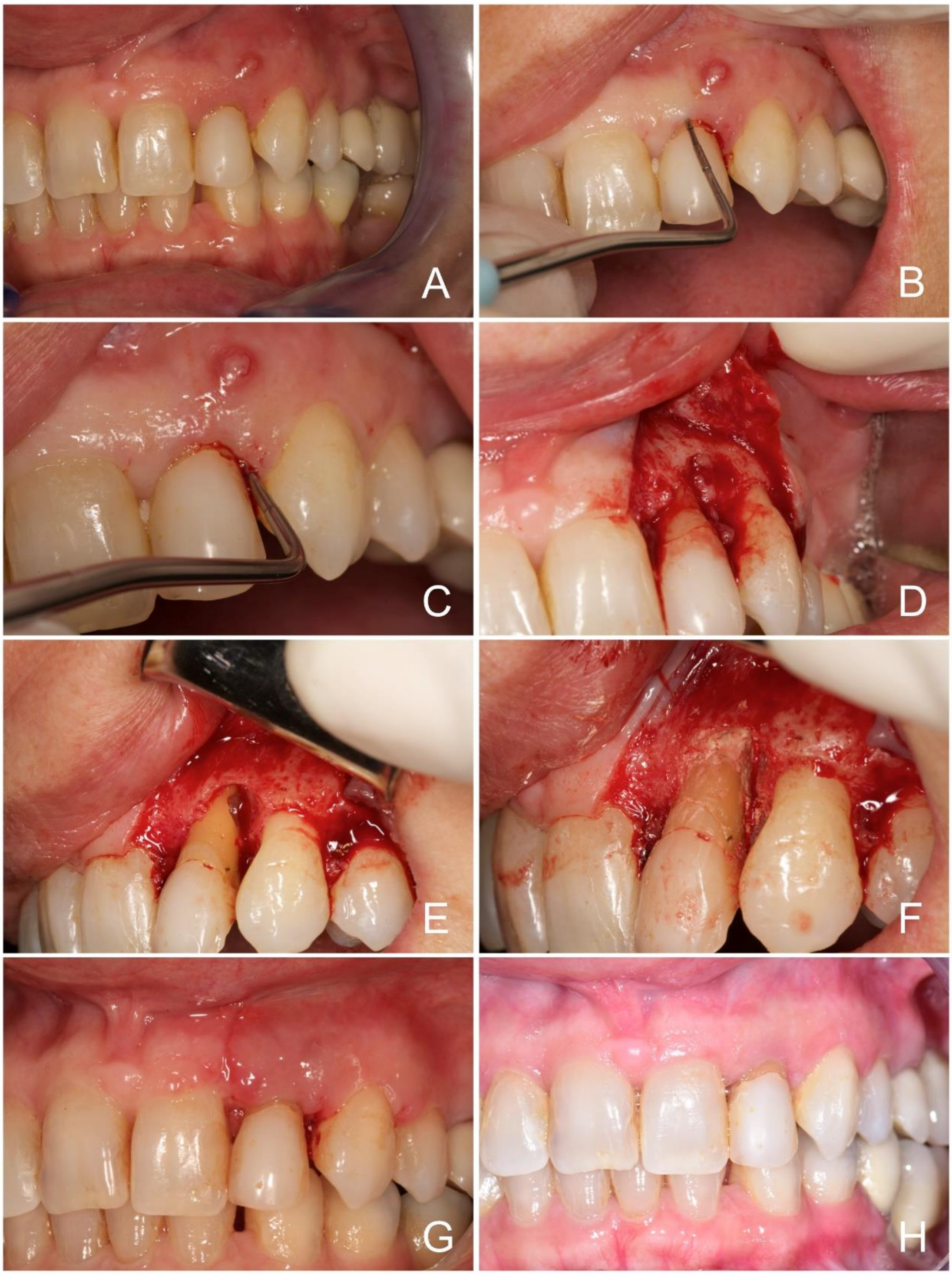

Figure VI.6: Operative photographs of case report #4. (**A**) Preoperative, with observation of a buccal fistula; (**B**) 2mm buccal probing; (**C**) 9mm distal probing; (**D**) distal resorption with granulation tissue; (**E**) resorption after granulation tissue removal; (**F**) Biodentine obturation; (**G**) after suture removal, without visible fistula; (**H**) six-month follow-up.

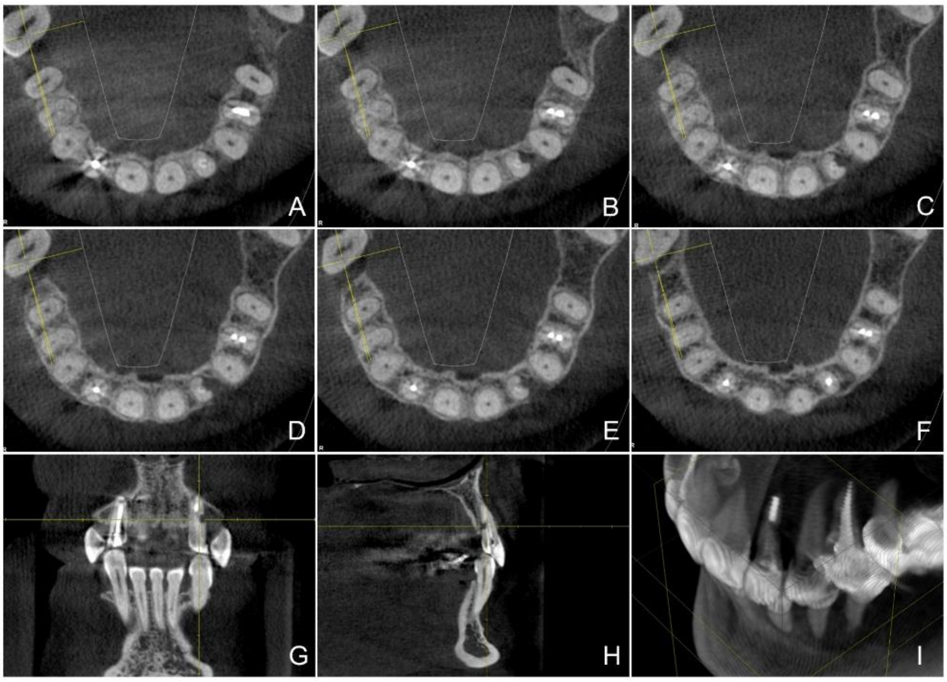

Figure VI.7: CBCT images of case report #4. (**A** to **F**) Axial images from coronal (A) to apical (F); (**G**) coronal view; (**H**) sagital view from right; (**I**) 3D reconstruction.

Case report 5: Palatogingival groove with external resorption

A 53-year-old male patient presented with complaints of pain and swelling on the maxillary left lateral incisor.

Facial clinical examination revealed an intact crown devoid of any carious lesions or fracture lines. Palatal examination showed a localized circumscribed swelling. Periodontal probing revealed 6mm on the mid palatal aspect of the root. After sensibility tests, vital pulp was diagnosed. Tenderness on percussion was mild with absence of mobility. Radiographic examination showed a radiolucent image, below the cement enamel junction similar to an external root resorption (Figure VI.8A).

Figure VI.8: Periapical radiographs of case report #5. (**A**) Preoperative radiograph; (**B**) after endodontic treatment; (**C**) resorptive defect restored with Biodentine; (**D**) postoperative radiograph, after restoration with composite; (**E**) fifteen-month follow-up.

After the patient provided informed consent, an access to the resorption lesion was performed through the crown. A pulp exposure occurred almost in the base of the resorption. The cavity was restored with DuoTEMP™ and endodontic treatment was performed. After rubber dam application, the access cavity was performed and the canal was shaped with ProTaper™ Universal F2 and filled with gutta-percha lateral condensation and AH Plus™ cement (Figure VI.8B).

Figure VI.9: Operative photographs of case report #5. (**A**) Resorptive defect restored with Biodentine; (**B**) partial removal of Biodentine; (**C**) postoperative after restoration with composite.

On the next visit, the cavity was cleaned and restored with Biodentine (Figures VI. 8C and 9A). After one week, part of Biodentine was removed until 1mm below the gingival margin (Figure VI.9B). and restored with composite Filtek™ Z250 (3M ESPE AG, Germany) (Figures VI.8D and 9C). After a 2-year follow-up, there was no symptomatology and no radiolucent image, with a healthy gum, predicting a good evolution (Figure VI.8E).

Case report 6: Indirect pulp capping

A 28-year-old male patient presented with history of recent trauma. Facial clinical examination revealed an upper lip laceration and an extended enamel-dentine tooth fracture without pulp exposure of the maxillary right and left central incisors (Figure VI.10A, B and H1). An indirect pulp capping with Biodentine and a composite provisional restoration was proposed. After the patient provided informed consent, anesthesia was performed and rubber dam was applied. A pink image could be seen in the center of the teeth, suggesting the pulp beneath (Figure VI.10C). The teeth were restored with Biodentine in the center of fracture (Figure VI.10D). Etching was performed only in the enamel (Figure VI.10E) and adhesive was applied at the entire surface (Figure VI.10F), also over Biodentine. Provisional restoration was performed with composite Filtek™ Z250 (Figure VI.10G).

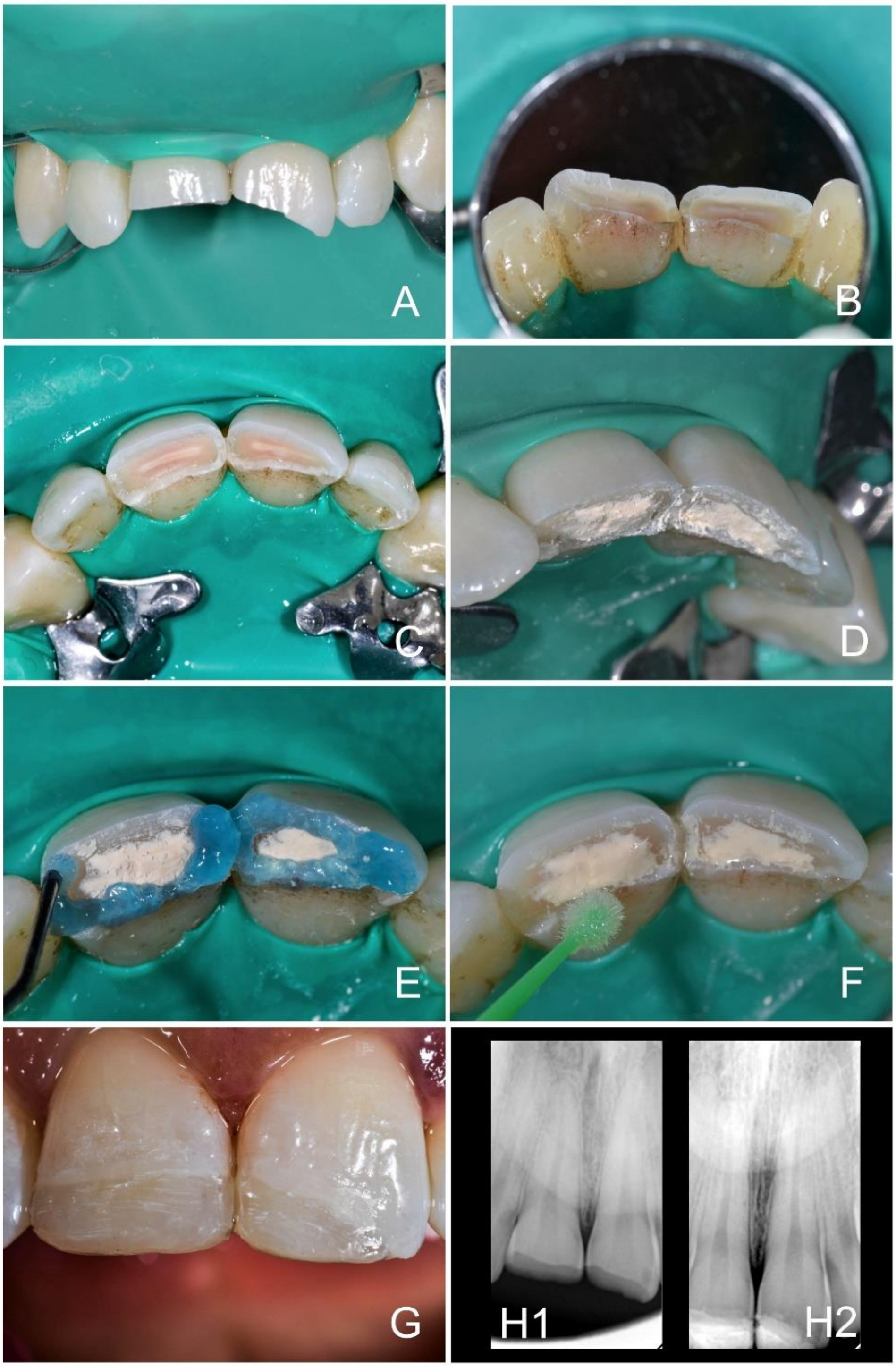

Figure VI.10: Operative photographs and radiographs of case report #6. (**A**) Coronal fracture (buccal view); (**B**) coronal fracture (palatal view); (**C**) coronal fracture (occlusal view); (**D**) indirect pulp capping with Biodentine; (**E**) selective etching; (**F**) bonding application; (**G**) provisional composite restoration; (**H1**) preoperative radiograph; (**H2**) six-month follow-up.

After 1, 3 and 6 months follow-up, the sensibility tests revealed that both teeth presented vitality and in the control radiograph (Figure VI.10H2) no lesion was detected, predicting a good evolution.

Case report 7: Indirect pulp capping / Apexogenesis

An 8-year-old female patient presented with complaint of recent trauma. Clinical examination revealed soft tissue injuries on the upper lip and an extended enamel-dentine tooth fracture without pulp exposure of the maxillary right central incisor. On radiographic examination the tooth revealed incomplete root formation (Figure VI.11A). An indirect pulp capping with Biodentine and a composite provisional restoration were proposed to preserve the tooth vitality and allow apexogenesis.

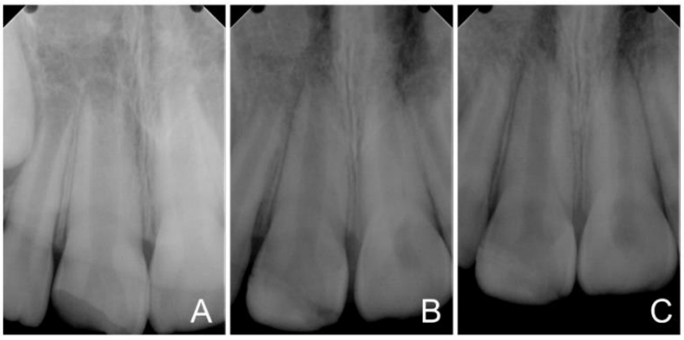

Figure VI.11: Periapical radiographs of case report #7. (**A**) Preoperative radiograph; (**B**) postoperative radiograph; (**C**) fifteen-month follow-up.

After informed consent was provided, anesthesia was performed. The tooth was restored with Biodentine in the center of fracture. After etching and adhesive application a provisional restoration was performed with composite Filtek™ Z250 (Figure VI.11B).

After 1, 3 and 6 months, the sensibility tests revealed that the tooth presented vitality and at the control radiograph the apexogenesis presented a good evolution. At the 15-month follow-up, the root was almost completely formed and the tooth was asymptomatic and vital, maintaining the function (Figure VI.11C).

Case report 8: Pulp capping

A 46-year-old female patient presented at the University Clinic (Universidade Católica Portuguesa) for a routine appointment. On radiographic examination the second mandibular right molar presented a carious lesion below an old restoration. During the carious lesion removal a pulp exposure occurred. Calcium hydroxide was applied and a provisional restoration with IRM™ was performed (Figure VI.12A).

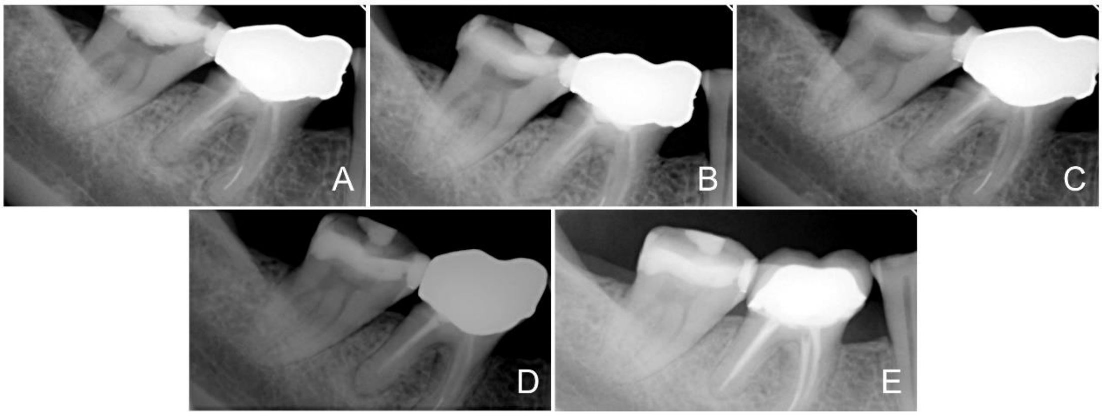

Figure VI.12: Periapical radiographs of case report #8. (**A**) Preoperative radiograph with IRM restoration; (**B**) after Biodentine restoration; (**C**) Biodentine after 1 month; (**D**) postoperative radiograph after restoration with composite; (**E**) three-year follow-up.

After one month, the vitality was confirmed; the provisional restoration was removed and calcium hydroxide was dislodged with pulp exposure (Figure VI.13A and B). The patient was reported to the endodontic department group and a restoration with Biodentine was performed (Figures VI.12B and 13C). After 45 days, the radiograph (Figure VI.12C) was normal and the sensibility tests revealed tooth vitality. Biodentine was partially removed and a composite restoration was done (Figure2 VI.12D and 13D).

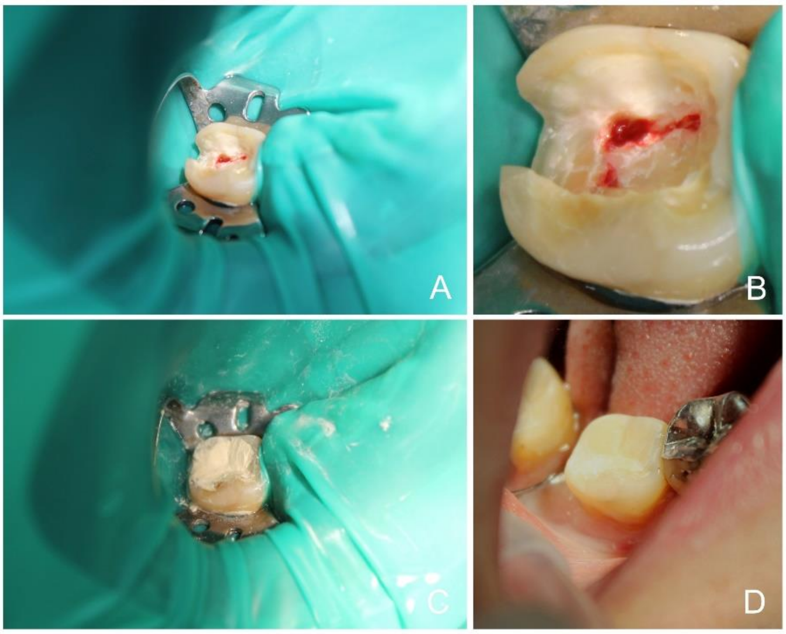

Figure VI.13: Operative photographs of case report #8. (**A**) Pulp exposure; (**B**) extensive pulp exposure; (**C**) Biodentine restoration; (**D**) postoperative, after restoration with composite.

After 3 years, the tooth remained vital and there was no symptomatology. The radiograph (Figure VI.12E) was normal, predicting a good evolution even in this case with an extensive pulp exposition.

Case report 9: Deciduous tooth pulpectomy

A 13-year-old female patient presented at our dental clinic with the deciduous second right mandibular molar with a deep carious lesion approaching pulp.

On orthopantomography (Figure VI.14A), agenesis of the second right mandibular premolar was noticed.

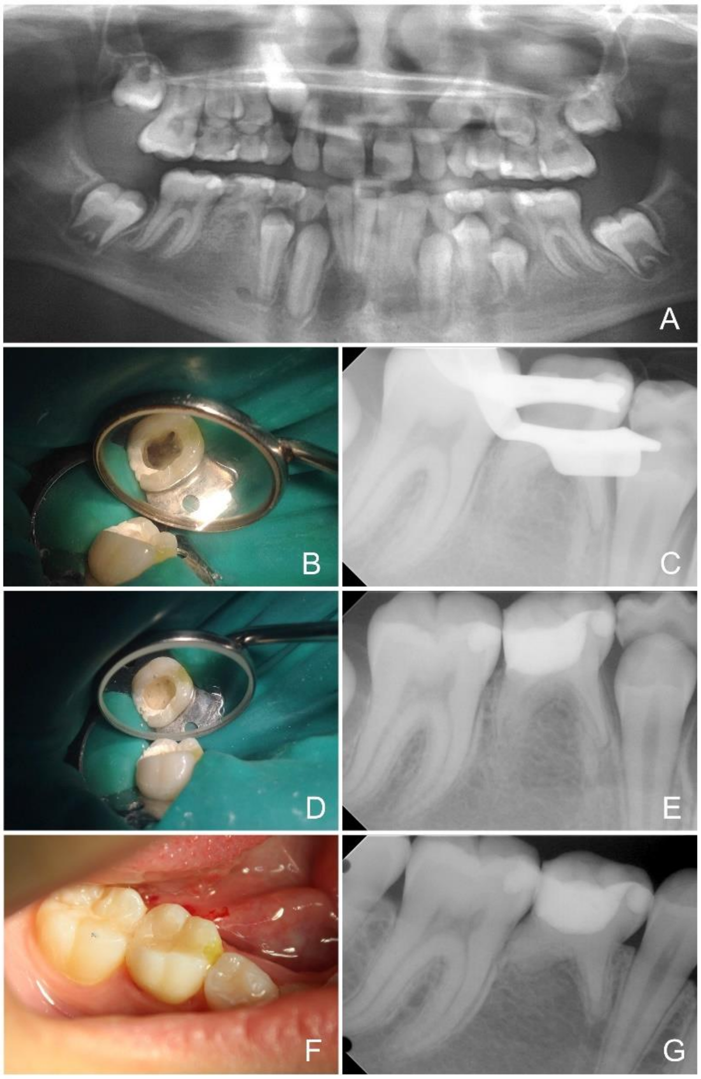

Figure VI.14: Operative photographs and radiographs of case report #9. (**A**) Preoperative orthopantomography; (**B** and **C**) access cavity; (**D**) Biodentine obturation; (**E**) postoperative radiograph; (**F**) composite restoration; (**G**) three-year follow-up.

After planning with an orthodontist, the maintenance of the deciduous molar was decided. After the patient provided informed consent, the carious lesion was removed and an access cavity was performed (Figure VI.14B and C). The working length was determined using an electronic apex locator (mesiobuccal: 13mm; mesiolingual: 14mm; distal: 13mm). All canals were prepared with ProTaper™ Universal F1 file. The canals and pulp chamber were filled with Biodentine (Figure VI.14D).

After one week, Biodentine was partially removed and a composite restoration was performed with Synergy™ D6 (Coltène/Whaledent, United Kingdom) (Figure VI.14E and F).

After 3 years, the tooth maintained the function and there was no symptomatology. The radiograph was normal, predicting a good evolution (Figure VI.14G).

Case report 10: Root perforation

A 21-year-old male patient was referred to our clinic with a history of root perforation on the buccal middle third of the upper right central incisor. On clinical examination a fistula was observed on buccal side of the referred tooth (Figure VI.15A); a radiograph showed a previous endodontic treatment (Figure VI.15C).

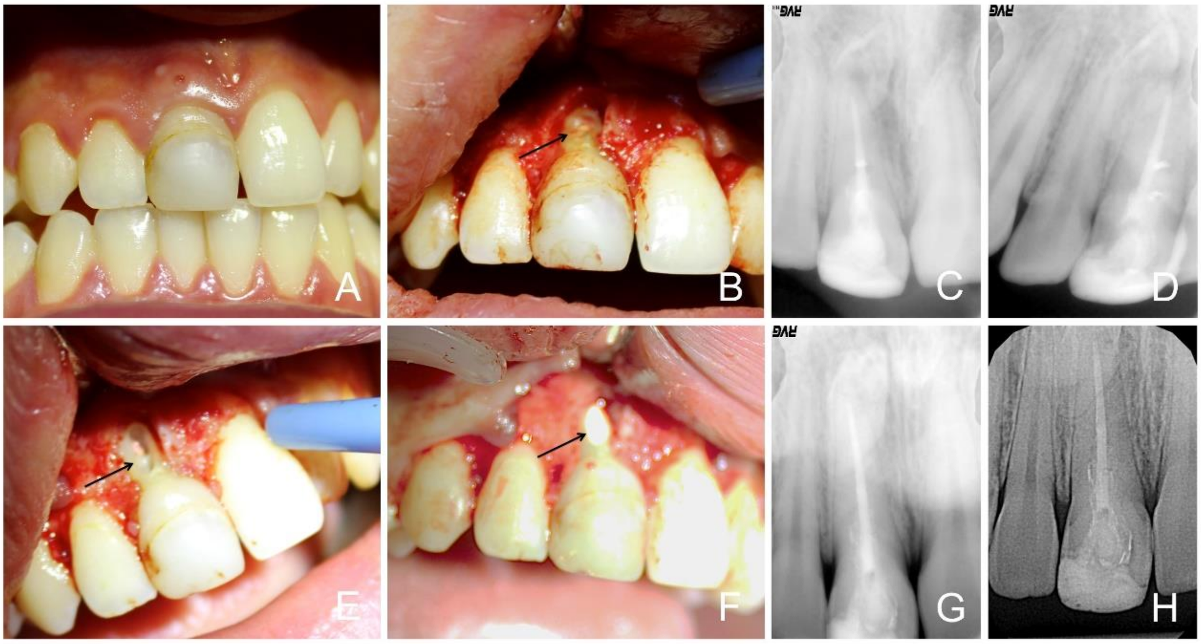

Figure VI.15: Operative photographs and radiographs of case report #10. (**A**) Preoperative photograph; (**B**) perforation (arrow); (**C**) preoperative radiograph; (**D**) postoperative radiograph; (**E**) perforation preparation; (**F**) perforation repair with Biodentine; (**G**) one-year follow-up; (**H**) two-year follow-up.

Periodontal probing revealed values of 1mm on the palatal aspect, 2mm on mesiobuccal and distobuccal aspects and 5mm on the buccal aspect. After the patient provided informed consent, a surgical repair of the root perforation was performed. After anesthesia, a sulcular flap was performed, with the incision between 13 and 22 teeth. After removing the granulation tissue (Figure VI.15B), the perforation site was regularized (Figure VI.15E) and restored with Biodentine (Figure VI.15D and F). The suture was performed with Surgycril™ PGA 5/0.

At the 1st and 2nd -year follow-ups, the tooth maintained the function and there was no symptomatology. The radiographs were normal (Figure VI.15G and H), predicting a good evolution.

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Case report 11: Apical surgery

A 29-year-old female patient presented with an abscess and referred pain on the second left mandibular premolar. Radiographic examination (orthopantomography and periapical radiograph) (Figure VI.16A and B) showed a periapical lesion, a non-treated canal and a fiber post. A CBCT (Figure VI.17) confirmed the presence and the localization of the second canal. A fenestration on the apical area of the tooth was also observed.

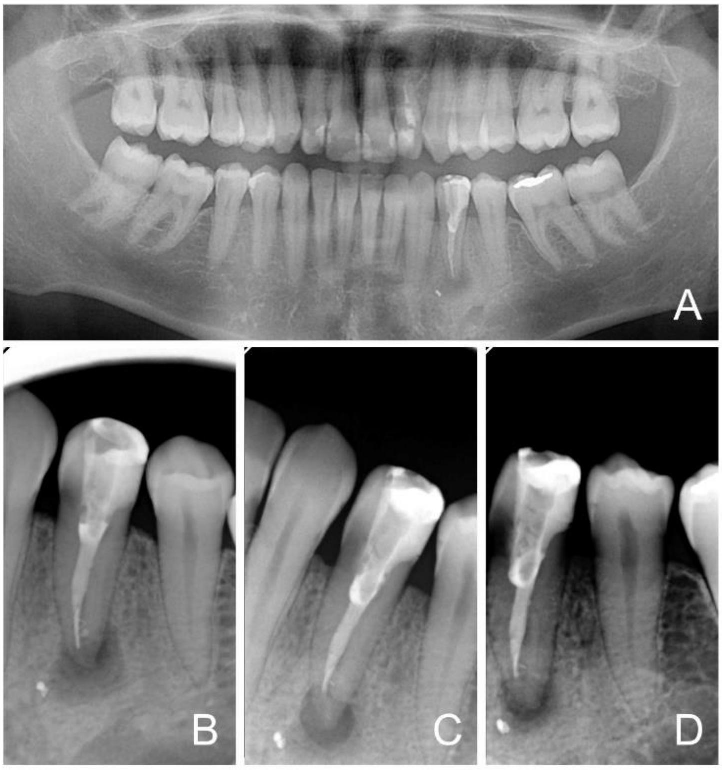

Figure VI.16: Radiographs of case report #11. (**A**) Preoperative ortopantomography; (**B**) preoperative, periapical; (**C**) postoperative, periapical; (**D**) six-month follow-up.

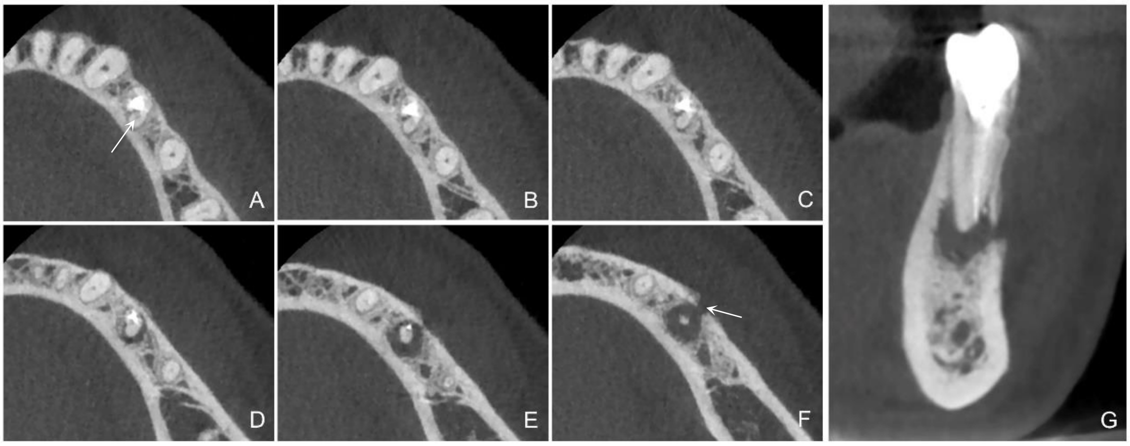

Figure VI.17: CBCT images of case report #11. (**A** to **F**) Axial images from coronal (A) to apical (F), with presence of a second canal (arrow) in a distolingual position and of an apical lesion in images D to F with buccal fenestration in F (arrow); (**G**) sagital view with buccal fenestration.

After the patient provided informed consent, an apical surgery with retrograde filling was performed with the aid of an operatory microscope (Figure VI.18). After anesthesia, a triangular flap was performed (Figure VI.18B, C and D) and a small osteotomy was done to access the apex.

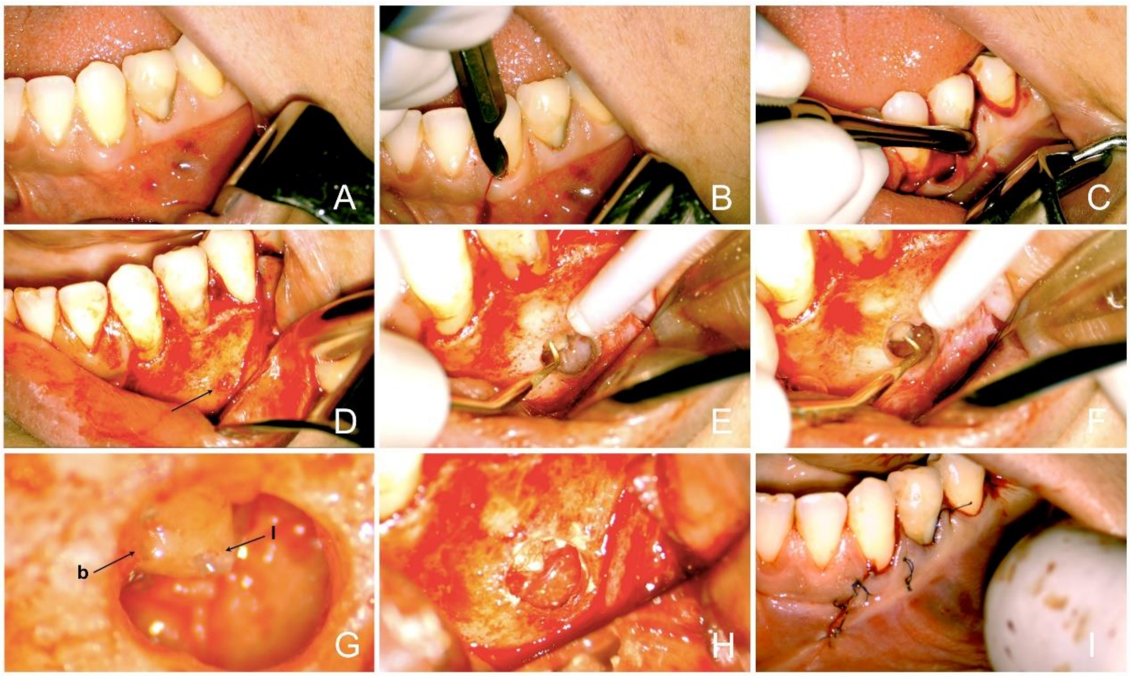

Figure VI.18: Operative photographs of case report #11. (**A**) Preoperative; (**B**) incision; (**C**) triangular flap; (**D**) apical buccal fenestration (arrow); (**E**) buccal canal preparation with ultrasonic tips; (**F**) lingual canal preparation with ultrasonic tips; (**G**) buccal (b) and lingual (l) canals after preparation; (**H**) retrograde filling with Biodentine; (**I**) suture with Surgycril™ PGA 5/0.

After removing granulation tissue, the canals were identified. The buccal canal contained a plastic core of a gutta-percha transporter and the lingual had not been prepared. Afterwards, the apical 3mm were prepared with ultrasonic tips (ProUltra™ #3 – Dentsply Maillefer, Switzerland) (Figure VI.18E, F and G) and obturated with Biodentine (Figure VI.18H). The flap was repositioned and sutured with Surgycril™ PGA 5/0 (Figure VI.18I). After 6 months, there was no symptomatology and the lesion diminished comparing with preoperative and immediate postoperative radiographs (Figure VI.16B, C and D), predicting a good evolution.

Further possible applications

The literature review and our experience emphasize the versatility of applications of Biodentine. There are some possible further applications, such as the reinforcement of the tooth structure, trying to minimize the tooth fracture as described by Hadrossek *et al.* in 2014, in an incomplete vertical root fracture repair. It can also be of great importance in teeth with developmental anomalies, such as gemination or fusion. We reported a case[71] where a nonsurgical retreatment in a rare case of teeth fusion with transposition was performed and where Biodentine could have been used. In this case, a 21-year old female patient presented with pain in the left maxilla and recurrent abscesses in this region. The patient's medical history was noncontributory, and her previous dental history included an extraction on the lower right quadrant and a root canal treatment in the upper left quadrant. The clinical examination revealed a tooth similar to a molar in the position of the first left premolar, which was adjacent to it on the distobuccal side. The tooth was sensitive to percussion. Periodontal probing revealed normal values of 1mm on the buccal, palatal, and mesial aspects and 3mm on the distal aspect. Orthopantomography and periapical radiography showed two crowns sharing the same root, with a root canal treatment and an associated periapical lesion (Figure VI.19).

Figure VI.19: Preoperative evaluation of fusioned teeth. (**A**) Preoperative orthopantomography; (**B** - **D**) preoperative periapical radiographs with different angulations.

After the patient provided informed consent, the tooth was anesthetized and isolated with a rubber dam. After opening the access cavity of the molar-like tooth and performing irrigation with 2.5% NaOCl, the gutta-percha was removed using ProTaper™ Universal retreatment files and the diagnosis of fused teeth was confirmed. An access cavity was opened on the premolar (Figure VI.20A) showing that the canal system was fused with that of the molar-like tooth (Figure VI.20B). The distobuccal and palatal canals were also fused (Figure VI.20C). After shaping with a finishing file ProTaper™ Universal F3, two canals persisted: a mesiobuccal canal and another canal with a shape similar to that of a C-shaped canal (Figure VI.20D). The root canals were dried with sterile paper points, a calcium hydroxide paste (Calcicur™) was applied, and the access cavity was temporarily sealed with IRM™.

Figure VI.20: Complexity of anatomy of fusioned teeth. (A) Access cavities; (B) communication between the canal systems of the molar and premolar; (C) fusion between distobuccal and palatal canals; (D) the remaining two canals after shaping.

After one month, the patient returned and the calcium hydroxide paste was removed. Irrigation was performed with 2.5% NaOCl, passive ultrasonic irrigation was performed with a size 15 file, and the area was dried with sterile paper points. AH Plus™ sealer was applied with a lentulo and the excess was removed with paper points (Figure VI.21A). The root canal filling was done with thermoplasticized gutta-percha systems, using Thermafill™ (Dentsply Maillefer, Switzerland) in the mesiobuccal canal and Thermafill™ followed by BeeFill™ with vertical condensation in the quasi-C-shaped canal (Figure VI.21B). A postoperative radiograph (Figure

VI.21C) confirmed that the root canal system filling was complete, with some lateral canals appearing in the image, showing the complexity of the anatomy. The crown was restored permanently with a ceramic onlay, improving the esthetics and strengthening the tooth (Figure VI.22).

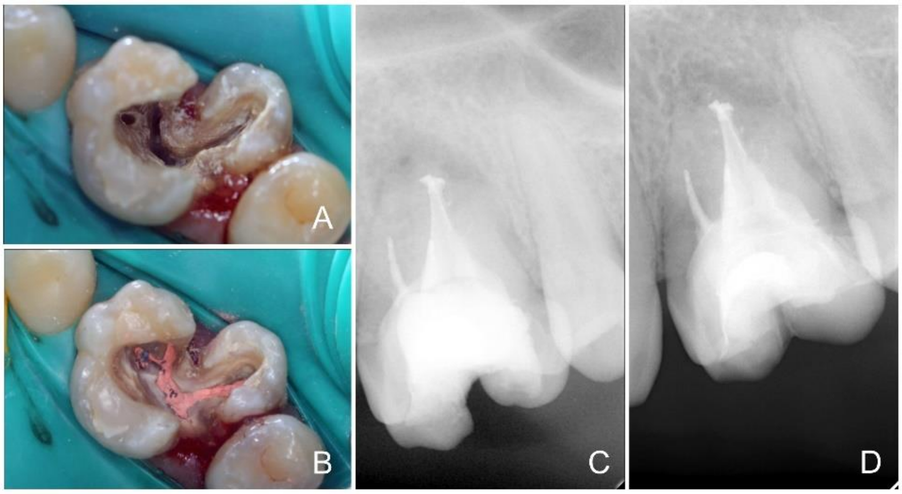

Figure VI.21: Filling procedure and postoperative evaluation of fusioned teeth. (**A**) Cement application; (**B**) root canal filling with Thermafill™ and BeeFill™; (**C**) postoperative periapical radiography; (**D**) four-year follow-up radiography.

Figure VI.22: Permanent restoration of fusioned teeth. (**A**) Initial intraoral lateral view of the patient in occlusion; (**B**) intraoral lateral view of the patient in occlusion after restoration; (**C**) initial intraoral occlusal view; (**D**) intraoral occlusal view after restoration.

At the four-year follow-up, there was no symptomatology and less radiolucency was observed around the apical region in comparison with the preoperative radiographs (Figure VI.21D).

Despite all precautions, due to its complex anatomy, in some instances complications may occur during treatment, such as perforations requiring the use of materials such as mineral trioxide aggregate or Biodentine to repair the communication between the pulp and periodontal tissues.

Furthermore, in these cases, where tooth is weakened, a calcium silicate cement may be applied in the obturation procedure to strengthen the tooth structure.

Complete tooth fracture incidence has been reported at 5 fractured teeth per 100 adults per year, with a vast majority of those being posterior teeth.[35] Clinicians have attempted to use various restorative methods to reinforce such weakened teeth and have reported these techniques and their observations in case reports.[57,247,248]

Seghi *et al.*[275] stated that, given what is known concerning crack propagation in dentine, it is reasonable to stiffen the root with a restorative method that is durable, in an attempt to minimize the cyclic strains that can occur in the tooth under normal function

The explanation of the enhanced fracture resistance of Biodentine could be attributed to the development of a hydroxyapatite-like layer between dentine and calcium silicate cements through the hydration reaction in the presence of tissue fluid.[94,126] The hybrid layer detected between calcium silicate cements and dentine suggests the formation of a chemical bond.[94]

DISCUSSION

According to the various reports found in literature and our findings and experience, Biodentine is a material that gives strong signs of being an effective material in different applications.

However, its use for FP repair has not yet previously been specifically addressed in literature regarding human use. Our personal series of different clinical applications of Biodentine includes a case report of its use for FP repair. In this latter case, we confirmed that Biodentine can be used successfully in this setting. Moreover, the lack of other similar cases in literature makes this report particularly relevant.

In conclusion, Biodentine is a promising material with wide-ranging applications in teeth rehabilitation, namely in FP repair.

Chapter VII - Final Conclusions

Final Conclusions

As widely proven in the medical literature, furcation perforations are pathological conditions of complex treatment and, currently, bioceramics are good options for FP repair. However, ProRoot MTA, the established gold standard, is not devoid of drawbacks, prompting the development of new calcium silicate cements. Among these, Biodentine has gained popularity and there are currently no limits to its clinical use since its approval by the FDA in 2009. Since then, multiple studies have confirmed the advantages of the physicochemical properties of Biodentine over an older similar biomaterial in the endodontic market, MTA; in addition, many other studies have confirmed the efficacy and safety of Biodentine in translational applications, which is in line with our own clinical experience reported in chapter VI of the present Thesis. As described, the clinical use of Biodentine may be considered in a wide variety of endodontic conditions, including FP. For instance, it may be used for the repair of a communication between the pulp and periodontal tissues occurring in the setting of treatment of complex situations, namely fused teeth with transposition. However, research is still in progress in pursuit of the best evidence that Biodentine is a material that can interact favourably with native dentine, thus ensuring the best results in daily clinical practice.

In the present Thesis, the main objectives were to evaluate the role of Biodentine as FP repair material and to improve both the methodology of experimental studies of FP and approach for canine endodontics.

A) In order to systematize the experimental FP models described in literature and to determine if there is evidence that a model is superior to others, we have carried out a review of the literature, presented in chapter II of this Thesis. This review established that although there is no model without disadvantages that can be stated as ideal and clearly higher than others for FP studies, dogs appear to present the most appropriate characteristics. We concluded that canine morphology and physiology make the dog a useful model for dental and medical research, with application in varied fields as toxicology, surgery or implantology. Although more expensive and harder to maintain than rodents, dogs' larger dental anatomy makes this species a more suitable model for FP studies.

B) To systematically summarize what is known about Biodentine´s results when used in FP repair, compared with currently used materials, a contemporary literature review was performed, which is described in Chapter III of our Thesis. As remarkable findings, first, we found no human studies or case reports in the literature. Biodentine performance in FP repair has been scarcely studied and available results were scattered and disorganized. Based on literature results we concluded that studies support that Biodentine is a good FP repair material, with overall better or equivalent performance when compared with currently used materials. However, research on Biodentine for repair of FP remains incomplete and larger clinical studies are still lacking. Finally, this review expands current knowledge because this is the first study to systematically address this issue.

C) Bringing benefit to the methodology of our experimental study, a new line of clamps specifically designed by the authors to better fit the dog's teeth and improve the isolation with rubber dam was developed for application in veterinary endodontics, as presented in Chapter IV of the Thesis. A similar device has been known for decades in human dental care with well-known clinical advantages, but the use of rubber dams is very unusual in veterinary dentistry and there was lack of a specific apparatus for this setting. The personal innovation of this study also includes the successful use, for the first time, of this new line of clamps in dog's teeth. Once a detailed description of the method of different rubber dams placement techniques has been given for the isolation of the dog's teeth, this could facilitate the spread of its use in veterinary endodontic treatment and research.

D) An *in vivo* study aiming to compare the histological, radiographic and microCT outcomes after FP repair with Biodentine or ProRoot MTA in dogs' teeth is analyzed in Chapter V. This is our original contribution to the study of Biodentine in experimental FP, in which the bioactivity of Biodentine was compared with ProRoot MTA after 4 months of FP repair using histologic and imaging methods. Dogs were selected for this study because of their well-documented physiological responses and dental anatomy, with a suitable furcation that provides good accessibility and visibility. The studied outcomes included histological assessment of inflammatory cell infiltration, hard tissue resorption, hard tissue repair and cementum repair. Immediate postoperative and after 4 months radiographs were compared for radiolucency in the furcation region. The volume of extruded material was quantified using microCT images. Biodentine presented tissue compatibility and allowed for mineralized tissue formation with similar morphology and integrity but greater cementum formation and lesser extrusion than prMTA. We concluded that, using an established large animal model, Biodentine's *in vivo* behavior was at least comparable to the gold standard. These results were consistent with other findings in literature, namely regarding the aspects of *in vivo* tissue compatibility and formation of new mineralized tissue, and complemented other favorable results obtained by different research methods. Taken together, our results and results from other studies attest that Biodentine shows substantial signs to be considered a good biomaterial to be used for the repair of furcation perforations.

E) As previously mentioned, Biodentine has been useful in a wide range of dental clinical conditions. However, its use for FP repair has not yet previously been addressed in literature regarding the human use, as shown by the summary of literature focused on case reports of Biodentine clinical use in human dentistry, presented in chapter VI of the Thesis. In addition, this chapter also documents a personal series of different applications of Biodentine to dentistry clinical practice, including a clinical case of Biodentine use for FP repair. In this latter case, we confirmed that Biodentine can also be used successfully for FP repair. Moreover, the lack of other similar cases in literature makes this report particularly relevant.

Altogether, the work presented under the scope of this Thesis consolidates investigation in the field of bioceramics and furcation perforation repair and enhances Biodentine's potential in dentistry.

Future research

Despite the advances brought forward by the present Thesis, there is still room to expand knowledge regarding the best bioceramic to be used in FP repair and if this place belongs to Biodentine.

Namely, there is need for a randomized clinical trial in this field. Moreover, the basis of Biodentine's interaction with surrounding tissues should be further explored to take full advantage of this material's properties and to identify and overcome possible drawbacks.

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