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REVIEW

Present status and future directions: Hydraulic materials for endodontic use

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Abstract

Background: Hydraulic materials are used in Endodontics due to their hydration characteristics namely the formation of calcium hydroxide when mixing with water and also because of their hydraulic properties. These materials are presented in various consistencies and delivery methods. They are composed primarily of tricalcium and dicalcium silicate, and also include a radiopacifier, additives and an aqueous or a non-aqueous vehicle. Only materials whose primary reaction is with water can be classified as hydraulic.

Objectives: Review of the classification of hydraulic materials by Camilleri and the literature pertaining to specific uses of hydraulic cements in endodontics namely intra-coronal, intra-radicular and extra-radicular. Review of the literature on the material properties linked to specific uses providing the current status of these materials after which future trends and gaps in knowledge could be identified.

Methods: The literature was reviewed using PUBMED, and for each clinical use, the in vitro properties such as physical, chemical, biological and antimicrobial characteristics and clinical data were extracted and evaluated.

Results: A large number of publications were retrieved for each clinical use and these were grouped depending on the property type being investigated.

Conclusions: The hydraulic cements have made a difference in clinical outcomes. The main shortcoming is the poor testing methodologies employed which provide very limited information and also inhibits adequate clinical translation. Furthermore, the clinical protocols need to be updated to enable the materials to be employed effectively.

KEYWORDS

antimicrobial properties, biological properties, clinical studies, extra-radicular, hydraulic calcium silicates, hydraulic cements, intra-coronal, intra-radicular, material characterization, physical characteristics

INTRODUCTION

Hydraulic cements are a group of materials, which hydrate in contact with water and also interact with environmental fluids. The term hydraulic derives from the Greek word hydra—meaning water. Hydraulic cements have a range of different chemistries and have been used for over a century in the construction industry. A number of names have been assigned to these materials. Mineral trioxide aggregate (MTA) was the first material in this category that was marketed and most refer to hydraulic cements as MTA or MTA-like. Bioceramics is also used to refer to hydraulic cements, but this term is vague and does not describe the chemistry and/or clinical behaviour of the materials. Thus, the term hydraulic, which is also used in the construction industry, is the best way to refer to these types of material.

Hydraulic cements are used almost exclusively for endodontic procedures. The same materials can be used for a range of procedures, but the interaction with the environment will be different as the environment will be specific to the clinical procedure in which the material is used. Most of the hydraulic cements used in Endodontics are based on tricalcium silicate. A recent classification of hydraulic cement used in Dentistry classifies them based on their chemistry and also on their clinical use (Camilleri, 2020). The one classification based on their chemistry is essential as although the hydraulic cements are assumed to be all calcium silicates, there are other types of hydraulic cements currently in use. Calcium aluminate is also a hydraulic cement; however, the material hydration is different to that of the hydraulic calcium silicates.

The hydraulic calcium silicates are also diverse. Although tricalcium silicate phase is the main phase for these cement types, it is not always the only phase. Furthermore, other phases such as dicalcium silicate and tricalcium aluminate modify the hydration of the material. Most of the newer materials include modifiers that can influence the chemical reaction, or modify the physical and mechanical properties. The materials can be mixed with water or else the powders can be dispersed in a non-aqueous vehicle, and the hydration occurs subsequently through interaction with the environmental fluids. These various types of hydraulic calcium silicates have been classified into five types depending on the cement and vehicle type and also whether they include modifiers or not (Camilleri, 2020). To be classified as a hydraulic cement, the primary reaction must be a hydration reaction. Thus, resin-modified materials and other hybrids cannot be classified as hydraulic.

The main component of hydraulic calcium silicate cement is tricalcium silicate. When tricalcium silicate reacts with water, it forms calcium silicate hydrate and calcium hydroxide (Camilleri, 2007, 2008; Camilleri et al., 2005), which had already been identified and studied extensively in the construction industry. The calcium silicate hydrate is responsible for forming the cement matrix whilst the calcium hydroxide is leached out and interacts with the environment in which the material is placed in. Portland cement includes dicalcium silicate and tricalcium aluminate, thus on hydration other reactions occur which is why Portland cement-based materials are classified in a different group. The release of calcium hydroxide is modified by reaction modifiers and other additives. The use of non-aqueous vehicles also modifies the release of calcium hydroxide since the material needs to absorb the environmental moisture and then commence the hydration reaction with the consequence that the release of calcium hydroxide is delayed. The interaction of the calcium hydroxide with the clinical environment and the improvement in material properties in contact with moisture renders the hydraulic cements unique.

Since the hydraulic cements interact with the clinical environment, a classification based on the material use was considered appropriate (Camilleri, 2020), and this classification will be used to evaluate the literature currently available on hydraulic cements. The aim of this review was to evaluate the physico-chemical, antimicrobial properties and biocompatibility of hydraulic calcium silicates and assess the information pertaining to the various types of cement and how this relates to the clinical data and influences the clinical use of hydraulic cements in Endodontics.

METHODOLOGY

A literature search was performed in PUBMED, and three sets of keywords were included. The keywords were subdivided into keywords relating to the material, the procedure and the type of article (Table 1). Keywords pertaining to each group were separated by OR whilst those in different groups were separated by AND. Eligibility criteria for inclusion were all publications needed to have full text available and be in English, and a publication range dating from January 1993 to May 2021.

The materials included were those which were available on the market and had the hydraulic calcium silicate chemistry. Materials that do not have a hydration reaction as the main hardening reaction but were primarily resin-based were excluded. The procedures were grouped by location of clinical use based on the classification by Camilleri (2020). These included

- Intra-coronal—pulp protection and barrier for regenerative endodontic procedures;

TABLE 1 Keywords used for the PUBMED searches using three categories namely material, procedure and article type

		Type of article			
Material	**Procedure**	**Physico-chemical**	**Biology/animal**	**Microbiology**	**Clinical**
Bioaggregate	Apexification	Alkalinity	Cell differentiation	Agar	Case control
Bioceramic	Apexogenesis	Colour stability	Pulp fibroblast	Antibacterial	Comparative studies
Biodentine	Apical plug	Ion release	Animal model	Antimicrobial	Controlled clinical trial
Bioroot	Apicectomy	Porosity	Bioactivity	Bacteria	Randomized clinical trial
Calcium silicate	Minimal invasive	Sealing ability	Biocompatibility	Biofilm	
Endosequence	Obturation	Setting time	Biological activity	Candida	
Hiflow	Perforation	Solubility	Biomineralization	Confocal	
Hydraulic cement	Pulp capping	Tubular Penetration	Cell culture	E faecalis	
Mineral trioxide aggregate	Regenerative endodontics		Cell proliferation	Fungi	
Portland cement	Remineralization		Cytotoxicity	Inhibition zone	
Totalfill	Resorption		MTT assay	Intratubular infection	
	Revascularization		Odontoblast	Lactobacillus	
	Revitalization		Remineralization	Leachate	
	Root canal treatment		Stem cell	Microbiology	
	Root filling		Toxicity	Planktonic	
	Stepwise excavation		XTT assay	S epidermidis	
	Vital pulp therapy			S mutans	
				Streptococcus	
				Surface	

Note: For article type, the search was further split into microbiology, biology/animal, physico-chemical and clinical.

- intra-radicular—root canal sealing and apical plugs; and
- extra-radicular—root-end fillers and perforation repair materials.

The study designs were either experimental laboratory ones including assessment of physico-chemical properties, biological, microbiological and animal studies or clinical including human experimental studies (randomized controlled trials—RCTs, controlled clinical trial—CCTs) and longitudinal comparative observational studies (prospective/retrospective cohort studies and case–control studies). Case reports were excluded. For all studies that were included in the searches, the methodology used, and the study and control groups were noted.

RESULTS

The outputs for each search are shown in Figure 1a–d. For the article search on physico-chemical properties (Figure 1a), 620 records were identified, with 75 being duplicates thus were excluded. Only 214 full-text articles were screened as 331 were excluded due to the publications being on non-hydraulic calcium silicates, review articles or topics not in the scope of the review such as investigating aspects of the cement not related to their physico-chemical properties. Other exclusions after screening the full texts were due to assessment of experimental or modified materials, publications not investigating physico-chemical properties, clinical articles or unsuitable methodological designs using artificial teeth. The intra-coronal and extra-radicular materials were further grouped together and the setting time, ion release, solubility and colour stability were assessed. The setting time and colour stability are more pertinent for the intra-coronal materials and solubility for the extra-radicular materials. The intra-radicular materials were reviewed for solubility, porosity, sealing ability, ion release and tubular penetration.

For the searches on biocompatibility and bioactivity, 392 records were identified in the database search, amongst which 339 were cellular studies, 55 were

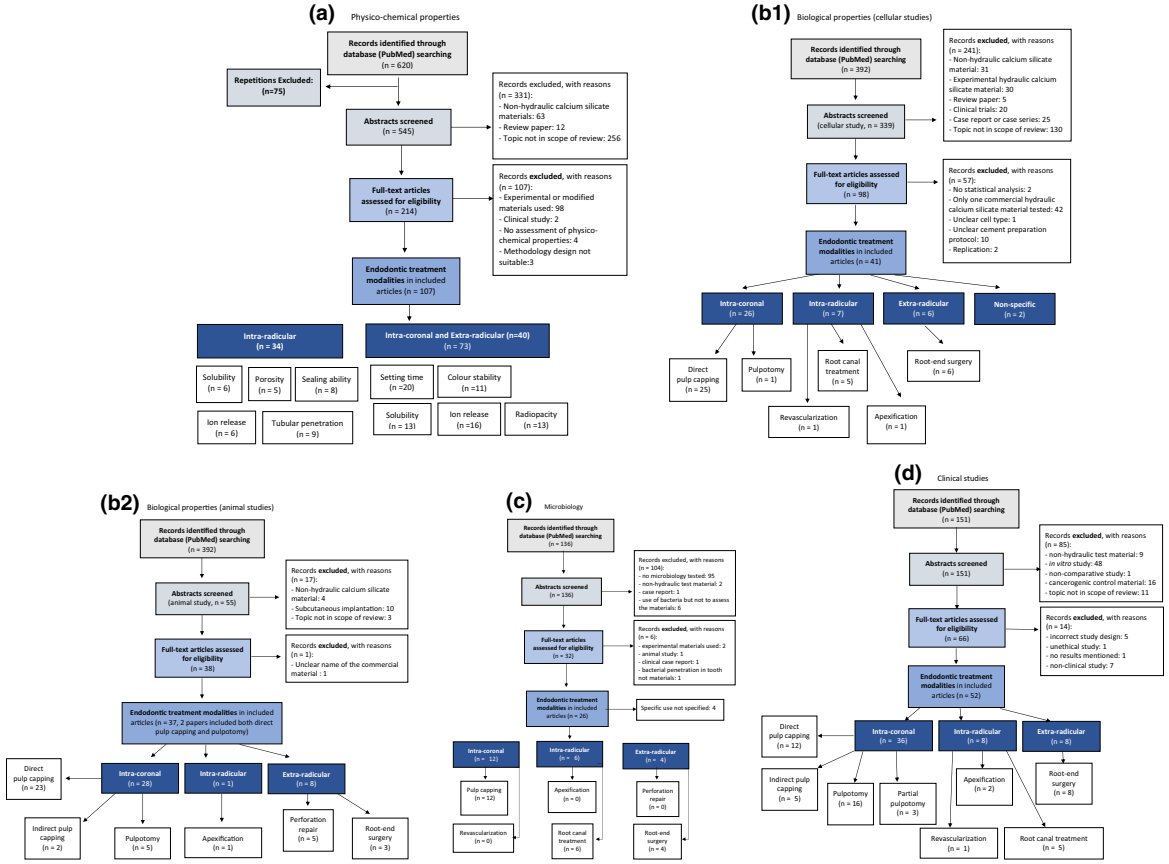

FIGURE 1 Flow diagrams for the different applications of hydraulic cements for endodontic use (a) physico-chemical properties (b) biological properties and animal studies (c) microbiology (d) clinical studies (n = number of articles)

animal studies and two articles (Liu et al., 2015; Park et al., 2014) contained both cellular and animal studies. Amongst 339 cellular studies (Figure 1b), 241 were excluded due to the publications being on non-hydraulic calcium silicates, experimental hydraulic calcium silicate cements, review articles, clinical trials, case report, case series or topic not in the scope of the review for instance not related to endodontic uses. Considering the numerous variations in experimental methodology for cellular studies, such as the cell type, direct or indirect contact of the cells with materials, cement preparation (freshly prepared or set materials), exposure time and the concentration of the cement extracts/eluates tested, it was not easy to compare the results amongst different cell culture studies. The results from 42 studies which tested only one commercial hydraulic calcium silicate cement lacked a reference and were therefore further excluded after the full-text screening. Moreover, another 15 studies were excluded due to the unclear cell type used, unclear cement preparation protocol, lack of statistical analysis and replication. In total, 41 articles were included after screening and all studies are listed in Table 2. A number of studies investigated the effect of materials on cell function but without specific clinical use (Dahake et al., 2020; Zhou et al., 2013).

For animal studies (Figure 1b), 10 studies used subcutaneous implantation of the materials into the connective tissue of animal models to evaluate the biocompatibility and biomineralization potential of calcium silicate cements (Bueno et al., 2016; Cintra et al., 2013; Hinata et al., 2017; Masuda et al., 2005; Mondelli et al., 2019; Mori et al., 2014; Saidon et al., 2003; Santos et al., 2021; Simsek et al., 2015; Sousa et al., 2004). They mainly focused on the mineralizing potential and the interfacial interaction of the materials with host subcutaneous tissues, which are not specific to clinical use and were excluded. Four studies focused on the effect of ethylene diamine tetracetic acid (EDTA; Liu et al., 2021), bone marrow-derived cells (Frozoni et al., 2020), a triple antibiotic paste (Quispe-Salcedo et al., 2020) or sclerostin (Frozoni et al., 2020) for endodontic use were also excluded. Furthermore, three studies which assessed the involvement of glucose supply during pulpal wound healing (Tohma et al., 2020), evaluated the bone tissue reaction of hydraulic cements in femur of rats (Quintana et al., 2019) or focused on the validation of an *in vivo* animal for furcal perforation (Silva

TABLE 2 Detail of cellular studies collected for hydraulic cements for endodontic use

First author, year published	Cell type	Materials tested	Direct/indirect contact	Assessment methods	Results
Intra coronal					
Direct pulp capping					
Rodrigues NS, 2021	HDPSCs	ProRoot MTA (Dentsply), Biodentine (Septodont)	Using a tooth-on-a-chip model	Cell viability and proliferation (live and dead stain)	
Widbiller M, 2016			Direct contact (24-h SET cements)	Cell morphology (SEM), cell viability (MTT assay), mineralization (ALP activity and RT-PCR)	No significant difference between two materials, both materials were biocompatible towards the cells
Manaspon C, 2021	HDPCs		Cement extracts (24-h SET cements)	Cell cytotoxicity and proliferation (MTT assay), migration (*in vitro* scratch assay), and the odonto/osteogenic differentiation (ALP, Von Kossa, and ARS staining, RT-PCR)	
Kang S. 2020			Cement extracts (24-h SET cements)	Cell viability (MTT assay) and mineralization-inducing potentials (ARS staining and ALP assays)	
Youssef AR, 2019			Direct contact (48-h SET cements)	Cell viability (MTT assay), osteogenic, odontogenic and angiogenic differentiations (RT-PCR)	Both materials were cytotoxic
Paula A, 2019	MDPC-23 (odontoblast-like mouse cell line)		Cement extracts (24-h SET Biodentine cement and 72-h SET ProRoot MTA cement)	Cell metabolic activity (MTT assay), cell viability (sulforhodamine B assay, flow cytometry), ALP activity and immunocytochemistry, mineralization (ARS colorimetric assay and spectrophotometry)	Both materials were biocompatible
Poggio C, 2014		ProRoot MTA (Dentsply), MTA Angelus (Angelus), Biodentine (Septodont)	Indirect contact (via Transwell inserts using FRESH cements)	Cytotoxicity tests (Alamar blue test and MTT assay), confocal laser scanning microscope	All three materials were biocompatible
Corral Nuñez CM, 2014	3T3 fibroblast cells	ProRoot MTA (Dentsply), Biodentine (Septodont)	Direct contact (24-h SET cements)	Cell viability (Alamar blue dye), cell morphology (SEM), and cytokine expression (RT-PCR)	Both materials were biocompatible
Pérard M, 2013	3D cell culture model developed from two mouse cell lines: MDPC-23 (odontoblast-like mouse cell line) and Od-21 (a undifferentiated pulp cell line)		Cement extracts (24-h SET cements)	Cell viability (acid phosphatase test), cell morphology (SEM)	Both materials generated cell line-dependent effects

(Continues)

TABLE 2 (Continued)

First author, year published	Cell type	Materials tested	Direct/indirect contact	Assessment methods	Results
Önay EO, 2018	HDPCs	ProRoot MTA (Dentsply), MM-MTA (Micro-Mega)	Indirect contact (via Transwell inserts using 24-h SET cements)	Cell odontoblastic differentiation (ALP activity, RT-PCR)	Both materials exhibited similar expression levels of odontogenic markers
Chang SW, 2015		ProRoot MTA (Dentsply), MM-MTA (Micro-Mega), RetroMTA (BioMTA)	Direct contact (24-h SET cements)	Odontoblastic differentiation (ALP activity, ARS staining and RT-PCR), and the inflammatory response (RT-PCR and ELISA)	All materials enhanced odontoblastic differentiation
Park SJ, 2014		ProRoot MTA (Dentsply), Endocem (Maruchi)	Cement extracts (24-h SET cements)	Cell viability (MTT assay), cell morphology (SEM) and odontoblastic differentiation (ALP activity and ARS staining, RT-PCR, Western blotting, and immunofluorescence analysis)	Both materials were biocompatible
Kim KA, 2015		ProRoot MTA (Dentsply), Endocem (Maruchi), EndocemZr (Maruchi)	Cement extracts (24-h SET cements)	Cell viability (MTT assay), cell morphological observation (SEM), mineralized nodule formation (ARS staining), odontogenic differentiation (CLSM)	All three materials were biocompatible
Chung CJ, 2016		ProRoot MTA (Dentsply), Endocem Zr (Maruchi), RetroMTA (BioMTA)	Direct contact (both FRESH and SET cements)	Cell viability (XTT assay) and angiogenic effects	All materials appeared biocompatible towards HDPCs
Liu S, 2015		ProRoot MTA (Dentsply), iRoot BP Plus (Innovative Bioceramix)	Direct contact (24-h SET cements)	Cell proliferation (MTT assay)	Both materials were biocompatible
Zhu L, 2014		ProRoot MTA (Dentsply), BioAggregate (Innovative Bioceramix)	Cement extracts (72-h SET cements)	Cell viability (cell counting Kit-8), cell adhesion, cell migration (*in vitro* scratch wound healing), double-labeling immunofluorescence assay and cell morphology (SEM)	BioAggregate was biocompatible while ProRoot MTA displayed suppressed viabilities at 72 h
Zhang S, 2013		ProRoot MTA (Dentsply), BioAggregate (Innovative Bioceramix), iRoot BP Plus (Innovative Bioceramix)	Cement extracts (4-h SET cements)	Cell proliferation (cell counting Kit-8), cell differentiation (ALP activity, RT-PCR)	BioAggregate induced a slight decrease in cell viability
Machado J, 2016	Mouse dental pulp cells	ProRoot MTA (Dentsply), EndoSequence BC RRM (Brasseler)	Direct contact (24-h SET cements)	Cell apoptosis (flow cytometry), cell proliferation (WST-1 proliferation assay, ELISA), and mineralization (ALP assay)	All materials were biocompatible and no significant differences were noted

TABLE 2 (Continued)

First author, year published	Cell type	Materials tested	Direct/indirect contact	Assessment methods	Results
Omidi S, 2020	HDPCs	MTA Angelus (Angelus), Biodentine (Septodont)	Cement extracts (48-h SET cements)	Cell viability (MTT assay), cell migration (Transwell migration assay), cytokine secretion (ELISA)	No cytotoxic effects were observed
Rodrigues EM, 2017		MTA Angelus (Angelus), MTA Plus (PREVEST DenPro)	Cement extracts (15-h SET cements)	Cell viability (MTT assay, flow cytometry), osteogenic bioactivity (ALP assay and ARS staining, RT-PCR)	No cytotoxic effects were observed
Chang SW, 2014		MTA Angelus (Angelus), OrthoMTA (BioMTA), Biodentine (Septodont)	Direct contact (24-h SET cements)	Biocompatibility (MTT assay), inflammatory response (RT-PCR, ELISA, western blotting), and odontoblastic potential (ALP activity, ARS staining and RT-PCR)	All materials enhanced odontoblastic differentiation
Hirschman WR, 2012	Human dermal fibroblast	MTA Angelus (Angelus), EndoSequence BC RRM (Brasseler)	Cement extracts (1-week SET cements)	Cytotoxic effects (MTT assay)	No significant differences were observed between two materials
Pinheiro LS, 2018	3T3 (fibroblasts)	MTA Angelus (Angelus), NeoMTA Plus (Avalon Biomed), Biodentine (Septodont)	Cement extracts (FRESH and 24-h SET cements)	Cell cytotoxicity (MTT assay and sulforhodamine B assay)	A higher viability was observed in cells cultured with cement extracts from set cements than that off the fresh cement
Tomás-Catalá CJ, 2018	HDPCs	MTA Repair HP (Angelus), NeoMTA Plus (Avalon Biomed), Biodentine (Septodont)	Cement extracts (48-h SET cements)	Cell viability (MTT assay) and migration assay (wound healing assay), cell morphology and attachment (SEM)	No cytotoxic effect was observed
Abou ElReash A, 2021		MTA Repair HP (Angelus), iRoot BP Plus (Innovative BioCeramix)	Cement extracts (24-h SET cements)	Cell proliferation (MTT assay), adhesion (SEM) and osteogenic differentiation (ARS staining)	Both materials were biocompatible
Pulpotomy					
Collado-González M, 2017	SHEDs	MTA Angelus (Angelus), Biodentine (Septodont)	Cement extracts (48-h SET cements)	Cell viability (MTT assay), cell apoptosis and changes in cell phenotype (flow cytometry), cell migration (*in vitro* scratch wound-healing assay), cell morphology and attachment (SEM), mineralization (ARS staining)	Biodentine showed a significantly higher rate of proliferation than MTA Angelus from 48-h incubation onwards

(Continues)

TABLE 2 (Continued)

First author, year published	Cell type	Materials tested	Direct/indirect contact	Assessment methods	Results
Intra-radicular					
Root canal treatment					
Oh H, 2020	HPDLCs	CeraSeal (MetaBiomed) EndoSeal TCS (MARUCHI)	Cement extracts (FRESH cements)	Cell viability (cell counting kit-8), inflammatory response (ELISA), osteogenic potential (RT-PCR, ARS and ALP activity), cell attachment and morphology (SEM)	Differences in biocompatibility and bioactivity were observed between fresh and set material extracts in cell viability
López-García S, 2020		CeraSeal (MetaBiomed), Endoseal MTA (MARUCHI), EndoSequence BC Sealer (Brasseler)	Cement extracts (48-h SET cements)	Cell metabolic activity (MTT assay) and cell migration (cell wound healing assay), cell morphology and attachment (SEM), bioactivity (RT-PCR and mineralization assays)	A higher mineralization capacity was observed in EndoSequence BC Sealer group compared with CeraSeal, while Endoseal MTA induced no mineralization
Giacomino CM, 2019	IDG-SW3 (a murine osteoblast precursor cell line)	EndoSequence BC Sealer (Brasseler), ProRoot ES (Dentsply)	Cement extracts (FRESH cements)	Cell viability (luminescence assay), osteogenic potential (fluorescence microscopy, ARS staining, and RT-PCR)	Both sealers influenced the osteoblast survival in a concentration-dependent manner
Tu MG, 2019	RAW 264.7 (a mouse macrophage cell line)	ProRoot MTA (Dentsply), iRoot SP (Innovative Bioceramix)	Cement extracts (FRESH cements)	Cell cytotoxicity and proliferation (MTT assay, cell hemocytometer, and western blotting), cell autophagy and osteoclast differentiation (western blotting), pro-inflammatory effect (RT-PCR)	Both sealers showed cytotoxicity in a dose-dependent manner
Wongwatanasanti N, 2018	SCAPs	ProRoot MTA (Dentsply), RetroMTA (BioMTA), Biodentine (Septodont)	Indirect contact (via Transwell inserts using 24-h SET cements)	Cell proliferation (MTT assay) and differentiation (ARS staining and RT-PCR)	All materials promoted cell proliferation
Apexification					
Liu Y, 2020	SCAPs	ProRoot MTA (Dentsply), iRoot FS (Innovative BioCeramix)	Cement extracts (SET cements)	Cell proliferation (MTT assay and BrdU labelling assay), migration (wound healing assay and Transwell assay) and osteo/odontogenic differentiation (RT-PCR, western blot and ARS staining)	Both materials were biocompatible
Revascularization					
Bortoluzzi EA, 2015	HDPCs	MTA Angelus (Angelus), Biodentine (Septodont)	Direct contact and cement extracts (24-h SET cements)	Cell viability (XTT assay and flow cytometry), osteogenic potential (RT-PCR, ALP activity, ARS staining and TEM)	The cytotoxicity was time- and concentration-dependent

TABLE 2 (Continued)

First author, year published	Cell type	Materials tested	Direct/indirect contact	Assessment methods	Results
Extra-radicular					
Root-end filling					
Sequeira DB, 2018	Apical papilla cells	ProRoot MTA (Dentsply), Biodentine (Septodont), PulpGuard (COLTENE)	Cement extracts (48-h SET cements)	Cell viability and proliferation (Alamar Blue assay), cell mobility (a wound-healing test), cell morphology (SEM)	The cytotoxicity was concentration dependent
Lv F, 2017	MC3T3-E1 cells (osteoblast cell line)	ProRoot MTA (Dentsply), iRoot BP Plus (Innovative Bioceramix), iRoot FS (Innovative BioCeramix)	Cement extracts (7-day SET cements)	Cell viability (cell counting kit-8), cell apoptosis (flow cytometry), cell attachment (SEM)	The survival rate of the cells treated with iRoot FS was significantly higher than those of the cells treated with the two other materials
De-Deus G, 2009	Human primary mesenchymal cells	ProRoot MTA (Dentsply), BioAggregate (Innovative BioCaramix)	Cement extracts (FRESH cement)	Cytotoxicity (XTT, neutral red, and crystal violet dye elution assays)	No significant difference in cytotoxicity was observed between two materials
Scelza MZ, 2017	Human primary osteoblasts	MTA Angelus (Angelus), Biodentine (Septodont)	Cement extracts (42-h SET MTA Angelus cement and 24-h SET Biodentine cement)	Cell viability (mitochondrial activity, membrane integrity and cell density)	Both materials were biocompatible
Tanomaru-Filho M, 2017	Saos-2 (human osteoblast-like cell lineage)	MTA Angelus (Angelus), NeoMTA Plus (Avalon Biomed)	Cement extracts (15-h SET cements)	Cell viability (MTT assay and neutral red cytotoxicity assay), mineralization potential (ALP activity and ARS staining)	Both materials were biocompatible
Ma J, 2011	Human gingival fibroblasts	EndoSequence BC RRM Putty (Brasseler), EndoSequence BC RRM Paste (Brasseler)	Cement extracts (2- and 7-day SET cements)	Cell viability (MTT assay), cell adhesion assay (SEM)	The cytotoxicity of the materials were time-dependent
Non-specific					
Dahake PT, 2020	SHEDs	MTA Angelus (Angelus), Biodentine (Septodont)	Cement extracts (FRESH cements)	Cell viability (MTT assay), mineralization potential (ARS staining)	Biodentine showed higher cell viability and proliferation than that of MTA Angelus
Zhou HM, 2013	Human gingival fibroblasts	ProRoot MTA (Dentsply), Biodentine (Septodont)	Cement extracts (48-h SET cements)	Cytotoxicity (flow cytometry), cell adhesion (SEM)	No significant difference was observed between two materials

Abbreviations: ALP, alkaline phosphatase; ARS, Alizarin Red S; CLSM, confocal laser scanning microscope; EndoSequence BC RRM, EndoSequence BC Root Repair Material; ELISA, enzyme-linked immunosorbent assay; HDPCs, human dental pulp cells; HDPSCs, human dental pulp stem cells; HPDLCs, human periodontal ligament cells; iRoot FS, iRoot Fast Set root repair material; iRoot SP, iRoot SP Injectable Root Canal Sealer; MTA, mineral trioxide aggregates; MTT assay, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay; RT-PCR, reverse-transcriptase polymerase chain reaction; SEM, scanning electron microscopy; SCAPs, stem cells from the apical papilla; SHEDs, stem cells from human exfoliated deciduous teeth; TEM, transmission electron microscopy.

et al., 2009) are not within scope of this review. Another study was excluded due to the unclear commercial name of the material tested (Yildirim et al., 2005). Therefore, 37 papers were included after full-text screening (Table 3).

Regardless of the specific chemistry, the hydraulic cements generated an initial mild or no inflammation with responses consisting of the infiltration of lymphocyte and macrophage on implantation which subsided in the long term (Mondelli et al., 2019; Simsek et al., 2015; Sousa et al., 2004). The mineralizing potential and interfacial activity indicated new bone tissue formed at the material-tissue interface and healthy bone containing osteoblasts in close contact with the material (Hinata et al., 2017; Sousa et al., 2004). The precipitates were mainly composed of calcium, phosphorus and silicon with the presence of a calcium and phosphorus rich area along the material-tissue interface, which indicated the biomineralization potential of hydraulic cements in contact with connective tissue (Hinata et al., 2017).

For the searches on microbiological assessments (Figure 1c), from the 136 records identified in the database search, only 31 were included after abstract screening. Most of the records did not include any microbiological testing at all with a few that used bacteria but mostly for leakage studies which is not the scope of this review. Publications investigating hybrid materials that do not set primarily by the hydraulic cement reaction were excluded. Most of the papers included the clinical use of the materials being investigated with pulp capping, root canal and root-end filling. Four publications did not specify the clinical use of the materials tested and used a range of bacteria in the tests undertaken. These studies tested a number of brands of MTA and CEM using the agar diffusion test and concluded that the different brands have different antimicrobial activity (Kim et al., 2015b). This is disputed by another research group who found negligible antimicrobial activity in all brands of MTA tested (Morita et al., 2021). The different types of MTA also behaved differently when using three types of the same bacterium and under anaerobic and aerobic conditions with none of the brands being associated with any microbial inhibition under anaerobic conditions (Ribeiro et al., 2010).

For the searches on clinical assessments, the following eligibility criteria were included additionally: 'abstract', 'full text' and 'clinical study'. Despite this, from the 151 records identified in the databases, a third were not clinical trials (Figure 1d). Furthermore, not in all clinical studies, the design was correct (which might have biased the statistics and the reported outcomes), for example RCTs lacking stratification or a 2 × 2 factorial design (Alsulaimani, 2016; Awawdeh et al., 2018; Bardini et al., 2021; Eghbal et al., 2020; Yang et al., 2020). Beslot-Neveu et al. (2011) described only a study protocol.

Studies assessing test materials that did not set in a hydraulic manner and protocols using formocresol in the control group were excluded. The International Agency for Research on Cancer of the World Health Organization has classified formaldehyde (a component of formocresol) as a known human carcinogen WHO (2019).

The only clinical trial record regarding perforation repair was excluded due to lack of good clinical practice (unethical, Figure 1d): patients lost bone and gingival tissue pre-implant placement only for experimental reasons (Tirone et al., 2018). Hence, after full-text screening, only 51 were included, from which the data are partially mentioned in Table 3 and partially described here below.

Intra-coronal materials

The materials used for intra-coronal procedures are most often the same as those used for root-end filling and perforation repair. In a number of publications, the intended use was not always divulged particularly during laboratory-based testing. For the physical and chemical characteristics, the setting time, ion release, solubility, colour stability and radiopacity were reviewed.

Physical and chemical properties

Setting time

The initial setting time for powder-liquid cements such as MTA ProRoot (Dentsply), MTA Angelus (Angelus) and Biodentine (Septodont Saint-Maur-des-Fossés, France) was reported in the ranges of (17–228) min, (9–30) min and (7–13) min respectively (Acris De Carvalho et al., 2021; Butt et al., 2014; Dawood et al., 2015; De Souza et al., 2018; Gandolfi et al., 2009; Grech et al., 2013; Jiménez-Sánchez et al., 2019; Kang et al., 2021; Kaup et al., 2015; Pelepenko et al., 2021; Vivan et al., 2010; Wang et al., 2014). The final setting time ranged between (122–241) min, (23–84) min and (20–45) min respectively (Dawood et al., 2015; Gandolfi et al., 2009; Grech et al., 2013; Guimarães et al., 2018; Jiménez-Sánchez et al., 2019; Kang et al., 2021; Pelepenko et al., 2021; Vivan et al., 2010; Wang et al., 2014). These variations are due to differences amongst the cements in their chemical formulations, purity of their constituents, the presence of setting accelerators (such as calcium chloride) and the means of hydration (El-Elaouni & Benkaddour, 1997; Kjellsen & Justnes, 2004; Lee et al., 2017). It must be pointed out that these values are based on *in vitro* testing that followed, in most of the studies, certain standards such as the International Standards

TABLE 3 Detail of animal studies collected for hydraulic cements for endodontic use

First author, year published	Animal model	*n* (teeth) per group per period	Materials tested	Assessment methods	Follow-up periods	Type of teeth	Coronal restoration
Intra-coronal							
Indirect pulp capping							
Choung HW, 2016	Beagle dogs	6	ProRoot MTA (Dentsply)	Histological evaluation (mineral tissue formation), SEM (remaining original dentin and dentinal tubules)	N.A	Premolars	Light-cured glass-ionomer cement (Fuji II LC, GC)
Tziafa C, 2015	Miniature swine	Biodentine (n = 12 for 3 weeks and n = 9 for 8 weeks), Dycal (n = 6 for each period)	Biodentine (Septodont), Dycal (Dentsply)	Histological assessment (pulpal inflammatory and reparative tissue responses) and SEM (interfaces of material with post-operatively formed mineralized matrix)	3 and 8 weeks	Permanent teeth (premolars, canines and incisors)	Biodentine (Septodont)
Direct pulp capping							
Amin LE, 2021	Male Wistar albino rats	8	Biodentine (Septodont), Dycal (Dentsply), no control	Histological examination according to ISO 7405 (inflammation reaction, tissue necrosis and hard tissue formation)	1, 3 and 5 weeks	N.A	Resin-modified glass-ionomer restorative material (Fuji II LC, GC)
Guerrero-Gironés J, 2021	Male Sprague–Dawley rats	16	ProRoot MTA (Dentsply), no control	Histological examination (inflammation, pulp necrosis, dentin bridge and reparative dentin formation, odontoblastic layer and fibrotic tissue)	30 days	Maxillary molars	A thin base of zinc oxide–eugenol (IRM, Dentsply) and silver amalgam (Tytin 29945, Kerr) on top
Pedano M, 2020	Female Göttingen minipigs	8	Biodentine (Septodont)	Micro-CT (radiodensity change in exposed pulps and continuity of mineralized tissue), histological analysis (inflammatory response and mineralized tissue formation)	7 and 70 days	Incisors, canines, premolars and molars	A resin-based glass-ionomer cement (Fuji II LC, GC), a universal adhesive (G-Premio Bond, GC) and light-cure composite (G-aenial Posterior, GC)
Trongkij P, 2020	Male Wistar Albino rats	10	Bio-MA (M-Dent/SCG), ProRoot MTA (Dentsply), control (uncapped pulp exposure, n = 3)	Histopathological evaluation (inflammatory cell infiltration and reparative dentin formation, defective area or cell inclusion)	1, 7 and 30 days	Maxillary first molars	A light-cured glass-ionomer cement (Fuji II LC, GC)

(Continues)

TABLE 3 (Continued)

First author, year published	Animal model	*n* (teeth) per group per period	Materials tested	Assessment methods	Follow-up periods	Type of teeth	Coronal restoration
Li X, 2018	Female Göttingen minipigs	8	ProRoot MTA (Dentsply)	Micro-CT (radiodensity change in exposed pulps and continuity of mineralized tissue), histological analysis (inflammatory response and mineralized tissue formation)	7 and 70 days	Incisors, canines, premolars and molars	A resin-based glass-ionomer cement (Fuji II LC, GC), a universal adhesive (G-Premio Bond, GC) and light-cure composite (G-aenial Posterior, GC)
Trongkij P, 2018	Wistar rats	5	ProRoot MTA (Dentsply), Bio-MA (M-Dent/SCG), control (no capping material, $n = 3$)	Histological analysis (inflammatory cell infiltration and reparative dentin formation)	1 and 7 days	Molars	Light-cured glass-ionomer cement (Fuji II LC, GC)
Lin HP, 2017	Male Wistar rats	6	ProRoot MTA (Dentsply), control (no capping materials)	Histologic evaluation (tertiary dentin, dentinal bridge, dystrophic calcification and inflammation)	2 and 4 weeks	First maxillary molars	A resin composite (Filtek Z350 XT; 3 M ESPE) with a self-etch adhesive (Single Bond Universal, 3 M)
Liu S, 2015	Male Wistar rats	4	iRoot BP Plus (Innovative Bioceramix), ProRoot MTA (Dentsply)	Histologic features (inflammatory cell response and hard tissue formation)	1 and 4 weeks	Maxillary first molars	Glass-ionomer cement (Fuji IX, GC)
Louwakul P, 2015	Male Wistar rats	10	Dycal (Dentsply), ProRoot MTA (Dentsply), no control	Histological evaluation (inflammatory cell response and hard tissue formation)	8 and 30 days	Maxillary first molars; the dental pulps were left opened for 48 h before pulp capping to induce inflamed exposed pulp tissue	Light-cured glass-ionomer cement (Fuji II LC, GC)
Kim KA, 2015	Male Wistar rats	6	ProRoot MTA Dentsply), Endocem (Maruchi), EndocemZr (Maruchi)	Histological evaluation (reparative dentin formation)	4 weeks	Upper first molars	Light-cured glass-ionomer cement (Fuji II LC; GC)
Park SJ, 2014	Male Wistar rats	8	Endocem (Maruchi) and ProRoot MTA (Maruchi)	Histological evaluation (tertiary dentin formation and pulp inflammation)	4 weeks	Upper first molar	Light-cured glass-ionomer cement (Fuji II LC, GC)

TABLE 3 (Continued)

First author, year published	Animal model	*n* (teeth) per group per period	Materials tested	Assessment methods	Follow-up periods	Type of teeth	Coronal restoration
Moazzami F, 2014	Sprague-dawley rats	10	ProRoot MTA (Dentsply)	Histologically evaluation (degree of inflammation, odontoblasts or odontoblast-like cells, reparative dentin formation)	2 weeks and 2 months	Maxillary molars	Light-cured restorative glass ionomer (GC)
Obeid M, 2013	Dogs	14	ProRoot MTA(Dentsply), no control	Cone-beam radiography (the thickness and continuity of the reparative calcific bridge), histological evaluation (calcific bridge formation)	12 weeks	Premolars and canines	Glass-ionomer cement
Tran XV, 2012	Male rats	6	Biodentine (Septodont), ProRoot MTA (Dentsply), no control	Immunohistochemistry (dentin sialoprotein and osteopontin), histomorphometry (the number of PCNA + cells, the porosity of the dentin bridge)	7, 14 and 30 days	Maxillary first molars	Glass-ionomer cement (GC Fuji IX, GC)
Orhan EO, 2012	Wistar albino rats	24	Dycal (Dentsply), ProRoot MTA (Dentsply)	Histological analysis (odontoblast-like cell count and reparative dentine thickness)	7 and 28 days	Incisors	Reinforced zinc oxide–eugenol cement (IRM, Dentsply)
Danesh F, 2012	Male beagle dogs	8	ProRoot MTA (Dentsply), control (exposed pulps without capping and remained open to oral microflora)	Histological evaluations (hard tissue bridge in terms of continuity, morphology, thickness and localization)	7 and 70 days	Canines	Intermediate restorative material (IRM, Dentsply)
Al-Hezaimi K, 2011	Baboons	8	Dycal (Dentsply), ProRoot MTA (Dentsply), control (uncapped exposed pulps)	Histomorphometric analysis (the nature of reparative hard tissues) and micro-computed tomography (the thickness of the formed reparative hard tissues)	4 months	Premolars	Amalgam
Asgary S, 2008	Male beagle dogs	8	Dycal (Dentsply), ProRoot MTA (Dentsply)	Histological assessment (pulp inflammation, hard tissue formation, pulp calcification or necrosis, and odontoblast cell layer)	8 weeks	Canines	Glass-ionomer cement (Fuji II, GC)
Asgary S, 2006	Beagle dogs	6	ProRoot MTA (Dentsply), calcium hydroxide (LD Caulk)	SEM (basic morphology of dental pulp reaction and dentin bridge formation)	2 months	Canines	Glass-ionomer cement (Fuji II, GC)

(Continues)

TABLE 3 (Continued)

First author, year published	Animal model	*n* (teeth) per group per period	Materials tested	Assessment methods	Follow-up periods	Type of teeth	Coronal restoration
Queiroz AM, 2005	Mongrel dogs	13	ProRoot MTA (Dentsply)	Histopathological analysis (dentin bridge, pulp tissue changes, intensity of the inflammatory infiltrate, type of inflammatory infiltrate, periodontal ligament thickness, resorption of mineralized tissues and changes at the periapical region)	90 days	Premolars	Adhesive system (Prompt L-Pop, 3 M) and a microhybrid resin composite (Z-100, 3 M)
Faraco Junior IM, 2004	Dogs	15	White MTA (Loma Linda University), no control	Histomorphological evaluation (hard tissue bridge and dental pulp)	60 days	N.A	Zinc oxide–eugenol cement (SSW Artigos Dentário)
Dominguez MS, 2003	Male mongrel dogs	N.A	ProRoot MTA (Dentsply)	Histological evaluation (pulp inflammation, tissue reaction to the material, impaction of particles of pulp-capping agents, location of dentin bridge, presence of dentin chips, dentin bridge formation, quality of dentin formation in the bridge and connective tissue in the bridge) and SEM analysis	50 and 150 days	N.A	Glass ionomer (Ketac Silver, 3 M)
Ford TR, 1996	Cynomolgus monkeys	6	Calcium hydroxide (Dycal), MTA (Loma Linda University)	Histological analysis (dentin bridge, inflammation and bacteria)	5 months	Incisors	Amalgam
Pulpotomy							
El-Zekrid MH, 2019	Male mongrel dogs	48	Biodentine (Septodont)	Histologic evaluation (inflammatory cell infiltration, tissue necrosis and hard tissue formation)	1 and 9 weeks	Posterior teeth	A resin-modified glass-ionomer restorative material (Fuji II LC, GC)
Liu S, 2015	Male Wistar rats	4	iRoot BP Plus and control group using only glass-ionomer cement	Histologic features (inflammatory cell response and hard tissue formation)	1 and 4 weeks	Maxillary first molars	Glass-ionomer cement (Fuji IX, GC)

TABLE 3 (Continued)

First author, year published	Animal model	*n* (teeth) per group per period	Materials tested	Assessment methods	Follow-up periods	Type of teeth	Coronal restoration
De Rossi A, 2014	Beagle dogs	Biodentine ($n = 36$), ProRoot MTA ($n = 24$)	Biodentine (Septodont), ProRoot MTA (Dentsply)	Periapical radiographs and histomicrobiological analysis (the remaining radicular pulp tissue, mineralized tissue bridge and apical and periapical tissues)	120 days	Premolars	A glass-ionomer base (Vidrion; S. S. White), silver amalgam (Velvalloy; S. S. White)
Kramer PR, 2014	Male Sprague-Dawley rats	12	Quick-Set (Avalon Biomed), ProRoot MTA (Dentsply) and MTA Plus (Avalon Biomed), untreated pulpotomy as a control	ELISA (pro-inflammatory cytokines), histology (dentin bridging, presence of bacteria, pulp vitality)	30 and 60 days	Maxillary molars	A self-adhering flowable composite resin (VertiseFlow; Kerr)
Dominguez MS, 2003	Male mongrel dogs	N.A	ProRoot MTA (Dentsply)	Histological evaluation (pulp inflammation, tissue reaction to the material, impaction of particles of pulp-capping agents, location of dentin bridge, presence of dentin chips, dentin bridge formation, quality of dentin formation in the bridge and connective tissue in the bridge) and SEM analysis	50 and 150 days	Premolars	Glass ionomer (Ketac Silver, 3 M)
Intra-radicular							
Apexification							
Altaii M, 2017	Sheep at the two-tooth age	N.A.	ProRoot MTA (Dentsply)	Radiographic measurement (root length, dentin thickness and apical diameter), micro-CT (root length, dentinal wall thickness), histological analysis (dentin with regular or irregular dentinal tubules, cementum or bone)	6 months	Immature first incisors; the pulps were mechanically exposed and supra-gingival plaque was scaled from the sheep's own teeth and introduced in the pulp chambers	Cavit (3 M) and glass-ionomer cement (Fuji IX, GC)

(Continues)

TABLE 3 (Continued)

First author, year published	Animal model	*n* (teeth) per group per period	Materials tested	Assessment methods	Follow-up periods	Type of teeth	Coronal restoration
Extra-radicular							
Root-end surgery							
Wälivaara DA, 2012	Mongrel dogs	6	MTA Angelus (Angelus)	Histologic examination (inflammatory infiltrate, the height of the retrograde seal and the healing status of the buccal access site), radiographic analysis and SEM analysis	120 days	Mandibular premolars	N.A.
Tawil PZ, 2009	Beagle dogs	12	ProRoot MTA (Dentsply)	Radiographic assessment and histological evaluation (inflammation with/without the presence of periodontal ligament)	6 months	Premolars; supra-gingival plaque scaled from the dog's autologous teeth was mixed with saline and was introduced into the canals to generate apical periodontitis	Glass-ionomer cement (Fuji IX GP, GC)
Economides N, 2003	Dogs	N.A.	ProRoot MTA (Dentsply)	Histological evaluation and SEM	1, 2, 3 and 5 weeks	Premolars	N.A.
Perforation repair							
Abboud KM, 2021	Adult mongrel dogs	8	NeoMTA Plus (Avalon BioMed), MTA Angelus (Angelus), no material as the control	Histopathological evaluation (inflammatory cell count, calcific bridge, configuration of fibrous tissue formed, tissue surrounding the furcation area, intra-radicular bone and the inflammatory nature of tissues)	1 week, 1 and 3 months	Permanent premolars, the coronal access was left open for 3 weeks to develop an osseous defect surrounding the perforation site, this as an infection period	Chemical cured glass-ionomer filling material

TABLE 3 (Continued)

First author, year published	Animal model	*n* (teeth) per group per period	Materials tested	Assessment methods	Follow-up periods	Type of teeth	Coronal restoration
Mahmood TR, 2020	Male Wistar albino rats	4	Biodentine (Septodont), MM-MTA (Micro-Mega), EndoSequence BC RRM putty (Brasseler)	Histological analysis (inflammatory response, odontoblast differentiation, dentin bridge formation, calcified bone formation and continuity and organization of periodontal ligament)	1 and 4 weeks	Lower incisors	N.A
Alazrag MA, 2020	Adult mongrel male dogs	10	MTA Angelus (Angelus), Biodentine (Septodont) and perforation without sealing as a control	Radiographic evaluation and histopathological evaluation (inflammatory cell count)	1 and 3 months	Premolars	Chemical cured glass-ionomer cement (Riva Light Cure LC/ Southern Dental Industries SDI)
Bakhtiar H, 2017	Mixed-breed dogs	6	ProRoot MTA (Dentsply) and no material as the control group	Histological examination (inflammation, dentin, cementum, connective tissue, foreign body reaction, new bone formation)	3 months	Premolars	Amalgam (SDI)
Silva Neto JD, 2012	Male mongrel dogs	20	MTA Angelus (Angelus)	Histological analysis (newly formed bone, inflammatory infiltrate)	120 days	Premolars	Light-cured composite resin

Abbreviations: ELISA, enzyme-linked immunosorbent assay; EndoSequence BC RRM, EndoSequence BC Root Repair Material; Micro-CT, Micro-computed tomography; MM-MTA, Micro-Mega mineral trioxide aggregate; N.A, not available; SEM, scanning electron microscopy.

Organization guidelines ISO 6876 (2012, ISO 9917, 2007) or the American National Standards Institute/American Dental Association ANSI/ADA Specification No. 57 2012 guidelines. This may have reflected on the variations in the testing conditions across the different studies and their results, which may question the relevance of these standards to the clinical performance of tested materials.

Solubility

Solubility of cements is evaluated in a number of ways. The most frequent method employed is the measurement of the change in their mass after storage in water over different durations. This method is described in ISO 4049, 2019 (standard is for polymeric restorative materials), and also allows the assessment of water sorption. After different storage durations in water, MTA ProRoot, MTA Angelus and Biodentine exhibited solubility in the rages of (0.88%–10.89%), (0.6%–15.5%) and (0.31%–7.34%) respectively (Acris De Carvalho et al., 2021; Al-Sherbiny et al., 2021; Dawood et al., 2015; De Souza et al., 2018; Gandolfi et al., 2015a, 2015b; Grech et al., 2013; Guimarães et al., 2017; Kang et al., 2021; Kaup et al., 2015; Torabinejad et al., 1995a; Torres et al., 2018; Vivan et al., 2010). In all cements, solubility was reported to drop over time (Kaup et al., 2015; Torres et al., 2018, 2019). Similar to the setting time, different standardization guidelines were followed to assess the solubility including ISO 6876, 2012, ISO 4049, 2019 and the American Society for Testing and Materials (ASTM) specification C 266, 2021. The methods described in each standard are different as whilst the ISO 6876, 2012 measures the percentage of mass loss of a 20-mm specimen in water, and the ISO 4049, 2019 enables the longitudinal testing of the materials and also the measurement of water sorption from the same specimens. For all standards, water was the immersant. Thus, the values obtained may not be clinically relevant since the hydraulic cements interact differently in water in a laboratory setting than in the clinical environment (Meschi et al., 2019).

Ion release

The pH of the soaking solution ranges from 7.1 to 8.2, 8.0–9.13 and 9.3–10.7 for the hydraulic cements ProRoot MTA, MTA Angelus and Biodentine respectively (Al-Sherbiny et al., 2021; Atmeh, 2020; Gandolfi et al., 2015a, 2015b; Guimarães et al., 2018; Pelepenko et al., 2021). Calcium ion release was reported to reduce with time (Acris De Carvalho et al., 2021; Al-Sherbiny et al., 2021; Atmeh, 2020; Dawood et al., 2015; De Souza et al., 2018; Gandolfi et al., 2013a, 2015a, 2015b; Guimarães et al., 2018; Han et al., 2015; Kang et al., 2021; Koutroulis et al., 2019a; Mahmood Talabani et al., 2020; Natale et al., 2015; Vivan et al., 2010). Despite the variations in the methodologies and designs used to quantify calcium, which makes comparisons difficult, studies reported a 50%–70% drop in calcium release over 4 weeks following cement hydration (Al-Sherbiny et al., 2021; Atmeh, 2020; Gandolfi et al., 2013, 2015a, 2015b; Guimarães et al., 2018; Koutroulis et al., 2019a; Mahmood Talabani et al., 2020).

Radiopacity

The ISO 6876; 2012, or its previous version published in 2001 (ISO 6876; 2001) was used to test and compare the radiopacity of hydraulic calcium silicate-based cements. In general, Biodentine exhibited a lower radiodensity in the range of (1.5–5.0) mm Al in comparison with MTA ProRoot and MTA Angelus that demonstrated radiodensities in the ranges of (5.0–8.5) mm Al and (4.5–7.0) mm Al respectively (Camilleri & Gandolfi, 2010; Camilleri et al., 2013b; Cavenago et al., 2014; Chng et al., 2005; Corral et al., 2018; Grech et al., 2013; Guimarães et al., 2017; Kang et al., 2021; Kaup et al., 2015; Kim et al., 2008; Laghios et al., 2000; Łuczaj-Cepowicz et al., 2019; de Souza et al., 2015; Tanalp et al., 2013; Vivan et al., 2009). The MTA Angelus changed from the use of bismuth oxide to calcium tungstate, but the exact date of the change is unclear. Not all studies evaluating the material physical properties characterize the tested specimens, and thus, whilst a lower value may have been obtained for MTA Angelus as indicated in the studies cited, the MTA Angelus may have been of the newer generation as calcium tungstate is less radiopaque than bismuth oxide (Camilleri & Gandolfi, 2010).

Colour stability

Studies looking at the colour changes associated with hydraulic calcium silicate cements varied in the substrate tested. Some examined the change in the cement itself (Camilleri, 2014; Keskin et al., 2015; Palma et al., 2019; Vallés et al., 2013), whilst others looked at discoloration in natural teeth from humans (Chen et al., 2020; Felman & Parashos, 2013; Kang et al., 2015b; Shokouhinejad et al., 2016; Vallés et al., 2015) or animals (Dettwiler et al., 2016; Lenherr et al., 2012; Marciano et al., 2014; Sobhnamayan et al., 2017; Yoldaş et al., 2016). Findings supported a correlation between the composition of the cements and their potential to discolour with time. Cements containing bismuth oxide as a radiopacifier, such as MTA ProRoot and an earlier version of MTA Angelus, had significantly more severe discoloration compared with Biodentine, Endosequence, Bioaggregate and newer formulations of MTA Angelus that contain alternative radiopacifiers (Alsubait et al., 2017; Camilleri 2014; Keskin et al., 2015; Vallés et al., 2013, 2015). Bismuth oxide is unstable and converts to sodium bismutate in contact with sodium hypochlorite and bismuth subcarbonate, which is light sensitive in contact with water and saline (Camilleri et al., 2020).

The change in colour was also assessed in different conditions. Upon exposure to blood, all hydraulic calcium silicate cements were reported to be associated with discolouration, regardless of their composition (Chen et al., 2020; Felman & Parashos, 2013; Kang et al., 2015b; Lenherr et al., 2012; Palma et al., 2019; Shokouhinejad et al., 2016; Yoldaş et al., 2016). Irrigation solutions such as sodium hypochlorite and chlorhexidine were also reported to cause discolouration of cements and tooth structure in contact with them (Camilleri, 2014; Keskin et al., 2015; Marciano et al., 2015; Sobhnamayan et al., 2017; Trusha & Banga, 2019).

Biological characteristics

Cellular studies

Direct pulp capping. Table 2 shows the research undertaken on materials used for pulp-capping procedures using different cell types. The most predominant were the human dental pulp stem cells (HDPSCs), human dental pulp cells and fibroblasts. The range of materials tested was varied very much with different brands of MTA, Biodentine and even root repair materials investigated. Set cements after 24–72 h after setting and extracts were investigated with only one study investigating fresh materials (Pinheiro et al., 2018). A number of tests as indicated in Table 2 were employed to assess cell proliferation, viability, mineralizing potential and morphology.

Most of the studies indicate a better cell proliferation and expression in contact with extracts rather than when direct contact tests are undertaken. Using a tooth-on-chip model, ProRoot MTA and Biodentine in culture with HDPSCs exhibited similar percentages of live cells (Rodrigues et al., 2021) and scanning electron microscopic (SEM) imaging revealed the adhesion of HDPSCs with elongated processes onto the surface of both materials (Widbiller et al., 2016). Viability of cells in direct contact with ProRoot MTA was significantly lower than Biodentine for the first 7 days, with no difference at 10 and 14 days (Widbiller et al., 2016). When both materials were tested using 3T3 fibroblast cells, they had similar viability to untreated control cells with SEM, revealing cells were generally spindle-shaped with flattened and extended cellular processes that attached to material surfaces (Corral Nuñez et al., 2014). Other direct contact tests indicated a significant decrease in cell viability on Day 3, which was 53% and 26% viability, respectively, in comparison with controls, suggesting a cytotoxicity (Youssef et al., 2019). The observed cytotoxicity is inconsistent with the previously mentioned studies which reported the cytocompatibility of the cements towards HDPCs. Regardless of the cytotoxicity reported by MTT assays, the expressions of odontoblastic differentiation markers ALP, osteopontin (OPN) and dentine sialophosphoprotein (DSPP) as well as angiogenic factor vascular endothelial growth factor (VEGF) were increased by both materials (Youssef et al., 2019).

Research undertaken on cement extracts revealed no difference between ProRoot MTA and Biodentine with both groups exhibiting improved mineralization (Manaspon et al., 2021; Paula et al., 2019) whilst other studies reported a greater viability of HDPCs exposed to Biodentine cement extract in comparison with ProRoot MTA after 1 and 3 days (Kang, 2020). After 72 h, Biodentine had a significantly greater number of viable cells compared with MTA Angelus and ProRoot MTA using the Alamar Blue assay. However, MTT assay revealed no significant differences in cell viability amongst the three materials and the negative control after 72 h (Poggio et al., 2014), suggesting the specific evaluation methods may also influence the result. Alkaline phosphatase (ALP) activity was lower in cells cultured with Biodentine compared with ProRoot MTA, whilst a similar pattern of mineralization-related gene expressions was observed (Widbiller et al., 2016) and the opposite was observed in another study (Kang, 2020). Both materials upregulated the expression of DSPP at 14 and 21 days (Widbiller et al., 2016).

Using a three-dimensional (3D) spheroid culture developed from two mouse cell lines (MDPC-23 cells and Od-21 cells) to evaluate the biological effects of the materials (Pérard et al., 2013) indicated that ProRoot MTA and Biodentine generated cell line-dependent effects. Both materials were not significantly different in terms of cell viability of MDPC-23 cells on Day 3, ProRoot MTA induced a higher cell viability than that of Biodentine on Day 7 (Pérard et al., 2013). For Od-21 cells, cell viability decreased from Day 3 to Day 7 in culture with Biodentine or ProRoot MTA extracts (Pérard et al., 2013), and this may be due to the undifferentiated nature of Od-21 pulp cells. The morphology of MDPC-23 and Od-21 spheroids cultured with both materials resembled those cultured in the control group with culture medium, suggesting a cytocompatibility (Pérard et al., 2013). Thus, the results obtained show that not only the results are specific to the test employed but are also dependent on the cell type.

Different brands of MTA namely ProRoot MTA, MM-MTA (Micro-Mega mineral trioxide aggregate, Micro-Mega) and RetroMTA (BioMTA) all enhanced ALP activity and mineralized nodules formation (Chang et al., 2015). In addition, all materials upregulated the expression of odontogenic markers such as osteonectin (ON), OPN, osteocalcin (OCN), DSPP and dentine matrix protein-1 (DMP-1) at 14 days (Chang et al., 2015). A similar inflammatory effect was also observed for all materials,

in the protein expression of inducible NO synthase and cyclooxygenase-2 (COX-2) as well as the expressions of pro-inflammatory cytokines tumour necrosis factor alpha, interleukin (IL)-1b, IL-6 and IL-8 (Chang et al., 2015). In another study, HDPCs were in indirect contact with MM-MTA or ProRoot MTA extracts at 1, 7 and 14 days (Önay et al., 2018). Both materials exhibited similar expression levels of odontogenic markers for instance alkaline phosphatase liver/bone/kidney (ALPL), DSPP, runx-related transcription factor 2 (RUNX2) and distal-less homeobox 3 (DLX3; Önay et al., 2018).

The biocompatibility of a MTA-derived pozzolan cement Endocem (Maruchi) was similar to that of ProRoot MTA towards HDPCs (Park et al., 2014). ALP activity and mineralized nodule formation increased in ProRoot MTA and Endocem-treated cells compared with culture medium control group (Park et al., 2014). The expression of odontogenic-related markers was also significantly higher in the cells exposed to two cement extracts, and no significant difference was detected (Park et al., 2014). Another study also confirmed the biocompatibility of ProRoot MTA, Endocem (Maruchi) and as another calcium silicate cement containing ZrO_2 as a radiopacifier EndocemZr (Maruchi) extracts towards HDPCs (Kim et al., 2015a). Well-spread and flattened cells were also observed on material surfaces (Kim et al., 2015a). There was also no significant difference in the mineralization potential of the three materials with regard to the mineralized nodule formation as well as the expression of odontogenic-related markers (Kim et al., 2015a).

HDPCs in direct contact with both fresh and set cements discs of ProRoot MTA, Endocem Zr and RetroMTA (BioMTA) revealed no significant difference in cell viability between fresh and set materials, and all materials appeared biocompatible towards HDPCs (Chung et al., 2016). There was also no significant difference in VEGF levels between set and fresh ProRoot MTA groups, but there were differences between set and fresh cements for RetroMTA and Endocem Zr (Chung et al., 2016). The Endocem Zr groups had significantly lower concentrations of VEGF and angiogenin levels compared with ProRoot MTA and RetroMTA groups (Chung et al., 2016).

iRoot BP Plus (Innovative Bioceramix) and ProRoot MTA both enhanced the proliferation of HDPCs (Liu et al., 2015). BioAggregate (Innovative Bioceramix) was introduced for use as a root canal filling material; a previous study reported that BioAggregate extracts induced a high viability of HDPCs at 24 and 48 h, whereas cells exposed to ProRoot MTA extracts displayed suppressed viabilities at 72 h (Zhu et al., 2014). BioAggregate extracts also enhanced cell adhesion and migration in a concentration-dependent manner, which was superior to the effects induced by ProRoot MTA (Zhu et al., 2014). Immunofluorescence staining indicated that both materials optimized focal adhesion formation and stress fibre assembly (Zhu et al., 2014). Furthermore, SEM analysis revealed that HDPCs attached onto BioAggregate were more flattened and exhibited better spreading than that of ProRoot MTA (Zhu et al., 2014). Whilst Zhang et al. (2013) reported BioAggregate induced a slight decrease in the viability of HDPCs. Regarding the mineralization potential, ALP activity of HDPCs when exposed to BioAggregate, iRoot BP Plus and ProRoot MTA was significantly higher than that of the negative control group up to 7 days, and reverse-transcriptase polymerase chain reaction reverse-transcriptase polymerase chain reaction (RT-PCR) indicated that both BioAggregate and iRoot BP Plus groups were associated with a higher up-regulation of mineralization and odontoblastic differentiation-associated gene expressions as compared to ProRoot MTA (Zhang et al., 2013).

For mouse dental pulp cells, ProRoot MTA and EndoSequence BC Root Repair Material (EndoSequence BC RRM, Brasseler USA) had similar cell viability and proliferation and no statistically significant differences were noted (Machado et al., 2016). Both materials also expressed significantly higher levels of odontoblastic/osteogenic differentiation marker ALP than the control group (Machado et al., 2016).

For HDPCs, MTA Angelus and Biodentine extracts had no cytotoxic effects, and Biodentine significantly enhanced cell migration and induced a higher expression of chemoattractant molecules of monocyte chemoattractant protein-1 (MCP-1), transforming growth factor-β1 (TGF-β1; Omidi et al., 2020), which may be associated with increased cell migration and angiogenesis, therefore, leading to a higher regenerative potential (Hayashi et al., 2015). Similar results were reported in another study which revealed that extracts of MTA Angelus and a tricalcium silicate-based cement MTA Plus (PREVEST DenPro) were not cytotoxic in culture with HDPCs, nor did they induce apoptosis (Rodrigues et al., 2017). After 14 days of culturing, both materials exhibited a stimulatory effect on the mineralized nodule formation (Rodrigues et al., 2017).

For HDPCs which were cultured directly on the material surface for an observation period of 14 days, MTA Angelus, OrthoMTA and Biodentine all enhanced ALP activity, the formation of mineralized nodules and the expressions of odontogenic markers such as DSPP, DMP-1, ON, OPN and bone sialoprotein (BSP) as well as transcriptional factors such as RUNX2 and osterix, and no significant difference was detected amongst the materials (Chang et al., 2014). Furthermore, all tested materials downregulated the expression of pro-inflammatory mediators including nitric oxide, prostaglandin E2, inducible nitric oxide synthase and cyclooxygenase-2 (Chang et al., 2014).

MTA Angelus and EndoSequence BC RRM putty (Brasseler) had similar cytotoxicity towards human dermal fibroblasts (Hirschman et al., 2012). Another study assessed the cytocompatibility of MTA Angelus, NeoMTA Plus (Avalon Biomed) and Biodentine towards 3T3 fibroblasts using cement extracts obtained from both fresh and 24-h set cement discs (Pinheiro et al., 2018). Both MTT and sulforhodamine B (SRB) assays had a higher viability in culture with cement extracts from set cements than that of the fresh cement (Pinheiro et al., 2018). From extracts obtained from fresh cements, MTT assay revealed that NeoMTA Plus and MTA Angelus were associated with higher cell viability compared with Biodentine, whilst the SRB assay revealed that NeoMTA Plus generated a higher cell viability than MTA Angelus and Biodentine (Pinheiro et al., 2018).

For HDPCs, NeoMTA Plus, MTA Repair HP (Angelus) and Biodentine had no cytotoxic effect, and Biodentine had a higher cell viability than the other two materials at 48 and 72 h (Tomás-Catalá et al., 2018). MTA Repair HP and NeoMTA Plus did not influence cell migration, Biodentine, however, significantly enhanced cell migration (Tomás-Catalá et al., 2018). HDPCs in culture with iRoot BP Plus and MTA Repair HP extracts exhibited a higher cell proliferation in comparison with the culture medium (Abou ElReash et al., 2021). The cells seeded directly on the material surface exhibited an elongated fibroblastic morphology, with projections of filopodia lamellipodia, microvilli and blebs from their surfaces, reflecting good attachment to the material (Abou ElReash et al., 2021). iRoot BP Plus increased mineralizing nodules formation compared with MTA Repair HP (Abou ElReash et al., 2021).

Pulpotomy. Biodentine extracts induced the proliferation of stem cells isolated from human exfoliated primary teeth (SHEDs) and had a significantly higher rate of proliferation than MTA Angelus from 48-h incubation onwards (Collado-González et al., 2017). Both materials promoted cell migration after 48 h compared with the culture medium control group (Collado-González et al., 2017). SEM revealed that SHEDs were well spread onto the surface of Biodentine and MTA Angelus discs (Collado-González et al., 2017). Moreover, cells cultured on Biodentine exhibited a significantly higher level of calcified matrix deposition compared with MTA Angelus after 21 days (Collado-González et al., 2017).

Revascularization. The cytotoxicity of MTA Angelus and Biodentine towards HDPCs was similar, was both time- and concentration-dependent (Collado-González et al., 2017). The expressions of osteogenic/dentiogenic-related genes ALP, OCN, BSP, DSPP and DMP-1 were significantly upregulated after exposure to MTA Angelus or Biodentine (Collado-González et al., 2017). Extracellular mineral nodules could be identified in HDPCs cultured with MTA Angelus and Biodentine for 21 days (Collado-González et al., 2017).

Animal studies

This review mainly focuses on the pulpal responses generated by different pulp-capping agents, and other factors for instance the site and size of exposure as well as the animal species which may also influence the pulpal responses will not be discussed. To evaluate the pulp-capping potential of hydraulic calcium silicate cements, the histological features of the pulp and the periapical tissues, more specifically the inflammatory pulpal response, as well as the extent, distribution and the nature of the reparative dentin are the most critical and commonly used criteria.

Indirect pulp capping. ProRoot MTA, as an indirect pulp-capping agent, generated the formation of irregular tertiary dentine beneath the remaining dentine, in premolars from beagle dogs (Choung et al., 2016). The formed dentine revealed an atubular morphology with cellular components in the calcified matrix (Choung et al., 2016) with some dentine tubules were slightly occluded, whilst most dentinal tubules remained open (Choung et al., 2016). In permanent teeth from miniature swine which were indirectly capped first with Dycal and subsequently with Biodentine, a continuous mineralized matrix was formed post-operatively after 3 and 8 weeks (Tziafa et al., 2015). The maximum thickness of the hard tissue increased over time (Tziafa et al., 2015). The mineralized tissue exhibited a homogenous structure of atubular tertiary dentine with a few scattered defects and tunnels at the first deposited zone, limited short dentinal tubules associated with odontblast layer were observed (Tziafa et al., 2015). Using Biodentine solely as the indirect pulp-capping agent, the formation of tertiary dentine with a similar morphology was also observed in most of the specimens (Tziafa et al., 2015).

Direct pulp capping. A total of 23 animal studies which evaluated the direct pulp capping potential of hydraulic cements were included. In the majority of the studies, healthy pulps were mechanically exposed, whilst in one study, inflamed pulps were used to mimic a caries-related exposure (Louwakul & Lertchirakarn, 2015). Briefly, exposed pulps were left opened for 48 h before pulp capping to induce inflammation (Louwakul & Lertchirakarn, 2015).

To evaluate the pulp-capping capacity of calcium silicate cements, the histological features of the pulp and the periapical tissues, more specifically the inflammatory

pulpal response, as well as the extent, distribution and the nature of the reparative dentine are the most critical and commonly used criteria. Considering the fact that various scoring methods have been used, it is therefore not possible to quantitatively compare the behaviours of different hydraulic cements amongst different studies. Instead, only the general findings will be presented.

The inflammatory responses of uncapped exposed pulps with no capping material show conflicting results. A mild-to-moderate chronic inflammation was observed in uncapped pulps at the observation period of 2 or 4 weeks (Lin et al., 2017). Whilst in another study which sealed the exposed pulps with a sterile piece of Teflon tape, mild-to-moderate acute inflammation was seen at Day 1 (Trongkij et al., 2019). After 30 days, severe inflammatory responses with all of the coronal pulp infiltrated with inflammatory cells were observed (Trongkij et al., 2019). A similar severe acute pulpal inflammation with necrosis was detected in uncapped exposed pulps which were left open to the oral microflora (Danesh et al., 2012).

When using calcium hydroxide which is the material that all the hydraulic cements are compared to, results derived from *in vivo* animal studies using Wistar rats (Amin & Montaser, 2021) and beagle dogs (Asgary et al., 2008) were similar with a moderate-to-severe inflammatory reactions including inflammatory cell infiltration, congested blood vessels and areas of necrotic tissue seen at an early stage (Amin & Montaser, 2021; Asgary et al., 2008). The inflammatory reaction reduced over time (Amin & Montaser, 2021), and no inflammation was seen in capped pulps from mongrel dogs after 3 months (de Queiroz et al., 2005). In pulps from baboon premolars, a minimal acute or chronic inflammatory reaction was observed 4 months after pulp capping (Al-Hezaimi et al., 2011). For monkeys, however, severe pulp inflammation dominated by polymorphonuclear leukocytes was seen after post-operative period of 5 months (Pitt Ford et al., 1996). For inflamed rat pulps, the inflammation also decreased over time, and acute inflammation was found in 80% of the pulps after 8 days, whilst after 30 days, a moderate inflammation was detected in 67% of samples (Louwakul & Lertchirakarn, 2015).

The pulp-capping potential of ProRoot MTA has been extensively investigated. In animal studies using rats, it induced a mild-to-moderate inflammatory response at Day 1. Most specimens exhibited focal accumulation of polymorphonuclear leukocytes, congested blood vessels and local disruption of odontoblastic layer (Trongkij et al., 2019). After 7 and 30 days, most specimens had no pulpal inflammation at the exposure site (Trongkij et al., 2019). This is in agreement with the result from two other studies, which also reported the decrease in the inflammatory response generated by ProRoot MTA over time (Lin et al., 2017; Liu et al., 2015). First, a mixed acute and chronic inflammation was noted in 67% of the exposed pulps after 2 weeks (Lin et al., 2017). This was followed by a mild chronic inflammatory reaction detected in 83.3% of the teeth (Lin et al., 2017). ProRoot MTA and iRoot BP Plus generated a similar mild inflammatory cell response with very few or limited inflammatory cells 1 week after pulp capping, and no inflammation was detected after 4 weeks (Liu et al., 2015). Similar inflammatory responses were observed in exposed pulps capped with another calcium silicate cement, Bio-MA (M-Dent/SCG), which is probably due to the similarity in their main ingredients including tricalcium silicate, dicalcium silicate and tricalcium aluminate (Trongkij et al., 2019). In exposed pulps from beagle dogs, ProRoot MTA also generated a mild chronic or no inflammation 7 days, 2 or 3 months after pulp capping (Asgary et al., 2008; Danesh et al., 2012; de Queiroz et al., 2005). A moderate inflammation was also observed in 37.5% of the exposed pulps from minipigs 7 days after pulp capping (Li et al., 2018). No inflammatory reaction was seen in the remaining capped pulps, and the pulp tissue adjacent to the exposure site appeared richer in cells, collagen fibres and capillaries, and numerous irregular stromal cells were observed in the matrix (Li et al., 2018). After 70 days, no obvious inflammation was detected (Li et al., 2018). For Biodentine, similar mild-to-moderate inflammatory reaction was observed in a majority of the exposed pulps after 1 week, which also reduced after 3 weeks (Amin & Montaser, 2021). In capped pulps from minipigs which were capped with Biodentine for 7 days, mild inflammatory reaction with scattered inflammatory cells or inflammatory cells with small focal grouping was observed in 62.5% of the specimens (Pedano et al., 2020). The pulp tissue immediately adjacent to the exposure appeared richer in cells and collagen fibrils (Pedano et al., 2020). Moreover, in some cases, the presence of prominent capillaries could be observed in the matrix (Pedano et al., 2020).

The formation of reparative dentine bridge in the absence of the pulp-capping agent has been previously reported in studies using exposed pulps from Wistar rats (Lin et al., 2017; Trongkij et al., 2019); this confirmed the self-repair potential of rat pulps. The formed dentine bridges were discontinuous and had an immature morphology of an atubular structure with cell inclusions (Lin et al., 2017; Trongkij et al., 2019). In uncapped pulps from baboons, however, no reparative hard tissue formation was noted (Al-Hezaimi et al., 2011).

Dycal induced the formation of incomplete calcific bridge in exposed pulps from Wistar rats, this for both healthy (Amin & Montaser, 2021; Orhan et al., 2012) and inflamed pulps (Louwakul & Lertchirakarn, 2015). Amorphous and atubular calcified bridges were also

formed in exposed pulps from beagle dogs, which were capped with Dycal for 8 weeks (Asgary et al., 2008). After 3 months, the exposure site was completely sealed by the dentine bridge, and a layer of normal odontoblasts was observed under the dentin bridge (de Queiroz et al., 2005). Minimal and incomplete reparative hard tissues were also formed in pulps from baboon premolars which were capped with Dycal for 4 months (Al-Hezaimi et al., 2011). The reparative hard tissues were atubular, of uneven thickness and exhibited porosities and tunnel defects (Al-Hezaimi et al., 2011). In exposed pulps from monkeys which were capped with Dycal for 5 months, dentine bridges were detected in 33.3% of the samples (Pitt Ford et al., 1996).

For ProRoot MTA, no early sign of hard tissue formation was seen in rat pulps 1 day after pulp capping (Trongkij et al., 2019). At 1 week, however, a layer of newly generated collagen fibrils was detected subjacent to the material and this structure was strongly immunolabelled for OPN (Tran et al., 2012). After 2 weeks, a homogenous reparative dentine was observed directly at the injury site, which was in continuity with primary dentine; the cells associated with the reparative dentine formation presented a polarized morphology and were immunolabelled for dentin sialoprotein and OPN (Tran et al., 2012). After 1 month, the dentine bridge completely covered the exposure site (Tran et al., 2012; Trongkij et al., 2019). The pulp tissue demonstrated a normal morphology with re-organization of pulpal cells and odontoblast-like cells under the newly formed dentine bridge (Trongkij et al., 2019). Moreover, the formed dentin bridge had a tubular structure with limited cell inclusion (Lin et al., 2017; Trongkij et al., 2019). Similar dentine bridge formation with the morphology of tubular dentine was reported in other studies, which capped the exposed pulps with ProRoot MTA for 2 or 4 weeks (Guerrero-Gironés et al., 2021; Lin et al., 2017). Moreover, the majority of the bridges were complete and continuous, and a regular odontoblastic layer could be detected (Guerrero-Gironés et al., 2021). The thickness of most formed dentine bridge in rat incisors was reported to be more than 200 μm after 4-week pulp capping (Orhan et al., 2012). Another calcium silicate cement Bio-MA (M-Dent/SCG) showed similar reparative dentine formation (Trongkij et al., 2019). Two other calcium silicate cements Endocem and EndocemZr also generated the formation of reparative dentine with complete continuity in exposed pulps from rats 4 weeks after pulp capping (Kim et al., 2015a; Park et al., 2014). Reparative dentine bridge was also seen at the injury site in specimens capped with ProRoot MTA and iRoot BP Plus for 4 weeks, and the newly formed reparative dentine was connected to the primary dentine and contained homogenous dentinal tubule-like structures (Liu et al., 2015).

In exposed pulps from beagle dogs, ProRoot MTA generated the formation of incomplete and irregular hard tissues 7 days after capping (Danesh et al., 2012). At a later stage, hard tissue bridge completely obliterating the exposure was formed (Danesh et al., 2012; Faraco & Holland, 2004; de Queiroz et al., 2005), and tunnel defects were observed in a few specimens (Danesh et al., 2012; Faraco & Holland, 2004). A well-organized odontoblast cell layer was present in close relation to dentine bridges (Asgary et al., 2008; de Queiroz et al., 2005). In another study, radiophotographs together with histological analysis also confirmed the formation of calcific bridge in dog pulps being capped with ProRoot MTA for 12 weeks, and the bridge showed irregularities in some sections; however, no tunnel defects or soft-tissue inclusions were noted (Obeid et al., 2013). A similar irregular dentine bridge, without tunnel defects was noted in another study (Asgary et al., 2006). In exposed pulps from Beagle dogs which were capped with ProRoot MTA for 2 months, SEM was used to assess the dentine bridge (Asgary et al., 2006). The formed dentin bridge consisted of three aspects: the outer aspect was composed of ProRoot MTA in direct contact with the newly formed hard tissue; in the middle portion, a dentine-like bridge with irregular dentinal tubules was identified; and the inner aspect exhibited pre-dentine layer which was rapidly mineralized (Asgary et al., 2006). Amorphous crystals of hydroxyapatite were observed in the odontoblastic intercellular spaces, which was considered as a sign of rapid mineralization (Asgary et al., 2006). In exposed pulps from baboons, ProRoot MTA induced thicker reparative hard tissue formation with lower porosity than Dycal, this 4 months after pulp capping (Al-Hezaimi et al., 2011). Thick and continuous dentine bridge was formed in pulps from monkeys which were capped with MTA (Loma Linda University) for 5 months (Pitt Ford et al., 1996). For minipigs, complete reparative dentine bridge with a tubular structure was also observed in capped pulps after 70 days, and the underlying pulp tissue revealed a normal morphology (Li et al., 2018).

Biodentine generated the formation of mineralized dentine bridges of similar morphology to that of ProRoot MTA after 2 and 4 weeks in rat pulps (Tran et al., 2012). Incomplete or completely calcific bridge formation was detected in most of the exposed rat pulps being capped with Biodentine for 3 weeks (Amin & Montaser, 2021). After 5 weeks, a complete calcific bridge with well-organized pulp tissue was detected in 62.5% of the specimens (Amin & Montaser, 2021). For capped pulps from minipigs, a thick (200–500 μm) mineralized tissue was formed after 70 days, the area close to the material is composed of a disorganized pattern of mineralization without

or with scare and irregular tubules; whilst the area facing the pulp tissue is of a regular tubular morphology (Pedano et al., 2020). A layer of odontoblast-like cells aligning underneath the mineralized dentine bridge was observed (Pedano et al., 2020).

Pulpotomy. Quick-Set (Avalon Biomed), ProRoot MTA and MTA Plus (Avalon Biomed) all generated dentine bridge formation in rat pulps as early as 1 month after pulpotomy (Kramer et al., 2014). A previous study investigating pulpotomies in beagle dogs showed that after 4 months, a mineralized dentine bridge completely covered the pulp amputation site was formed in 72.2% and 96.8% of the roots capped with ProRoot MTA and Biodentine respectively (De Rossi et al., 2014). The integrity of the lamina dura and periapical region was preserved, and no internal/external root resorption was detected (De Rossi et al., 2014). The dentine bridges induced by ProRoot MTA and Biodentine seemed alike and revealed an irregular atubular or mixed (atubular and tubular) morphology, with encapsulation of cells and blood vessels (De Rossi et al., 2014). Only, the thickness of the newly formed dentine bridge for Biodentine was thicker (De Rossi et al., 2014). ProRoot MTA also generated dentin bridge formation at the pulp-exposure interface with a minimal pulp inflammation in pulps from mongrel dogs (Dominguez et al., 2003). Overall, 40% of the dentine bridges were complete, and 60% had a tubular morphology (Dominguez et al., 2003). In exposed pulps from mongrel dogs which were capped with Biodentine, a moderate inflammatory cell infiltrate and tissue necrosis were observed early at one week, whilst no sign of dentine bridge formation or pulp regeneration was noted (El-Zekrid et al., 2019). After 9 weeks, inflammatory cell infiltrate decreased and a complete thin osteodentin bridge with tunnel defects was formed (El-Zekrid et al., 2019). iRoot BP Plus induced the formation of hard tissues at the canal orifice in molars from rats, and most specimens exhibited no inflammatory cells, which presented a normal morphology (Liu et al., 2015). After 4 weeks, a thick layer of dentine bridge which completely sealed the canal orifice was detected, and polarizing odontoblast-like cells were observed below the dentine bridge (Liu et al., 2015).

Antimicrobial characteristics

The outputs for the microbiological testing are shown in Table 4. Only tests aimed to test pulp-capping materials have been published. No testing was performed for materials used for revascularization. From the 12 intracoronal procedures, one publication was for pulpotomy procedures in primary teeth. Eleven publications tested materials for pulp capping and one of these tested the antimicrobial characteristics of the materials when used in a clinical study. The range of bacterial species used included a number of streptococci and lactobacilli known in caries research. Enterococci and pseudomonas were also used. Enterococci are more well known for intra-radicular infections, whilst pseudomonas aeruginosa is found mostly in anaerobic conditions and most commonly associated with immunocompromised patients. The materials tested included MTA, Biodentine and Bioaggregate, whilst calcium hydroxide, resin-modified experimental materials and Theracal (Bisco) were comparators. The study testing materials for pulpotomies used formocresol and zinc oxide–eugenol as a control. Only in 2 papers, were the materials used characterized and only 4 included other testing which was mostly biological characterization. The most frequent test undertaken was agar diffusion test and direct contact test on materials with one study testing leachates. The clinical study (Neelakantan et al., 2012) sampled the materials used as pulp caps with lactobacilli being the micro-organism most frequently sampled.

The testing undertaken when using agar diffusion methods revealed that the hydraulic cements used for pulp capping exhibited some degree of antimicrobial properties against the cariogenic streptococci (Poggio et al., 2014, 2015), which was not as good as calcium hydroxide used as a control (Poggio et al., 2014, 2015) and also inferior to formocresol and zinc oxide–eugenol used for pulpotomy procedures. MTA Angelus exhibited larger inhibition zones when compared to Biodentine (Poggio et al., 2014, 2015); another publication comparing the same materials had the opposite result using enterococci and staphylococci (Özyürek & Demiryürek, 2016). The antimicrobial activity of Biodentine can be improved by addition of cetrimide and chlorhexidine (Deveci et al., 2019). When using the direct contact method (Yalcin et al., 2014), the hydraulic cements had lower antimicrobial activity than calcium hydroxide and other resin-based cements with addition of quaternary ammonium salts with no difference between the calcium hydroxide and MTA (Yang et al., 2014). The lack of antimicrobial activity was shown in other research particularly in freshly mixed materials with the activity enhanced with ageing after 1 week. MTA Angelus had better antimicrobial activity than Biodentine (Koruyucu et al., 2015), and the latter was more antimicrobial than Theracal (Farrugia et al., 2018; Fathy et al., 2019). Leachate analysis also revealed inadequate antimicrobial activity (Arias-Moliz et al., 2017).

The other tests conducted in conjunction with the antimicrobial assays revealed no deterioration in compressive strength with the addition of cetrimide and chlorhexidine powders to Biodentine indicating the adequacy of enhancing the antimicrobial properties in this way (Deveci et al.,

TABLE 4 Detail of microbiological data collected for hydraulic cements for endodontic use

Material use	Monospecies/ multispecies	Species used	Test undertaken	Test material	Control	Characterization	Other tests
Intra-coronal							
Pulp capping	2 multispecies, 5 monospecies with multiple bacteria, 4 monospecies using 1 type of bacterium only, 1 clinical study sampling	*S. mutans, S. salivarius, S. Sanguis, S. aureus*, S. gordonii, *S. Sobrinus, S. acidophilus, E. coli*, *E. faecium, E. faecalis*, *P. aeruginosa*, *L. casei,* 1 sampling	5 agar diffusion test, 5 direct contact test, 1 minimum inhibitory test on leachates, 1 sampling	MTA, Biodentine, Bioaggregate	Calcium hydroxide, Theracal, experimental resin-based materials, modified Biodentine, polyantibiotic paste, formocresol, zinc oxide	10 without characterization, 2 characterized	4 included other tests (biological testing and physical testing), 8 no other tests
revascularization	No research						
Intra-radicular							
Apexification	No research						
Root canal treatment	3 monospecies, 3 multispecies	*E. faecalis*, *S. mutans*, *S. epidermidis, S. aureus*, *C. albicans*, *P. endodontalis*, P. gingivalis	Direct contact test on planktonic and biofilm, agar diffusion, intratubular infection	BioRoot, TotalFill/ Endosequence, Endoseal	Sealer 26, AH Plus, CRCS, GuttaFlow 2, Pulp canal sealer	2 characterization of material and 1 characterization of leachate, 3 no characterization	4 included other tests such as flow, interfacial assessment, setting time, wettability, surface roughness, biological activity
Extra-radicular							
Perforation repair	No research						
Root-end surgery	1 single species, 3 multispecies	*S. aureus*, epidermidis, *E. faecalis*, *P. aeruginosa*, *B. subtilis*, *E. coli*, *P. buccae*, *P. intermedia, P.melaninogenica*, *B. fragilis*, *F. necrophorum*, *F.nucleatum, P. anaerobius*, *M. luteus*, *C. albicans*	Agar diffusion, direct contact tests on materials, testing of dentine in contact with the materials	MTA	Amalgam, IRM, Superbond, Geristore, Composite resin	No characterization	No other tests
Unspecified use	4 multispecies	*S. mutans*, *E. faecalis*, *L. rhamnosus paracasei*, *P. gingivalis*, *E. coli*	Agar diffusion	MTA, Endocem, CEM	No controls	No characterization	1 paper testing mineralizing activity

2019). The pulp-capping materials had initial cytotoxic activity which subsided after 3 days (Arias-Moliz et al., 2017). The hydraulic cements exhibited lower cytotoxicity than calcium hydroxide (Poggio et al., 2014).

Clinical application

The clinical application of these materials in the intracoronal region of teeth comprises of vital pulp therapy (VPT). VPT is a broad field in (paedo-) Endodontology, that can be subdivided depending on the level of injury and hence the remaining amount of dentinal and nerve tissue (Duncan et al., 2019). As mentioned in Figure 1d and Table 5, the following VPT modalities have been assessed: (in)direct pulp capping, (partial) pulpotomy and revascularization.

Indirect pulp capping

Five publications have been assessed for this type of VPT (Table 5). The materials were applied in deeply carious, permanent and deciduous teeth with vital pulps without periapical pathosis nor signs of irreversible pulpitis. In all studies except for one (Koc Vural et al., 2017), rubber dam isolation was used. The preparation of the test materials is mentioned in Table 5. However, only two papers reported the clinical application of hydraulic calcium silicate cement: by means of an MTA gun (Leye Benoist et al., 2012) and with light pressure of moist-cotton pellets (Koc Vural et al., 2017; Leye Benoist et al., 2012).

For ProRoot MTA (Dentsply), the clinical success rates for recalls between 6 and 24 months and radiographic dentine bridge formation at 6 months were not significantly different than those for Dycal (Dentslpy) or GIC Fuji IX GP (GC) (Koc Vural et al., 2017; Leye Benoist et al., 2012; Mathur et al., 2016). Regarding adverse events of MTA treated teeth, in one study, irreversible pulpitis was reported for two teeth (Mathur et al., 2016), and in another study, two teeth failed to react on pulp vitality testing (Leye Benoist et al., 2012).

Initial and follow-up (24 months) studies compared Biodentine (Septodont) with GIC Fuji IX GP (GC; Hashem et al., 2015, 2019). Even if at 12 months the clinical outcome was more in favour of Biodentine, at 24 months, the clinical success rate was 72% for both groups and was related to the intensity of reversible pulpitis symptoms at baseline. At 12 and 24 months, no difference in radiographic (periapical RX) assessment and in the integrity of the resin composite was reported between both groups. Nevertheless, teeth with an initial cone-beam computed tomogram (CBCT) periapical lesion had a failure rate of 63% and teeth with no initial lesion on CBCT had a failure rate of 16% (Hashem et al., 2015). Furthermore, at 24 months, six teeth in the Biodentine group and nine in GIC group failed to maintain pulp vitality (Hashem et al., 2019).

Direct pulp capping

Twelve articles were reviewed where this technique was applied in primary and in (im)mature permanent teeth (Table 5). Carious, mechanical and traumatic pulp exposures were the main aetiological reasons to perform direct pulp capping. In one study, where 358 teeth were treated, up to 81% of the teeth did not receive rubber dam isolation at time of pulp exposure (Hilton et al., 2013). If the pulp-exposure site dimensions were mentioned, then this amounted between 1.2 and 2 mm. Next to MTA (ProRoot or Angelus) and Biodentine, Endocem (Maruchi) and an injectable treated dentine matrix hydrogel (TMDH) were applied as well as test material. In nine studies, the mixing and application of materials were mentioned, and in five studies, the thickness applied was described (1.5–3 mm). In only two studies, some explanations were given regarding the set of the materials (Brizuela et al., 2017; Parinyaprom et al., 2018), and in one study, the mixing device applied for Biodentine was mentioned (Katge & Patil, 2017). In only one study, Biodentine was left for 3 months in bulk as a temporary restoration and was subsequently covered with composite (Katge & Patil, 2017). The coronal restorations varied between studies: IRM, RM GIC, amalgam, composite, onlay, overlay, stainless steel crown and ceramic crowns. Regarding the studies with at least 12-month follow-up, the overall survival, clinical and radiographic outcomes for MTA and Biodentine were favourable and comparable to or even better than the test materials (Endocem and CH, respectively). Regarding the pain intensity during the early post-operative stage (1 week), the data were inconsistent, but in favour of MTA when compared to CH (Kundzina et al., 2017; Suhag et al., 2019). The most clinically significant adverse event was MTA discolouration. Parinyaprom et al. (2018) concluded that Biodentine did not cause discolouration and was non-inferior to ProRoot MTA.

Pulpotomy

Sixteen articles were reviewed where this technique was not only applied in mainly primary teeth (Alsanouni & Bawazir, 2019; Cardoso-Silva et al., 2011; Carti et al., 2017; Çelik et al., 2019; Cuadros-Fernández et al., 2016; Doyle et al., 2010; Elhamouly et al., 2021; Kang et al., 2015a; Liu et al., 2011; Lourenço Neto et al., 2015; Silva et al., 2019; Vilella-Pastor et al., 2021) but also immature permanent teeth (four studies, Table 5). Carious and traumatic pulp exposures were the main aetiological reasons. In one study, no rubber dam nor field isolation was applied

TABLE 5 Detail of clinical data collected for hydraulic cements for endodontic use

Material use	First author, year published	Study design	*n* teeth, age (range/mean)	Groups & materials applied	Healing assessment methods	Follow-up period	Type of teeth and aetiology	Application of materials	Coronal restoration
Intra-coronal									
Indirect pulp capping									
	Leye Benoist F, 2012	RCT	60, 16–34 y	Test: ProRoot MTA (Dentsply) control: Dycal (Dentsply)	Clinical and radiographic	3 and 6 m	Molars and premolars with active deep carious lesion on either the occlusal or proximal surface	MTA: powder mixed with sterile water in a 3:1 ratio, placed with MTA Gun, light pressure with moist-cotton pellets Dycal: mixed according to the manufacturer's instructions, placed with ball-ended instruments	Until 6 m: GIC, final restoration: amalgam/composite
	Hashem D, 2015	RCT	72, 18–76 y	Test: Biodentine (Septodont) control: GIC Fuji IX GP (GC)	Clinical: sensitivity radiographical: periapical + CBCT	Clinical and PR: 0, 6 and 12 m; CBCT: 0 and 12 m	Deep carious lesion, at least 3/4 dentine penetration (PR assessed), clinically mICDAS score 4 (Banerjee et al. 2011); reversible pulpitis; + on sensitivity test; no AP	'following manufacturer's instructions'	1 m later: resin composite veneer ('closed sandwich' technique)
	Mathur VP, 2016	RCT	109, 7–12 y	Test: ProRoot MTA (Dentsply) controls: Dycal Ivory (Dentsply) and GIC (GC Fuji VII)	American Association of Paediatric Dentistry clinical criteria CBCT (dentine bridge thickness)	Clinical: 8 w, 6 and 12m CBCT: direct post-op and 6 m	Primary second molar and permanent first molar	'following manufacturer's instructions'	Composite
	Koc Vural U, 2017	RCT	100, mean age: 20.93 y ± 3.48 y	Test: MTA (Dentsply) control: Dycal (Dentsply)	Clinical and radiographic	6, 12 and 24 m	Permanent molars/premolars with deep carious lesions involving 75% or more of the dentine, without pulp exposure	Passive application on the deepest part of the cavity, light pressure with wet cotton pellet	Light-cured GIC + composite
	Hashem D, 2019	RCT	72, 18–76 y	Test: Biodentine (Septodont) control: GIC Fuji IX GP (GC)	Clinical and radiographic	12 and 24 m	Deep carious lesion, at least 3/4 dentine penetration (PR assessed), clinically mICDAS score 4 (Banerjee et al. 2011); reversible pulpitis; + on sensitivity test; no AP	'following manufacturer's instructions'	1 m later: resin composite veneer ('closed sandwich' technique)

(Continues)

TABLE 5 (Continued)

Material use	First author, year published	Study design	*n* teeth, age (range/mean)	Groups & materials applied	Healing assessment methods	Follow-up period	Type of teeth and aetiology	Application of materials	Coronal restoration
Direct pulp capping									
	Tuna D, 2008	RCT, split mouth	25 pairs, 5–8 y	Test: ProRoot MTA (Dentsply) control: Dycal (Dentsply)	Clinical and radiographic	1, 3, 6, 9, 12, 18 and 24 m	Symmetrical pairs of primary molars with deep occlusal caries	MTA powder mixed with sterile water in 3:1 ratio, placed on the exposure sites with plastic amalgam carriers and light pressure with moist-cotton pellets Dycal mixed according to the manufacturer's instructions, applied to the exposure sites with ball-ended instruments	2 mm of resin-bonded ZOE (Kalzinol; Dentsply) and amalgam
	Accorinte ML, 2009	RCT	40, 25–42 y	Test: grey MTA (Angelus) control: grey ProRoot MTA (Dentsply)	Histology + clinical	1 and 2 m	Intact, caries-free premolars (mechanical traumatic exposures)	Not mentioned	IRM (Dentsply)
	Hilton TJ, 2013	RCT	358, 8–90 y	Test: white ProRoot MTA (Dentsply) control: Life CH (Kerr)	Clinical: sensitivity radiographical: PR	6, 12, 18 and 24 m	Permanent teeth exhibiting pulp exposure (carious, traumatic, or mechanical), no AP and no pain	'Used according to the manufacturer's clinical directions'	RM GIC + 'The teeth were restored as deemed appropriate by the dentist'
	Nowicka A, 2013	Comparative study	28, 19–28 y	Test: Biodentine (Septodont) control: group 1: ProRoot White MTA (Dentsply); group 2: intact teeth	Clinical, radiographic and histology	6 w	Caries-free third molars (pulp mechanically exposed (diameter: 1.2 mm))	Biodentine: capping and as temporary filling; MTA: 2 mm, moistened cotton pellet + GIC	Both groups after 1 w: composite
	Song M, 2015	RCT	46, 19–79 y	Test: Endocem (Maruchi) control: ProRoot MTA (Dentsply)	Clinical and radiographic	1, 2, 4 and 12 w	Mature permanent teeth with pulp exposure (carious or traumatic)	Endocem: mixed with distilled water or saline (2:1 powder-to-liquid ratio), 3-mm over exposure site with a Centrix syringe gun (Centrix, Shelton,CT), after 5 min MTA: mixed in a 3:1 powder-to-water ratio using sterile water, 3-mm incrementally placed over exposure site, cotton pellet moistened with sterile saline (for 2 days to set), IRM	RM GI

TABLE 5 (Continued)

Material use	First author, year published	Study design	*n* teeth, age (range/mean)	Groups & materials applied	Healing assessment methods	Follow-up period	Type of teeth and aetiology	Application of materials	Coronal restoration
	Jang Y, 2015	RCT	46, 19–79 y	Test: Endocem (Maruchi) control: ProRoot MTA (Dentsply)	Clinical and radiographic	3 and 12 m	Mature permanent teeth with pulp exposure (carious or traumatic)	Endocem: mixed with distilled water or saline (2:1 powder-to-liquid ratio), 3-mm over exposure site with a Centrix syringe gun (Centrix, Shelton,CT), after 5 min MTA: mixed in a 3:1 powder-to-water ratio using sterile water, 3 mm incrementally placed over exposure site, cotton pellet moistened with sterile saline (for 2 days to set), IRM	RM GI for 3 m (to assess complications), final restoration with direct resin filling, an inlay/overlay, or a single crown
	Brizuela C, 2017	RCT	169, 7–16 y	Test: ProRoot MTA (Dentsply) and Biodentine (Septodont) control: CH (Hertz Pharmaceutical)	Clinical: sensitivity (thermal and electrical) + percussion radiographical: PR (with a positioner)	Clinical: 1 w, 3, 6 and 12 m PR: baseline, 6 and 12 m	(im)mature permanent molar with less than 2 mm of carious exposure, and with pulpal testing compatible with normal pulp or reversible pulpitis; exposure site: 2 mm × 2 mm	CH: mixed with saline on a sterile glass slab; MTA and Biodentine: mixed in accordance with the manufacturer's instructions; all: applied with MTA gun + wet cotton pellet. Set: for MTA: wait until the material was partially set, expected that the humidity of the pulp would finish the setting of the material. for other materials: set not mentioned	RM GIC + composite
	Katge FA, 2017	RCT, split mouth	58, 7–9 y	Test: Biodentine (Septodont) control: ProRoot MTA (Dentsply)	Clinical and radiographic	6 and 1 2 m	Bilateral asymptomatic carious first permanent molars	Biodentine and MTA: applied to the pulp-exposure site with a plastic filling instrument Biodentine: manipulated with CapMix (3 M ESPE, Seefeld, Germany) and filled in the entire cavity. MTA: manipulated according to the manufacturer's instructions. RMGI on top	After 3 m: Biodentine was partially removed, leaving 1-mm layer of Biodentine intact MTA: RMGI partially removed Both groups: composite

(Continues)

TABLE 5 (Continued)

Material use	First author, year published	Study design	*n* teeth, age (range/mean)	Groups & materials applied	Healing assessment methods	Follow-up period	Type of teeth and aetiology	Application of materials	Coronal restoration
	Kundzina R, 2017	RCT	70, 18–55 y	Test: white ProRoot MTA (Dentsply) control: Dycal (Dentsply)	Clinical, QoL and radiographic	1 w, 6, 12, 24 and 36 m	First or second permanent molar with a proximal carious lesion (primary or secondary caries)	Dycal: thin layer on pulpal exposure and left to set; MTA: mixed according to the manufacturer's instructions, 2 mm layer placed over the pulpal exposure and the surrounding dentine, leaving at least 2 mm of dentine and enamel available circumferentially, a water-moistened cotton pellet was placed directly over the material	Temporary filling: GIC After 1 w: partially removed for Dycal and totally removed for MTA (to check the set); composite
	Suhag K, 2019	RCT	64, 15–40 y	Test: white ProRoot MTA (Dentsply) control: CH (Prevest)	Clinical, radiographic and post-operative pain	7 d (pain) and 3,6 and 12 m (RX and clinical)	Mature permanent molars, reversible pulpitis, occlusal deep caries penetrating more than half the thickness or more into dentine	CH: powder was mixed with saline according to the manufacturer's instructions MTA: mixed according to the manufacturer's instructions (1:3 water/powder ratio), applied to the exposure site with a sterile carrier, cotton pellet soaked with saline, IRM	RM GIC + composite timing: CH immediately, MTA 24 h later
	Parinyaprom N, 2018	RCT	55, 6–18 y	Test: Biodentine (Septodont) control: grey MTA (Angelus)	Clinical and radiographic	Every 6 m, mean follow-up of 18.9 ± 12.9 m	Cariously exposed permanent teeth, including teeth with diagnosis of normal pulp, reversible pulpitis, or irreversible pulpitis, early periapical involvement and exposure size of up to 2.5 mm	Cements mixed according to the manufacturer's instructions MTA: 1.5 mm thickness pulp dressing, RM GIC base Biodentine: as pulp dressing and base, 12 min. set	Depending on the remaining tooth structure: composite, amalgam or stainless steel crown
	Holliel AA, 2021	RCT	45, 18–40 y	Group 1: injectable treated dentine matrix hydrogel (TMDH) group 2: Biodentine (Septodont) group 3: white MTA (Angelus)	Clinical and radiographic (PR and CBCT)	3, 6, 12, 18 and 24 m	Permanent posterior teeth (mechanical traumatic exposures within an estimated size of 1–2 mm^2)	Freshly mixed TDMH: injected to seal the exposed area using a single syringe (5 mm chamber) other materials: not mentioned	RM GI + composite

TABLE 5 (Continued)

Material use	First author, year published	Study design	*n* teeth, age (range/mean)	Groups & materials applied	Healing assessment methods	Follow-up period	Type of teeth and aetiology	Application of materials	Coronal restoration
Pulpotomy									
	El-Meligy OA, 2006	Comparative study	30, 6–12 y	Test: MTA (Dentsply Caulk) control: CH + saline	Clinical and radiographic	3, 6 and 12 m	Traumatized or carious immature permanent teeth	CH: powder mixed with saline to a thick consistency, 1–2 mm thickness placed on the pulp stump, ZOE MTA: powder mixed with sterile water according to the manufacturer's directions, placed on the pulp stump surface and patted with a moist-cotton pellet, damp cotton pellet + IRM for 1 w	Anterior teeth: composite posterior teeth: amalgam
	Doyle TL, 2010	RCT	112 mean age: 47 m	Tests: ProRoot MTA (Dentsply), FS + MTA controls: FS, eugenol-free FS	Clinical and radiographic	12, 24 and 36 m	Carious primary molars where caries removal was likely to produce vital pulp exposure	FS: 15.5% aequous FS solution (Adstringedent, Utradent Products Inc.) on pulp stumps +IRM; eugenol-free FS: FS + Cimpat S (Septodont) MTA: powder + sterile water in 3:1 ratio, 'according to manufacturer's instructions', cover exposed radicular pulp and a margin of at least 1 mm beyond the pulp dentine interface, IRM MTA + FS: see FS and MTA groups	Stainless steel crowns cemented with polycarboxylate cement (Durelon, 3 M)
	Liu H, 2011	RCT, split mouth	17 pairs of teeth, 4–9 y	Test: ProRoot MTA (Dentsply) control: 50%CH +50% Iodoform +2g tetracaine +0.5 g sodium benzoate in 100 ml distilled water	Clinical and radiographic	Each 6 m	Primary molars	Not described	GI-liner + composite
	Cardoso-Silva C, 2011	Comparative study	210, not mentioned	Test: white ProRoot MTA (Tulsa Dental Products) control: grey ProRoot MTA (Tulsa Dental Products)	Clinical and radiographic	6–84 m (monitored every 6 m)	Carious primary molars	Mixing of the MTA with sterile water on a glass slab following the manufacturer's instructions. Pressing the MTA to the walls and floor of the pulp chamber with a cotton pellet moistened in sterile water	Filling the pulp chamber with light-curing GIC. Cementation of the stainless steel crown with self-curing GIC

(Continues)

TABLE 5 (Continued)

Material use	First author, year published	Study design	*n* teeth, age (range/mean)	Groups & materials applied	Healing assessment methods	Follow-up period	Type of teeth and aetiology	Application of materials	Coronal restoration
	Nosrat A, 2013	RCT	51, 6–10 y	Test: CEM cement (BioniqueDent) control: ProRoot MTA (Dentsply)	Clinical and radiographic	6 and 12 m	Carious-exposed vital immature permanent first molars	MTA and CEM: prepared according to the manufacturer's instructions, 3 mm placed over the amputated pulps and gently adapted to the dentinal walls using a cotton pellet, wet cotton pellet + temporary filling (Cavite, Ariadent), post-op PR to check appropriate thickness of the hTCS	After 24 h: inspection of the setting of the hTCS, 2 mm self-cure GIC + permanent restoration
	Lourenço Neto N, 2015	RCT	30, 5–9 y	Test: PC + Iodoform (Biodinaˆmica Quı´mica eFarmaceˆutica LTDA), PC +Zroxide (Sigma-Aldrich Co.), control: PC (CAS 65997-15-1, Votorantim-Cimentos)	Clinical and radiographic	6,12 and 24 m	Primary mandibular molars with deep caries and vital pulp	All groups: cements prepared using as the measure parameter one MTA kit spoon (1 g) of power to two drops (0.3 ml) of distilled water and mixed in sterilized glass to obtain a paste consistency; materials were then placed into the pulp chambers with a spatula	IRM + RM GIC
	Kang CM, 2015	RCT	143, 3–10 y	Test: Ortho- and RetroMTA (BioMTA) control: ProRoot MTA (Denstply)	Clinical and radiographic	1, 3, 6 and 12 m	Primary molar with a deep caries, presenting a potential risk of pulp exposure during treatment	ProRoot and OrthoMTA: wet cotton pellet + temporary filling, 3 weeks later coronal restoration; RetroMTA: restored immediately after 5 min set	RM GIC + stainless steel crowns
	Cuadros-Fernández C, 2016	RCT	90, 4–9 y	Test: Biodentine (Septodont) control: MTA (not specified)	Clinical and radiographic	6 and 12 m	Vital, symptom-free, carious primary molars	MTA: the radicular tissue was covered with MTA paste obtained by mixing MTA powder with sterile saline in a ratio of 3:1 in accordance with the manufacturer's instructions Biodentine: the radicular tissue was covered with Biodentine obtained by mixing Biodentine powder with a single dose of liquid in accordance with the manufacturer's instructions.	IRM + stainless steel crowns cemented with GIC

TABLE 5 (Continued)

Material use	First author, year published	Study design	*n* teeth, age (range/mean)	Groups & materials applied	Healing assessment methods	Follow-up period	Type of teeth and aetiology	Application of materials	Coronal restoration
	Carti and Oznurhan, 2017	RCT	50, 5–9 y	Test: Biodentine (Septodont) control: MTA (not specified)	Clinical and radiographic	1, 3, 6 and 12 m	Primary molar with deep cavity lesions that exposed vital pulp during the removal of caries; no clinical symptoms, roots 2/3 present and no RX anomalies	Biodentine: capsule struck gently on a solid surface to mix the powder inside, mixed with 5 droplets of liquid for 30 seconds using a triturator, mixture was condensed to the pulp stumps with amalgam carrier and moistened cotton pellet. Placed in bulk in the entire cavity MTA: powder mixed with distilled water in a 3:1 ratio, condensed over the pulp stumps lightly with a moistened cotton pellet, pulp chamber filled with RM GIC	Stainless steel crowns cemented with GIC
	Alsanouni M, 2019	RCT	80, 4–8 y	Test: NeoMTA Plus (Avalon Biomed Inc.) control: ProRoot MTA (Dentsply)	Clinical and radiographic	3, 6 and 12 m	Primary molars with deep caries	Both cements: mixed following the manufacturer's instructions, with amalgam carrier, placed over the canal orifices with slight pressure using a wetted cotton pellet; thickness: 2–3 mm	IRM + stainless steel crowns cemented with GIC
	Silva LLCE, 2019	RCT	45, mean age: 6.5 y	Test: grey MTA (Angelus) controls: CH P.A. (Biodinâmica Química e Farmacêutica Ltda.) + saline and CH P.A.+PEG (MAPRIC)	Clinical and PR	3, 6 and 12 m	Deciduous mandibular molars	MTA: powder + distilled water 1:1 ratio CH + 0.9% saline: mixed 1:1 ratio CH + PEG: 1:1 powder/liquid ratio application of all groups: 1-mm thick layer	Base: 1-mm thick layer of cement-cured CH (Biodinâmica Químicae Farmacêutica Ltda.), restoration: RM GIC
	Çelik BN, 2019	RCT	44, mean age: 6.7 y	Test: Biodentine (Septodont) control: ProRoot MTA (Dentsply)	Clinical and PR	3, 6, 12, 18 and 24 m	Deciduous mandibular molars	MTA: prepared according to the manufacturer's instructions, moistened cotton pellet was placed over the MTA paste to set, IRM; Biodentine: prepared according to the manufacturer's instructions, condensed lightly with a condenser on the pulp stumps	Stainless steel crowns: after 24 h for MTA group, after 12 min set for Biodentine group

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TABLE 5 (Continued)

Material use	First author, year published	Study design	*n* teeth, age (range/mean)	Groups & materials applied	Healing assessment methods	Follow-up period	Type of teeth and aetiology	Application of materials	Coronal restoration
	Abuelniel GM, 2020	RCT	50, 7.5–9 y	Test: Biodentine (Septodont) control: ProRoot white MTA (Dentsply)	Clinical and radiographic	0, 6, 12 and 18 m	Traumatized anterior immature permanent teeth, reversible pulpitis, no AP	Both groups: prepared according to the manufacturer's instructions, MTA: 3-mm thick layer adapted with a wet cotton pellet	Self-curing GIC and composite
	Abuelniel GM, 2021	RCT, split mouth	60, 7–8 y	Test: Biodentine (Septodont) control: Endocem MTA (Maruchi)	Clinical and radiographic	6,12 and 18 m	Immature first permanent with carious exposure	'Cements mixed in accordance with the manufacturer's instructions'. MTA: 3-mm thick, over the amputated pulps, gently adapted to the dentinal walls using a wet cotton pellet Biodentine: 3-mm thick	Self-curing GIC and composite
	Elhamouly Y, 2021	RCT	70 patients, exact number of included teeth not mentioned, 5–9 y	Test: 70S30C-Bioactive Glass (BAG; Faculty of Dentistry, Alexandria University, Egypt) control: Biodentine (Septodont)	Clinical, histology	Clinical: 1, 3, 6, 9 and 12 m histology: 6 w	Primary teeth with clinically deep caries	Biodentine: mixed for 30 s in an amalgamator BAG: mixing 2–3 drops of sterile distilled water with 0.1–0.2 g of the powder containing 70 mol% silicon dioxide/30 mol% calcium oxide, polyethylene oxide, N acetic acid, tetramethyl orthosilicate, calcium nitrate tetrahydrate and 2.5 vol% hydrofluoric acid as a gelation catalyst Both materials: carried to the pulp chamber using a sterile amalgam carrier and condensed on the pulp stumps using sterile cotton pellets. Biodentine was left to set for 6–12 min.	RM GIC + pre-formed stainless steel crowns (cemented with GIC luting cement)
	Vilella-Pastor S, 2021	RCT	84, 4–9 y	Test: Biodentine (Septodont) control: ProRoot MTA (Dentsply)	Clinical and radiographic	6, 12, 18 and 24 m	Primary molars with pulpal caries and/or dental trauma leading to pulp exposure without clinical evidence of pulp degeneration (irreversible pulpitis or pulp necrosis)	'in accordance with the manufacturer's instructions'	IRM

TABLE 5 (Continued)

Material use	First author, year published	Study design	*n* teeth, age (range/ mean)	Groups & materials applied	Healing assessment methods	Follow-up period	Type of teeth and aetiology	Application of materials	Coronal restoration
Partial pulpotomy									
	Qudeimat MA, 2007	RCT	64, 6.8–13.3 y	Test: ProRoot grey MTA (Dentsply) control: Hypocal (Ellman International Inc.) and Dycal (Dentsply)	Clinical and radiographic	3, 6 and 12 m	Restorable, (im)mature, permanent first molars with carious pulp exposures	CH: the pulp was dressed first with a layer of non-setting CH (Hypocal) followed by a setting layer of CH (Dycal) MTA: mixed according to manufacturer's instructions on a glass mixing pad, gently placed against the wound with spoon excavators and plastic instruments, adapted to the wound with a wet cotton pellet and the excess MTA was scraped off	Both groups: Base: a layer of light-cured GIC (Vitrebond) extended onto dentine + a base of light-cured GIC (GC Corp). Final restoration: either amalgam or where grossly carious, with pre-formed metal crowns cemented with GIC
	Özgür B, 2017	RCT	80, 6–13 y	Group 1: 2.5% SH (haemorrhage control agent) + ProRoot white MTA (Dentsply) group 2: 0.9% SS +ProRoot white MTA group 3: 2.5% SH +CH (Merck) group 4: 0,9% SS +CH (Merck)	Clinical and radiographic	12, 18 and 24 m	Permanent first and second molars with incomplete root formation and deep occlusal carious lesions	Groups 1 and 2: haemorrhagic agent placed for 5 min + MTA prepared following the manufacturer's instructions, placed for 1 mm of the surrounding dentine, pressed with a moist-cotton pellet, moist-cotton pellet was placed over the MTA, GIC groups 3 and 4: haemorrhagic agent placed for 5 min. + freshly mixed paste of CH powder with SS at a 3:1 ratio, placed for 1 mm of surrounding dentine	In all groups: GIC base + composite timing: groups 1 and 2 after 24 h, in groups 3 and 4 directly
	Kang CM, 2017	RCT	104, mean age: 29.3 ± 14.8 y	Test: Ortho- and RetroMTA (BioMTA) control: ProRoot MTA (Denstply)	Clinical and radiographic	1, 3, 6 and 12 m	Permanent teeth with advanced caries or dental trauma with pulp exposure	ProRoot and OrthoMTA: wet cotton pellet +temporary filling, 2–3 d later coronal restoration RetroMTA: restored immediately after 5 min. set	RM GIC + composite/ onlay/crown

(Continues)

TABLE 5 (Continued)

Material use	First author, year published	Study design	*n* teeth, age (range/mean)	Groups & materials applied	Healing assessment methods	Follow-up period	Type of teeth and aetiology	Application of materials	Coronal restoration
Revascularization									
	Aly MM, 2019	RCT	26, 8–15 y	Test: Biodentine (Septodont) control: white MTA (Angelus)	Clinical and radiographic	3, 6, 9 and 12 m	Non-vital immature permanent anterior teeth with AP	Both materials: 3–4 mm with an amalgam carrier, packed lightly with a moistened cotton pellet	In third session (post set MTA): composite
Intra-radicular									
Root canal treatment									
	Graunaite I, 2018	RCT	112, mean age: 49.5 y	Test: Total Fill BC sealer (FKG) control: AH Plus (Dentsply)	Intensity of post-operative pain	1, 2, 3 and 7 d	Mature permanent teeth with asymptomatic AP	Root canal retreatment, single visit test: Total Fill sealer and a Total Fill BC point control: AH plus and GP point Both groups: sealer prepared according to the manufacturer's instructions, root canal drying with paper points, sealer introduced into the canal with a paper point, GP point was adapted, canal obturated by a warm vertical condensation (150°C AH Plus and 180°C Total Fill), depth of the plugger WL-5 mm	IRM
	Atav Ates A, 2019	RCT	78, 18–65 y	4 groups (vital and devital teeth, 2 materials): test: iRoot SP (Innovative BioCeramix Inc.) control: AH Plus (Dentsply)	Intensity of post-operative pain	6, 12, 24 and 72 h	(Non-)vital permanent teeth	Root canal treatment, single visit first paper point: coated root canal with sealer (AH Plus or iRoot SP) up to the middle third second paper point: distributed sealer third paper point: removed excess sealer Herofill™ Soft-Core obturators (Micro-Mega), heated for 35s in HEROfill® Oven, inserted to WL using firm apical pressure without rotation	Composite

TABLE 5 (Continued)

Material use	First author, year published	Study design	*n* teeth, age (range/mean)	Groups & materials applied	Healing assessment methods	Follow-up period	Type of teeth and aetiology	Application of materials	Coronal restoration
	Tan HSG, 2021	RCT	163, 21–70 y	Test: Total Fill BC sealer (FKG) control: AH Plus (Dentsply)	Intensity of post-operative pain	1, 3 and 7 d	Mature permanent teeth	Root canal treatment, two visits both groups: sealer carried to full WL using a fitted cone and in a slow pumping action control: non-standardized GP cones and a heated plugger test: a matched TotalFill BC point (FKG Dentaire SA) and a heated plugger both groups: additional accessory cones used if the canal was broad or irregularly shaped	Composite/amalgam/IRM/crown
	Aslan T, 2021	RCT	84, 18–60 y	Test: Endoseal MTA (Maruchi) and EndoSequence BC Sealer (Brasseler) control: AH Plus (Dentsply)	Intensity of post-operative pain	6, 12, 24 and 48 h and 3, 4, 5, 6 and 7 d	First or second molar with asymptomatic irreversible pulpitis	Root canal treatment, single visit single tapered GP cone adapted to the root canal +RX to check the fit, sealer application with suitable paper point cones (WL−1mm) first paper point to apply the sealer, second to distribute and third used to remove excess sealer, single cone root canal filling	Composite
Apexification									
	El-Meligy OA, 2006	Comparative study	30, 6–12 y	Test: ProRoot MTA (Dentsply) control: CH	Clinical and radiographic	3, 6 and 12 m	Necrotic permanent teeth with immature apices	MTA: carried with amalgam carrier and condensed with pluggers, moist-cotton pellet in session 1, session 2 set was tested CH: powder mixed with saline to a stiff paste, placed into the canal to WL with an amalgam carrier	IRM
	Bonte E, 2015	RCT	30, 6–18 y	Test: ProRoot MTA (Dentsply) control: CH	Clinical and radiographic	6 and 12 m	Immature necrotic permanent incisors	Not mentioned final root canal filling with warm GP	Adhesive coronal restoration

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TABLE 5 (Continued)

Material use	First author, year published	Study design	*n* teeth, age (range/mean)	Groups & materials applied	Healing assessment methods	Follow-up period	Type of teeth and aetiology	Application of materials	Coronal restoration
Extra-radicular									
Root-end surgery									
	Chong BS, 2003	RCT	221 adults	test: MTA (Loma Linda University) control: IRM (Dentsply)	Clinical and radiographic	12 and 24 m	Permanent teeth	'materials mixed according to the manufacturer's directions'	n.a.
	Lindeboom JA, 2005	RCT	100, 17–64 y	test: MTA (Dentsply) control: IRM (Dentsply)	clinical and radiographic	1 w, 3 and 12 m	single-rooted, permanent teeth	tooth apex bevel of 10°−25°, retrograde preparation: ultrasonic 2–3-mm apical preparation, application of cements not mentioned	n.a.
	Chong BS, 2005	RCT	100, adults	test: MTA (Loma Linda University) control: IRM (Dentsply)	QoL: VAS and questionnaire	3–5, 24 and 48 h	1 affected permanent tooth per patient: single-rooted anterior teeth, 1 root of premolar teeth, or the mesio-buccal root of a maxillary molar	not mentioned	n.a.
	Christiansen R, 2009	RCT	42, 30–68 y	test: white ProRoot MTA (Dentsply) control: GP	QoL: VAS and questionnaire	1 w	infected, single-rooted, root canal treated, permanent teeth	GP: smoothing of the GP root filling was performed with a heated droplet-shaped steel instrument MTA: 3-mm deep root-end cavity prepared using diamond-coated Surgical Endo Tips (ProUltra), MTA applied with delivery gun (Dentsply)	n.a.
	Christiansen R, 2008	RCT	52, 30–77 y	test: white ProRoot MTA (Dentsply) control: GP	clinical and radiographic	1 w and 1 y	infected, single-rooted, root canal treated, permanent teeth	GP: smoothing of the GP root filling was performed with a heated droplet-shaped steel instrument MTA: 3-mm deep root-end cavity prepared using diamond-coated Surgical Endo Tips (ProUltra), MTA applied with delivery gun (Dentsply)	n.a.

TABLE 5 (Continued)

Material use	First author, year published	Study design	*n* teeth, age (range/mean)	Groups & materials applied	Healing assessment methods	Follow-up period	Type of teeth and aetiology	Application of materials	Coronal restoration
	Song M, 2012	RCT	260, age limits not well indicated (between <20 and >60 y)	test: white MTA ProRoot (Dentsply) control: Super EBA (HJ Bosworth)	clinical and radiographic	3, 6 and 12 m	root filled permanent teeth with (a)symptomatic AP	Super EBA: slowly mixed in a 4:1 powder-to-liquid ratio until putty-like consistency, inserted incrementally into the dried cavity preparations, burnished MTA: mixed in a 3:1 powder-to-water ratio using sterile water; incrementally placed into the root-end preparations	n.a.
	Kruse C, 2016	RCT	39, 40–85 y	test: white ProRoot MTA (Dentsply) control: GP	clinical and radiographic	1–6 y	infected, single-rooted, root canal treated, permanent teeth	cfr Christiansen R et al. 2009	n.a.
	Zhou W, 2017	RCT	158, no age range mentioned	test: iRoot BP RRM (Innovative BioCeramix Inc.) control: ProRoot White MTA (Dentsply)	clinical and radiographic	1 w, 3, 6 and 12 m	permanent teeth	MTA: placed incrementally iRoot BP RRM: putty was rolled into small 2–3-mm cones and delivered into the root-end cavity in increments	n.a.

Note: AP, apical periodontitis; CBCT, cone-beam CT; CEM, calcium-enriched mixture; CH, calcium hydroxide; d, day(s); DPC, direct pulp capping; EMP, enamel matrix protein; FS, ferric sulphate; GP, gutta-percha; hTCS, hydraulic tricalcium silicate; IPC, indirect pulp capping; IRM, intermediate restorative material; m, month(s); MTA, mineral trioxide aggregate; PC, Portland cement; PCO, pulp canal obliteration; PEG, poly ethylene glycol; QoL, quality of life; RCT, randomized controlled clinical trial; rhPDGF, recombinant human platelet-derived growth factor; (RM) GIC, (resin-modified) glass-ionomer cement; RX, radiograph; SH, sodium hypochlorite; SS, sterile saline; w, week(s); WL, working length; y, year(s); ZOE, zinc oxide–eugenol.

(Liu et al., 2011). Next to Biodentine and MTA (ProRoot or Angelus), CEM cement (BioniqueDent), Portland cement, NeoMTA Plus (Avalon Biomed Inc.), Endocem and Ortho- and RetroMTA (BioMTA) were applied as well. The permanent teeth were restored with RM GIC (as a base) and composite or amalgam. However, a great variety exists in the coronal restoration of primary molars post-pulpotomy: IRM, RM GIC, and composite and stainless steel crowns.

Regarding survival and clinical and radiographic outcomes, MTA (ProRoot or Angelus) performed similar to Biodentine, CEM cement, NeoMTA Plus (Avalon Biomed Inc.) and Ortho-and RetroMTA (BioMTA). However, MTA (ProRoot or Angelus) was superior to CH and ferric sulphate. Not less than six papers compared Biodentine with MTA (ProRoot/Angelus/Endocem) in primary and immature permanent teeth with a follow-up of 12–24 months (Table 5). As mentioned above, no significant difference was found between these materials, except for the discolouration due to MTA.

Apexogenesis and further root development were seen in the studies where the materials were applied in immature permanent teeth (Abuelniel et al., 2020, 2021; Nosrat et al., 2013). Two studies emphasized the importance of a calcified bridge induced by the biomaterial, calling it 'a biologic response of the pulp' and 'a protective pulp barrier' (Cardoso-Silva et al., 2011; Nosrat et al., 2013). Regarding adverse events other than tooth discolouration, for primary teeth in all groups (without significant difference between the materials applied), internal and external root resorption occurred.

Partial pulpotomy

Three articles were reviewed where this technique was applied in deeply decayed, (im)mature permanent teeth with vital pulps (Table 5). One study reported that rubber dam isolation was only possible in 55% of the cases, hence in the 45% other cases, cotton rolls and salivary ejectors were placed (Qudeimat et al., 2007). MTA (ProRoot white and grey; Ortho-and RetroMTA) was used in all of the studies, mainly in comparison with CH. The manipulation and application of the materials were described in all the included studies. A great variety existed in the coronal restoration of teeth undergoing partial pulpotomy: amalgam, composite, onlay and (pre-formed metal) crowns.

For a follow-up until at least 12 months, the clinical and radiographic outcomes were favourable and comparable between the groups. The formation of a hard tissue barrier underneath the biomaterial was considered to be a positive outcome (Özgür et al., 2017; Qudeimat et al., 2007). Failures occurred in all studies and for all groups, in an insignificant number of cases. However, in case of failure in immature teeth, further root development was impeded as root canal treatment was performed in order to preserve the teeth (Özgür et al., 2017).

Revascularization

Here, only one study was assessed, where necrotic, infected pulps in immature permanent teeth were revitalized with Biodentine in one group and white MTA (Angelus) in the other (Aly et al., 2019). Until 12 months, both materials were successful clinically regarding the resolution of signs and symptoms associated with necrotic pulps. This was ascribed to their adequate coronal sealing ability. However, significantly more discolouration was seen in the MTA group. Radiographically, no difference in further root development was seen between groups.

Intra-radicular materials

The intra-radicular materials have a diverse presentation. The root canal sealers are a subtype, and for this clinical application, these materials need to have a specific flow, solubility, radiopacity and ion release to sustain the biological characteristics of the materials. The materials used for apexification have a different presentation, and materials used for intra-coronal and extra-radicular procedures are commonly used for management of immature permanent teeth.

Physical and chemical properties

For the intra-radicular use of the hydraulic cements, the physico-chemical properties reviewed included solubility, ion release, porosity, sealing ability, and flow and tubular penetration. It is important to note that the presentation of the sealer varies as they can either be mixed with water or alternatively be available in a single syringe system and the setting will occur in contact with the dentinal fluids. This changes the sealer and apical plug characteristics considerably and also makes the materials susceptible to the changes in the clinical protocol used.

Solubility

For hydraulic calcium silicate-based sealers, the solubility was reported in the range of 1.2% to 37.6% after 24 h of storage in water (Elyassi et al., 2019; Poggio et al., 2017; Prüllage et al., 2016; Siboni et al., 2017). This represents a wide scope of values, which may be difficult to correlate with the actual behaviour of these sealers. The discrepancy could be attributed to the variations in the testing conditions and designs following different guidelines (ISO 6876:2012, ADA 57, 2012). One important factor is the time between mixing the sealer and immersion in

the storage solution. In some studies, mixed sealers were immersed after 150% of their setting time (Elyassi et al., 2019; Siboni et al., 2017), compared with 300% of the setting time (Poggio et al., 2017), whilst others used a set duration of 24 h (Prüllage et al., 2016; Zhou et al., 2013) or 48 h (Urban et al., 2018) after mixing. It is noticeable that the reported solubility values were inversely related to the duration between mixing and immersion.

Ion release

A pH level of 10.9–12.1 was reached after ageing hydraulic calcium silicate sealers in water for 24 h, dropping down to 8.7 after 4 weeks (Lee et al., 2017; de Miranda Candeiro et al., 2012; Siboni et al., 2017). A higher pH of 11.0 was reported when BioRoot RCS was aged in Hank's balanced salt solution (Khalil et al., 2016). The release of calcium and hydroxyl ions was found to be significantly higher in hydraulic calcium silicate sealers in comparison with epoxy resin-based sealers (Lee et al., 2017; de Miranda Candeiro et al., 2012; Siboni et al., 2017).

Porosity and sealing ability

Using micro-computed tomography (micro-CT), the initial total porosity of hydraulic calcium silicate sealers was reported in the range 4.5%–5.5% (Guerrero et al., 2018; Milanovic et al., 2020; Viapiana et al., 2016). Using the water sorption method, the reported porosity was around 50% compared with 3% detected in epoxy resin-based sealers (Siboni et al., 2017). Higher percentages of interfacial gaps were also reported with hydraulic calcium silicate sealers (Al-Haddad et al., 2015; Arikatla et al., 2018; Chen et al., 2017). However, the sealing ability of hydraulic calcium silicate sealers was found comparable to epoxy resin-based sealers (Huang et al., 2018a; Park et al., 2020; Viapiana et al., 2016).

Flow and tubular penetration

Hydraulic calcium silicate-based sealers were reported to have comparable flowability to epoxy resin-based sealers, but the film thickness was higher in the former (Khalil et al., 2016; Lee et al., 2017; Zhou et al., 2013). No significant differences were also reported in the penetration depth of these sealers within the dentinal tubules (De Bem et al., 2020; Eid et al., 2021; Gunes et al., 2019; Reynolds et al., 2020; Wang et al., 2018). Other studies, however, reported conflicting results of lower (Chen et al., 2017; Kim et al., 2019; Yang et al., 2021) or higher (Akcay et al., 2016; Türker et al., 2018) penetration of hydraulic cement sealers in comparison with epoxy resin-based sealers. However, no correlation was reported between the depth of sealer penetration and the adaptation or bonding to dentine (Tedesco et al., 2019). A very important factor that may affect sealer penetration is the anatomical variations of the dentinal tubules amongst different teeth, or even locations, which may confound the results obtained (Reynolds et al., 2020).

Biological characteristics

Cellular studies

Root canal treatment. Differences in biocompatibility and bioactivity were observed between fresh and set material extracts in the viability of human periodontal ligament cells (HPDLCs; Oh et al., 2020). For material extracts obtained from freshly prepared and unset cements, CeraSeal (MetaBiomed) and EndoSeal TCS (MARUCHI) induced initially similar cell viability as the culture medium control group at 1 and 3 days, whereas at Day 7, cell viability of CeraSeal significantly increased compared with control and EndoSeal TCS (Oh et al., 2020). For set material extracts collected from cements which were incubated for 48 h to allow complete setting, cell viability of HPDLCs was not significantly different between materials over all periods (Oh et al., 2020). SEM imaging showed well-adhered HDPLCs on the surface of the both materials (Oh et al., 2020). Both sealers had statistically similar levels of pro-inflammatory cytokines IL-6 and IL-8 compared with the control group, confirming the biocompatibility towards HPDLCs (Oh et al., 2020). For the anti-inflammatory cytokine TGF-β, the expression level from sealers in the fresh extracts was significantly lower than that of the control; whilst for the set group, CeraSeal remained at statistically similar levels to the control and EndoSeal TCS was significantly lower (Oh et al., 2020). Regarding osteogenic differentiation, CeraSeal and EndoSeal TCS had similar ALP and ARS staining intensity to that of the positive control in which cells were cultured in osteoinduction media, suggesting the mineralization potential of both sealers. In another study, HPDLCs were also exposed to CeraSeal, EndoSeal MTA (MARUCHI) and EndoSequence BC Sealer extracts, cell migration and numerous flattened cells with prolongations adhering onto CeraSeal and EndoSequence BC Sealer surfaces were observed, whereas only few and rounded cells were detected on the surface of Endoseal MTA (López-García et al., 2020). After 21 days of culturing, a higher mineralization capacity was observed in EndoSequence BC Sealer group compared with CeraSeal, whilst Endoseal MTA induced no mineralization (López-García et al., 2020).

The biocompatibility and bioactivity of EndoSequence BC Sealer and ProRoot ES (Dentsply) were tested using a murine osteoblast precursor cell line (IDG-SW3) (Giacomino et al., 2019). Both sealer extracts influenced the osteoblast survival in a concentration-dependent

manner (Giacomino et al., 2019). Moreover, they promoted osteoblastic differentiation via the increase in DMP-1 expression, robust up-regulation of osteogenic marker gene expression ALP and superior mineral deposition (Giacomino et al., 2019).

For RAW 264.7 cells (a mouse macrophage cell line), freshly prepared ProRoot MTA and iRoot SP Injectable Root Canal Sealer (iRoot SP, Innovative BioCeramix) were associated with cytotoxicity in a dose-dependent manner, and iRoot SP was more cytotoxic than ProRoot MTA (Tu et al., 2019). RAW 264.7 cells treated with 300 mg/ml ProRoot MTA and iRoot SP had enhanced cell growth from Days 1 to 3, which then declined from Days 4 to 7, indicating an accumulated toxicity of both cements (Tu et al., 2019). Both materials inhibited RANKL/RANK signalling, thus suppressed the osteoclastogenesis of RAW 264.7 cells (Tu et al., 2019). At a high concentration of 1250–2500 μg/ml, both materials upregulated the expression of pro-inflammatory cytokines including tumour necrosis factor (TNF)-a, interleukin 6 (IL-6) and cyclooxygenase-2 (COX-2; Tu et al., 2019).

ProRoot MTA, RetroMTA and Biodentine all promoted the proliferation of stem cells from the apical papilla (SCAPs), significant differences was found on 7 and 14 days when compared to the control group (Wongwatanasanti et al., 2018). All three materials induced cell odontogenic/osteogenic differentiation, amongst which Biodentine generated the highest expressions of DMP-1, DSPP and extracellular phosphoglycoprotein (MEPE) on Day 21 (Wongwatanasanti et al., 2018).

Apexification. In culture with SCAPs, iRoot Fast Set root repair material extract (iRoot FS, Innovative BioCeramix) generated no significant effect on cell proliferation rate as similar results were observed with ProRoot MTA or the culture medium control group (Liu et al., 2020). iRoot FS promoted cell migration at a higher extent compared with ProRoot MTA, and the expression levels of osteo/odontogenic markers and mineralization nodule formation were significantly elevated this via the activation of Wnt/b-catenin signalling (Liu et al., 2020).

Animal studies

Apexification. A study assessed the responses of immature sheep teeth with open apices (apex diameter >1 mm) to a commonly used vitalization protocol containing ProRoot MTA as a barrier layer (Altaii et al., 2017). Infected root canal system was mimicked by the introduction of supragingival plaque scaled from the sheep's own teeth in the mechanically exposed pulp chambers (Altaii et al., 2017). This resulted in apical periodontitis and/or canal infection. Increases in root length (4%–23%) and dentine thickness (26%–53%) as well as a reduction in the apical diameters (38%–72%) were observed post-operatively after 6 months (Altaii et al., 2017). Histological analysis revealed the presence of vital tissues in the canal system, and mineralized tissue was formed on dentinal walls (Altaii et al., 2017). In the coronal aspect of the teeth, a row of stellate or spindle-shaped cells were attached to the dentinal wall next to ProRoot MTA plug and small blood vessels were seen (Altaii et al., 2017). More differentiated hard tissue with a mosaic-like matrix was noted underneath, and the canal system was filled with loose fibrovascular tissue (Altaii et al., 2017). The canal walls were covered by mature hard tissues with either one layer of cementum-like tissue or two layers of tubular dentine-like and cementum-like tissues (Altaii et al., 2017). The apical regions were well-developed with a narrow apical foramen (Altaii et al., 2017).

Antimicrobial characteristics

Only sealers were tested for antimicrobial characteristics. Materials used for apexification procedures are usually the same as the ones used for pulp capping and root-end surgery. The microbial flora in the root canal is different to that in close proximity to the dental pulp. Regardless of this, the materials used for apical plugs have not been assessed for their antimicrobial characteristics.

The hydraulic cement sealers tested ranged from ones mixed with water such as BioRoot (Type 4 materials) to ones provided in single-phase syringes that do not require any mixing and set when in contact with the environmental liquids classified as either Type 3 or 5 depending on their cement base (Camilleri, 2020). The testing undertaken was quite complex with more than one microbiological test used to assess the sealers and a range of bacterial also employed as indicated in Table 4. A degree of characterization was undertaken, and a number of other tests were used to elucidate the material characteristics.

Totalfill was shown to eliminate *E. faecalis* both in planktonic forms and as biofilm and also *C. albicans* (Zordan-Bronzel et al., 2019). The leachate of Endosequence BC sealer exhibited a weak antibacterial effect on all bacteria whilst Endoseal sealer showed antibacterial activity against not only the Gram-negative bacteria, but also against the Gram-positive bacteria, *E. faecalis*. The conventional root canal sealers tested (AH Plus, Tubliseal and Sealapex) exhibited strong antibacterial activity against the Gram-negative bacteria, *P. endodontalis* and *P. gingivalis* (Shin et al., 2018). The addition of drug-silica co-assembled particles enhanced the antimicrobial activity of Endosequence BC sealer over a 30-day period of testing when compared to modified AH Plus, which lost its enhanced properties after 24 h (Marashdeh et al., 2021). The modification of the latter also negatively affected its flow.

The sealers were also tested in clinically valid applications with the interaction of the final irrigating solution assessed. The use of chlorhexidine as a final irrigating solution enhanced BioRoot activity, but this was less than when using a zinc oxide–eugenol-based sealer. However, chlorhexidine retarded the setting time and surface roughness and rendered BioRoot more hydrophobic (Kapralos et al., 2021). On the contrary, the use of phosphate-buffered saline as the final irrigating solution reduced the antimicrobial action of BioRoot and AH Plus with AH Plus being more negatively affected (Arias-Moliz & Camilleri, 2016). The use of EDTA as the final rinse was shown not to be necessary as tubule penetration and antimicrobial action with the elimination of the intratubular bacteria was possible with BioRoot but not with AH Plus. Totalfill BC sealer had lower antimicrobial activity than BioRoot (Zancan et al., 2021). This may be related to the length of time that the material takes to set since it requires environment moisture for the setting that may delay the antimicrobial activity unlike materials such as BioRoot that are mixed with water.

Clinical application

The clinical application of these materials in the intraradicular region of teeth comprises filling the entire root canal after pulpectomy. As mentioned in Figure 1d publications regarding root canal treatment and apexification have been reviewed.

Root canal treatment

Four RCTs were reviewed where hydraulic calcium silicate sealers were compared with an epoxy resin-based sealer (AH Plus, Dentsply) regarding the intensity of pain during the early post-operative stage (6 h to 7 days) in root canal (re)treated mature permanent teeth. The test materials were Total Fill BC sealer (FKG), iRoot SP (Innovative BioCeramix Inc.), Endoseal MTA (Maruchi) and EndoSequence BC Sealer (Brasseler). The pulpal and periapical status, number of visits, root canal filling technique and coronal restoration differed amongst the studies (Table 5). No adverse events nor significant difference in post-operative pain between epoxy resin- and bioceramic sealer were reported. However, the data regarding analgesic intake post-operative were inconsistent. Atav Ates et al. (2019) reported that iRoot SP sealer was associated with less analgesic intake compared with AH Plus sealer. Nevertheless, Aslan and Dönmez (2021) reported no difference in analgesic intake between Endoseal MTA, EndoSequence BC Sealer and AH Plus. The coronal seal was case-dependent and varied between composite, amalgam, IRM or crown.

Apexification

Two studies were reviewed comparing MTA to CH apexification in immature permanent teeth with necrotic pulps for 12 months (Bonte et al., 2015; El-Meligy & Avery, 2006a). In both studies, an amalgam carrier was used to place the materials inside the cavity. In one study, the entire root canals were filled with the biomaterials (El-Meligy & Avery, 2006b) in the other only an apical plug was placed and the rest of the root canals were filled with warm gutta-percha (Bonte et al., 2015). The coronal seal was different: IRM or adhesive. At 12 months, in both studies, MTA was clinically and radiographically superior to CH and the failures occurred only in the CH group. More specifically, in Bonte et al. (2015), nearly 30% of the incisors treated with CH were associated with cervical root fracture.

Extra-radicular materials

Physical and chemical properties

These materials are the same as those used intra-coronally with a number of formulations developed for root repair specifically. These materials are presented in a single syringe and are called pre-mixed, such as Endosequence RRM supplied in paste or putty consistency. The setting time of Endosequence was investigated in two studies (Guo et al., 2016; Zamparini et al., 2019) with an evident difference in reported values. The initial and final setting times reported by Guo et al. (2016) were 62 and 208 min respectively. A longer initial and final setting times were reported by Zamparini et al. (2019) for the same putty material, which were 20 and 27 h respectively. They also reported 22.5 and 51 h initial and final setting times, respectively, for the paste consistency of Endosequence RRM. They attributed this difference to the variation in the ageing conditions by storing the samples in 95% relative humidity rather than immersion in water as Guo et al. (2016) reported. Endosequence was reported to have comparable calcium ion release to Biodentine (Talabani et al., 2020) with evident drop of more than 50% after 4-week ageing. The change in pH and hydroxyl ion release was also comparable to other hydraulic calcium silicate cements. The pH values of the paste and putty forms showed a major drop after 2 weeks of ageing but maintained alkaline conditions up to 4 weeks (Zamparini et al., 2019).

Biological characteristics

Cellular studies

Root-end filling. Apical papilla cells incubated in the presence of eluates extracted from set ProRoot MTA and

PulpGuard (COLTENE) showed similar viability as that of the culture medium control group; in contrast, undiluted Biodentine eluates induced a significant reduction in cellular viability, indicating a cytotoxicity (Sequeira et al., 2018). The wound-healing assay revealed that eluates from ProRoot MTA and PulpGuard allowed for unhindered cellular migration and proliferation (Sequeira et al., 2018). Cells in direct contact with all three materials were all well individualized, flattened and spindle-like in shape, with multiple prolongations (Sequeira et al., 2018).

In MC3T3-E1 cells (osteoblast cell line) which were cultured in ProRoot MTA or iRoot BP Plus extracts, the percentages of early apoptotic, late apoptotic and dead MC3T3-E1 cells were significantly higher than those in the control culture medium group, whilst iRoot FS was similar as the control group (Lv et al., 2017). Moreover, the survival rate of the cells treated with iRoot FS was significantly higher than those of the cells treated with the two other materials (Lv et al., 2017). No statistical difference in cytotoxicity was observed between ProRoot MTA and BioAggregate extracts in culture with human primary mesenchymal cells (De-Deus et al., 2009).

For human primary osteoblasts cultured with MTA Angelus or Biodentine 24-h extracts, cell metabolism, integrity and density have statistically equivalent levels to those of the control group with culture medium (Scelza et al., 2017). For 42-day cement extracts, both materials were associated with a cytotoxic effect (Scelza et al., 2017). However, the 42-day extracts represented a cumulative release of the cements without refreshing the culture medium, which may be an overestimation of the clinical situation as the diffusion of the surrounding tissues and blood vessels that refreshes the cement extracts was not taken into consideration (Scelza et al., 2017).

MTA Angelus and NeoMTA Plus extracts showed biocompatibility towards Saos-2 cells (a human osteoblast-like cell lineage) as a significant lower 24-h cell cytotoxicity was observed when compared to serum-free medium (negative control) (Tanomaru-Filho et al., 2017). After 21 days of cell exposure, ARS staining revealed both materials induced a greater production of mineralized nodules when compared to the negative control group and NeoMTA Plus produced more mineralized nodules than MTA Angelus, indicating a favourable mineralization potential (Tanomaru-Filho et al., 2017).

In another study, the effect of two calcium silicate cements EndoSequence BC RRM Putty (Brasseler) and EndoSequence BC RRM Paste (Brasseler) on cell viability of human gingival fibroblasts was also assessed (Ma et al., 2011). Cement extracts were obtained from cement discs which were allowed to set for 2 or 7 days (Ma et al., 2011). Cells in culture with EndoSequence BC RRM Paste extract obtained from 2-day set cement, presented a lower cell viability than that from an extend time of 7 days (Ma et al., 2011). SEM showed that human gingival fibroblasts attached to and spread out over the material surface overnight and increased number of cells with contacts via the processes formed a matrix-like overlay on the surface of EndoSequence BC RRM Paste and EndoSequence BC RRM Putty (Ma et al., 2011).

Animal studies

Root-end filling. Using ProRoot MTA as a root-end filling material in root-end resection of healthy dog teeth revealed the formation of soft tissue and thin layers of hard tissue at 2-, 3- and 5-week observation periods (Economides et al., 2003). New bone at the site of the resected apices was detected after 2–5 weeks (Economides et al., 2003). Similar favourable healing of the periapical tissue was also observed in beagle dogs, this for inflamed root canal system which was intentionally infected with supra-gingival plaque scaled from the dog's autologous teeth (Tawil et al., 2009). Furthermore, tooth location may significantly influence the outcome as the healing was better on posterior premolars compared with anterior premolars (Tawil et al., 2009). Using MTA Angelus as retrograde root-end filling material in premolars from mongrel dogs, a mild inflammatory infiltration in the connective tissue as well as adjacent to the material was seen after 4 months (Wlivaara et al., 2012). New hard tissues were formed in the periodontal space adjacent to material surface (Wlivaara et al., 2012). A re-establishment of the buccal cortical plate was observed, indicating bone healing potential (Wlivaara et al., 2012).

Perforation repair. Furcation perforation results in an artificial communication between the root canal system and the surrounding tissues including dentine, cementum, periodontal ligament and alveolar bone tissues (Sinai, 1977). The materials used for furcation repair are assessed with regards to the inflammatory response and the mineralization ability.

For furcation perforation from mongrel dogs which was sealed with Teflon instead of materials, numerous inflammatory cells scattering around the furcation perforation zone were detected early at 1 week (Abboud et al., 2021). Similar inflammation was observed later after 1 and 3 months (Abboud et al., 2021). The furcation defect was filled with a granulation tissue with no calcific bridge formation. The irregular silhouette of the inter-radicular bone at the granulation tissue interface was also seen besides the distorted thin discontinuous trabeculae (Abboud et al., 2021). After one month, still no hard tissue was formed, and the inter-radicular bone was clearly disfigured with reduced thickness, continuity and cellularity/vascularity (Abboud et al., 2021). Haphazard

calcifications were observed along the randomly oriented collagen fibres, and calcification was noticed around the blood vessel with dispersed inflammatory cells (Abboud et al., 2021). After three months, the furcation defect was repaired with a heavy scar tissue with no evidence of hard tissue formation (Abboud et al., 2021). The inter-radicular bone exhibited a resorption, this is regarded as a part of the remodelling process (Abboud et al., 2021). Similar moderate-to-severe inflammation reaction was confirmed in other studies using mongrel or mixed-breed dogs, this up to 3 months (Alazrag et al., 2020; Bakhtiar et al., 2017).

ProRoot MTA generated a mild-to-no inflammation, and new bone formation in mix-breed dogs after 3 months (Bakhtiar et al., 2017). A cellular and continuous cementum was seen and vascular connective tissue in periodontal ligament was the dominant morphology of connective tissue (Bakhtiar et al., 2017).

NeoMTA Plus (Avalon BioMed) generated a mild inflammatory reaction in mongrel dogs after 1 week, and the furcation was sealed with a prominent calcific tissue overlying areas of heavily deposited collagen bundles in different directions (Abboud et al., 2021). The inter-radicular bone trabeculae demonstrated a normal morphology, this in terms of thickness, outline and cellularity/vascularization (Abboud et al., 2021). After one month, no obvious inflammation was detected and the furcation defect was covered with a coherent fibrous tissue (Abboud et al., 2021). The inter-radicular bone revealed a normal morphology, and the uppermost trabeculae were restored in normal orientation and appearance (Abboud et al., 2021). A remarkable deposition of calcific tissue was noted around the blood vessels and along some collagen fibres (Abboud et al., 2021). After 3 months, the area of furcation defect was packed with a fibrous tissue, exhibiting hard tissue formation (Abboud et al., 2021). The inter-radicular bone almost regained its normal shape and the newly formed hard tissue was thought to be either a poorly cellular osteoid or osteodentine (Abboud et al., 2021).

MTA Angelus also generated initially a mild-to-moderate inflammatory reaction in premolars from mongrel dogs, and the inflammation tended to reduce over time (Abboud et al., 2021). After 1 week, a dense fibrous tissue with horizontally oriented collagen bundles was formed at the area close to furcation defect (Abboud et al., 2021). The inter-radicular bone exhibited a regular morphology with fairly calcified fibrous tissue and plump fibroblasts (Abboud et al., 2021). After 1 month, the condensed fibrous tissue was seen with variable thickness and completeness (Abboud et al., 2021). Some lacelike basophilic calcifications were also observed, and thread-like calcifications were surrounded by the material residues (Abboud et al., 2021). Three months later, hard tissue-like structure bridging the perforation defect was noticed (Abboud et al., 2021). Two other studies also reported a mild inflammatory reaction induced by MTA Angelus in premolars from mongrel dogs (Alazrag et al., 2020; Silva Neto et al., 2012), and newly formed bone was observed after 4 months (Silva Neto et al., 2012).

EndoSequence BC RRM putty induced a similar mild inflammation and a partial irregular mineralized dentine bridge (Mahmood Talabani et al., 2020). Furthermore, minimum periodontal ligament necrosis and disorganization was detected and partial calcified bone formation as an early sign of healing was detected in 25% of the specimens (Mahmood Talabani et al., 2020). After one month, no sign of inflammation was detected in majority of the specimens, and a thick, regular and complete dentine bridge was formed with a palisading layer of odontoblasts present underneath the dentine bridge (Mahmood Talabani et al., 2020). Calcified bone completely closing the exposure site was formed, and fully organized periodontal ligament with no necrosis or inflammation was observed (Mahmood Talabani et al., 2020).

Compared with MM-MTA (Micro-Mega) and EndoSequence BC RRM putty, a more intense inflammation was observed initially for Biodentine. In the first week, the pulp contained hyperaemia, numerous congested capillaries and an area of necrosis (Mahmood Talabani et al., 2020). Disorganized periodontal ligament was infiltrated by chronic inflammatory cells, and partial, incomplete bone deposition was formed (Mahmood Talabani et al., 2020). After one month, no obvious inflammation was detected, an incomplete and thin irregular dentin bridge was noticed in 75% of the specimens, and odontoblasts-like cells were also evident (Mahmood Talabani et al., 2020). The calcified bone formation remained incomplete (Mahmood Talabani et al., 2020). In premolars from mongrel dogs, Biodentine also induced a mild-to-moderate inflammatory reaction after 1 or 3 months (Alazrag et al., 2020).

Antimicrobial characteristics

MTA was developed for use as a root-end filling and perforation repair material (Torabinejad & White, 1993, 1995). The only material that seems to have been tested for antimicrobial characteristics when used extra-radicularly is MTA. It has been compared with a range of materials using a wide range of bacteria as shown in Table 4. The materials used were never characterized and no other tests were performed.

Using the direct contact test showed a delayed growth of *E. faecalis*, *S. aureus* and *P. aeruginosa* with the set MTA and IRM showing higher antimicrobial activity than amalgam, bonding agents and composite resin (Eldeniz

et al., 2006). Agar diffusion tests showed MTA to be less effective than zinc oxide–eugenol (Tanomaru-Filho et al., 2007). MTA had an antibacterial effect on some of the facultative bacteria and no effect on any of the strict anaerobic bacteria (Torabinejad et al., 1995b). The antimicrobial characteristics of the dentine in contact with MTA were also assessed and MTA led to a reduction in the number of *E. faecalis* cultured after 1 and 7 days of treatment, and this was similar to zinc oxide–eugenol-based cements with further reduction at Day 7 in MTA (Prestegaard et al., 2014). MTA prevents the adhesion and penetration of *E. faecalis* into root cementum preventing the possibility of a long-term nidus for subsequent infection (Halkai et al., 2016).

Clinical application

The clinical application of these materials in the extraradicular region of teeth comprises root-end surgery and perforation repair. As mentioned in Figure 1d only root-end surgery-related publications were reviewed as no research regarding perforation repair was included.

Eight articles were reviewed where permanent teeth of adult patients undertook root-end surgery procedures with hydraulic calcium silicate cement as test material versus IRM/gutta-percha/Super EBA as control material. The applied test materials were ProRoot (white) MTA (Dentsply) or iRoot BP RRM (Innovative BioCeramix Inc.). In 60% of the articles, single-rooted teeth were treated and the maximum follow-up period was between 1 and 6 years. The application of the biomaterials occurred mostly incrementally or by means of a delivery gun in 3-mm deep root-end cavities. The mixing and application of the biomaterials were in nearly 60% and 30% of the studies, respectively, not mentioned. Two studies assessed the patient's quality of life in the early post-operative stage (until 1 week; Chong & Pitt Ford, 2005; Christiansen et al., 2009). Chong and Pitt Ford (2005) reported no significant difference in quality of life between IRM and MTA. However, in Christiansen et al. (2009), three teeth of the gutta-percha group were re-operated due to pain and the prolongation of the operation time due to MTA placement did not significantly affect the patient's quality of life post-operatively. The six other studies evaluated clinical and radiographic success. At 12-month follow-up, favourable outcomes and no significant difference between groups were reported when MTA was compared with IRM, Super EBA or iRoot BP RRM. Gutta-percha on the contrary was reported to be inferior to MTA for a follow-up between 1 and 6 years, emphasizing the importance of placing a root-end filling after resection (Christiansen et al., 2008; Kruse et al., 2016). Root fractures occurred in all groups and were the most reported adverse events (Christiansen et al., 2008, 2009; Kruse et al., 2016; Song & Kim, 2012).

DISCUSSION

In the current review physico-chemical, antimicrobial, biological and clinical data were assessed to evaluate the present status and future directions of hydraulic cements used in Endodontics. A number of tests are undertaken, which are not targeted to any specific use of the materials.

The physico-chemical characteristics reviewed in this publication were targeted to the specific material use. Notwithstanding this, the data obtained may not have clinical relevance particularly for hydraulic cements due to their interaction with the clinical environment. Most of the physical testing undertaken follows international standards organization (ISO) guidelines specifically the ISO 6876; 2012, for root canal sealers. The ISO 4049; 2019, and ISO 9917-1; 2007, are also used for various tests in the absence of specific standards to test pulp capping and root-end filling materials. This results in non-conformity specifically due to the use of water as immersant which particularly affects the solubility testing where the change in immersing solution results in very diverse results for solubility of the same materials (Kebudi Benezra et al., 2017). Furthermore, the ISO testing is very basic as its scope is to compare materials for quality assurance purposes rather than to be used as a research tool.

Biocompatibility refers to the ability of a material to perform its desired function without eliciting any undesirable local or systemic effects in the recipient (Williams, 2008). Materials used in Endodontics should be biocompatible, and it is particularly necessary for pulp-capping agents, root-perforation repairing materials and root-end filling materials, which are placed in direct contact with the human tissues. For biocompatibility and bioactivity of calcium silicate cements, primary cell cultures or established cell lines have been used. The cellular effects for instance viability, proliferation, migration and odontoblastic differentiation are dose- and time-dependent (Collado-González et al., 2017; Giacomino et al., 2019; Tu et al., 2019; Zhu et al., 2014). The inconsistency in the results obtained from different studies (Kang, 2020; Manaspon et al., 2021; Youssef et al., 2019) should be attributed to different methodologies applied. In general, three main approaches have been used, the cells were either cultured directly or indirectly with material discs, or with material extracts obtained from freshly prepared or set cements. For studies using cement extracts, set materials were mostly prepared for extraction, which may represent the cellular responses at a later stage, studies using

cement extracts from freshly mixed and unset materials may mimic the initial responses. Differences in cellular responses were observed amongst fresh and set materials (Chung et al., 2016; Oh et al., 2020). A higher viability in 3T3 fibroblasts cultured with cement extracts from 24-h set MTA Angelus, NeoMTA Plus and Biodentine cements was observed than that from the fresh cement (Pinheiro et al., 2018). In some studies, the ratio of the surface area of the cement disc to the volume of cement extract was set to 150–600 mm^2/ml (Collado-González et al., 2017; Manaspon et al., 2021; Omidi et al., 2020; Paula et al., 2019; Tomás-Catalá et al., 2018), this in accordance with the guidelines of the ISO standard 10993-12, whilst in other studies the ratio of the surface area of the cement disc to the volume of cement extract was either not mentioned or varied from 1.96 to 62.8 mm^2/ml (Kang, 2020; Kim et al., 2015a; Park et al., 2014; Pérard et al., 2013; Pinheiro et al., 2018; Rodrigues et al., 2017; Zhang et al., 2013; Zhu et al., 2014). In addition, the clinical diffusion of the surrounding tissues and blood vessels that refreshes the cement extracts should be taken into consideration when preparing cement extracts. Previously, the cytotoxicity of MTA Angelus and Biodentine was tested using 24-h and 42-day cement extracts in culture with human primary osteoblasts (Scelza et al., 2017). Not surprising, both materials had a cytotoxic effect for the 42-day extracts, whilst 24-h extracts revealed biocompatibile (Scelza et al., 2017), as the 42-day extracts represented a cumulative release of the cements without refreshing the culture medium, which may be an overestimation of the clinical situation. Other factors for instance the extraction time, the filtering of the extracts and pH adjustment of the extract may also influence the results. Therefore, particular attention should be paid to interpret the results of studies with different methodologies.

Moreover, most cellular studies used monolayer cell culture model which had the limitation as for instance the cell-to-cell and cell-to-extracellular matrix interaction were not taken into consideration.

The most commonly used a *in vivo* laboratory animal models are rats (Amin & Montaser, 2021; Guerrero-Gironés et al., 2021; Hinata et al., 2017; Lin et al., 2017; Mahmood Talabani et al., 2020; Mondelli et al., 2019; Santos et al., 2021; Trongkij et al., 2019), dogs (Abboud et al., 2021; Alazrag et al., 2020; Bakhtiar et al., 2017; De Rossi et al., 2014; El-Zekrid et al., 2019), miniature pigs (Li et al., 2018; Pedano et al., 2020) and monkeys (Pitt Ford et al., 1996). Noteworthy is that the biological reactions of animal models are not necessarily predictive of the clinic outcomes (Moretton et al., 2000; Nyborg, 1955; Watts & Paterson, 1981). This is due to differences in anatomical, physiological and pathological characteristics between animal models and human tissues (Watts & Paterson, 1981). The biological reactions of the pulp tissues in rats are similar as those of human pulps, whilst with a faster metabolism (Moretton et al., 2000), which reduces the observation periods. The dentition of non-rodent mammals such as dogs, miniature pigs and monkeys resemble that of humans in terms of size and morphology, which facilitates the treatment procedures and the histology evaluation. In spite of the often limited clinical significance of scientific data obtained, it is important to point out that the results of these laboratory animal studies, if evaluated attentively and judiciously, may still contribute to the understanding of the data observed in clinical studies.

An innovative vital human tooth model has been developed to assess pulp-capping effectiveness as well (Téclès et al., 2008). This pre-clinical laboratory model, if effective, would be a good alternative for animal research and can so serve as basis for clinical trials in a next phase.

Intra-coronal materials

The setting reaction of hydraulic calcium silicate cements is initiated when the solid phases of the cement are exposed to water. It transforms the freshly mixed paste from a freely flowing plastic consistency into a set material that crumbles under pressure (Beaudoin & Odler, 2019). This may take place by adding water to the powder directly before application, or when the solid phase carried in a viscous vehicle is exposed to physiological fluids *in situ*. Depending on the degree of hardening, the setting time is described as initial or final (Ha et al., 2017). Any manipulation after the initial setting should be avoided until the cement is fully hardened. The initial and final setting time are arbitrary points, and bear no clinical relevance nor do they correspond to specific stages of the setting reaction. Biodentine exhibited the shortest initial and final setting times, whilst MTA ProRoot had the longest. This variation is attributed to the difference in formulation and composition. Biodentine and MTA Angelus contain no calcium sulphate, which is known to work as a setting retarder (Berger, 1929). Additionally, the calcium chloride in Biodentine's liquid acts as an accelerator that shortens the setting time further (Bortoluzzi et al., 2009). The setting reaction and resultant physical properties of Biodentine are fine-tuned further by the use of additives such as calcium carbonate which acts as a nucleating agent and the superplasticizing admixture that reduces the water/cement ratio, and this in turn leads to better material physical and mechanical properties (Grech et al., 2013). Clinically, prolonged setting time, especially in perforation repairs, may ease the washout of the cement risking the quality of the seal whilst the material is setting. For

dentine replacement materials, the extended setting time results in a limited use of the materials as the restoration of the tooth would not be possible on the same visit and layering of different materials has been shown to lead to deterioration of the hydraulic cement at the material interface (Camilleri, 2011).

Solubility values reported from laboratory tests may not reflect the actual behaviour of materials *in situ*, even under standardized conditions. Using water as an ageing medium may overestimate solubility (Ha et al., 2017), whilst physiological solutions could induce mineral deposition and increase rather than decrease in the mass (Grech et al., 2013; Kaup et al., 2015; Torres et al., 2018) leading to different values for solubility of the same material when tested using different immersant solutions (Kebudi Benezra et al., 2017). Therefore, careful interpretation is essential to perceive how these cements perform clinically. The solubility of the cements is strongly related to its composition, porosity and water dynamics that determine the elution of water-soluble components of the cement and the amounts of ions released (Atmeh & Watson, 2021; Kaup et al., 2015). Hence, this dictates the nature and extent of interaction between the cement and surrounding environment as a result of ion exchange (Huang et al., 2018b).

With the production of calcium hydroxide by the hydrating cement, both hydroxyl and calcium ions are dispersed through the water-filled pore system in the cement and released to the outer environment (Atmeh & Watson, 2021; Kjellsen & Justnes, 2004; Taylor, 1997). The hydroxyl ion is responsible for the alkaline nature of hydraulic calcium silicate material producing high pH levels that can reach up to 12.9 (Lee et al., 2017). Calcium ion is associated with the formation and deposition of calcium minerals, such as hydroxyapatite, depending on the surrounding environment (Koutroulis et al., 2019b). This is what characterizes the hydraulic calcium silicate cements compared with other materials by providing prolonged release of these ions. This ion release drops with ageing and is due to the progressive reduction in the hydration rate and porosity of the cement. This drop may reflect on the biological properties and performance of the cement as the highest level of activity is expected to be within the first few days of application (Beaudoin & Odler, 2019).

With a relatively longer setting time, and a sensitive powder to water ratio, hydraulic calcium silicate-based cements have been associated with challenges related to their handling (von Arx, 2011; Parirokh & Torabinejad, 2010). To ease their handling, different application systems have been introduced, such as the Micro Apical Placement System (MAP System; PD Dentaire; Dentsply-Maillefer), MTA applicator (Angelus Indústria de Produtos Odontológicos) or Lee's MTA pellet-forming (G. Hartzell & Son). However, the inconsistency of the mixed pastes remains a contributing factor to the difficult handling due to the uncontrolled proportions between the liquid and powder (Koutroulis et al., 2019). Therefore, later formulations are based on pre-proportioned containers of liquid and powder or pre-mixed formats, whether as a packable putty or injectable paste. Although Biodentine is supplied in capsules, it is still reported to be inconsistent. This has been attributed to the clinicians not following the manufacturer's recommendations precisely as small changes in the powder-to-liquid ratio and using an automatic mixer which is not at the correct speed can lead to changes in the material microstructure and physical and chemical properties (Domingos Pires et al., 2021).

Despite their merits, hydraulic calcium silicate cements have a bad reputation for discolouring teeth. This was noticed with the MTA formulas in which bismuth oxide was the radiopacifier used (Vallés et al., 2013). In recent years, other radiopacifier agents have been used, such as zirconium or tantalum oxides used in Biodentine and Endosequence respectively. These cements were found to maintain colour stability in comparison with hydraulic cements containing bismuth oxide (Alsubait et al., 2017; Keskin et al., 2015; Vallés et al., 2013, 2015). Although the discoloration was explained by the formation of metallic bismuth (Vallés et al., 2013), some findings are still debating the direct relation of bismuth oxide and discoloration (Dettwiler et al., 2016; Keskin et al., 2015), with a suggestion that involves other substrates, such as collagen to mediate this process *in situ* (Marciano et al., 2014). A correlation was also made between exposure to light, heat and anaerobic conditions, which would encourage further discolouration due to bismuth oxide dissociation (Kang et al., 2015b; Vallés et al., 2013). The bismuth oxide has always been assumed as inert but has been shown to be mobile when in alkaline environments (Camilleri, 2008). In contact with a number of solutions used in Endodontics, bismuth oxide undergoes a number of chemical changes, whereby it forms coloured complexes ranging from sodium bismuthate when interacting with sodium hypochlorite and bismuth subcarbonate, which is light sensitive in contact with water and saline (Camilleri et al., 2020). The discoloured bismuth complexes migrate across the tooth to material interface leading to tooth discoloration (Marciano et al., 2015). Using bovine teeth to study tooth discoloration induced by hydraulic cements may amplify this effect considering the larger diameter of dentinal tubules, which may allow more penetration of the cement and fluids (Chen et al., 2020). Discoloration associated with blood contamination of the cement could be related to the incorporation of blood products during setting. This could be facilitated by the high fluid uptake and exchange when the cement is initially placed on a bleeding tissue

(Felman & Parashos., 2013; Shokouhinejad et al., 2016; Yoldaş et al., 2016).

The direct pulp-capping potential has been intensively investigated in cellular studies, and the cytocompatibility of two most commonly used materials ProRoot MTA and Biodentine has been widely accepted, this in direct/indirect contact with HDPSCs (Rodrigues et al., 2021), HDPCs (Manaspon et al., 2021), MDPC-23 (Paula et al., 2019) and 3T3 fibroblasts (Corral Nuñez et al., 2014). In one study, human dermal fibroblast was selected as the cell type which is less clinically relevant (Hirschman et al., 2012). A three-dimensional (3D) spheroid culture model consisting of two mouse cell lines MDPC-23 and Od-21 cells was used previously to investigate the direct pulp-capping potential of ProRoot MTA and Biodentine, and cytotoxicity was observed for both materials towards Od-21 cells (Pérard et al., 2013). The mechanism underlying the higher toxicity of materials in 3D cultures compared with a monolayer model needs to be further explored. The possible reasons for this cytotoxicity are the change in the intercellular junctions within the spheroids, which may influence cohesion and cell viability, and secondly the cell differentiation (Pérard et al., 2013). Inconsistences in the cytotoxicity of BioAggregate were reported in literature, Zhu et al. (2014) showed that BioAggregate extracts enhanced the cell viability of HDPCs, according to another study, and BioAggregate induced a slight decrease in the viability of HDPCs (Zhang et al., 2013). This may be due to the differences in the setting time applied for the cements; in the latter study, cements were set for 4 h before exposing to culture medium, whilst in previous study, it was 3 days. The chemical reactions that occur during material setting could influence the biological effects, and a longer setting time applied before extraction would ensure a complete hydration and further optimize the biocompatibility.

Furthermore, ProRoot MTA and Biodentine generated similar cellular responses in terms of migration and odontoblastic differentiation, the latter was confirmed by enhanced ALP activity, the formation of mineralized nodules, and the up-regulation of odontoblastic differentiation marker expressions for instance ALP, OPN, DSPP and DMP-1 (Chang et al., 2015; Machado et al., 2016; Önay et al., 2018; Paula et al., 2019; Youssef et al., 2019).

The direct pulp-capping potential of calcium silicate cements has also been intensively investigated in animal models. Most studies used mechanically exposed healthy pulps, and only in one study, the exposed pulps from Wistar rats were left opened for 48 h before pulp capping to induce inflammation (Louwakul & Lertchirakarn, 2015). Further research of capped inflamed pulps is needed to further validate the pulp-capping potential of calcium silicate cements. In exposed pulps with no pulp-capping agents or sealed with a sterile piece of Teflon tape, an initial mild-to-moderate inflammation was observed (Lin et al., 2017; Trongkij et al., 2019). Considering the fact that sterile Teflon tape is inert, this inflammation may be attributed to the trauma induced by surgical procedures. For most calcium silicate cements, an initial inflammatory reaction with the presence of superficial pulp necrosis was mostly observed in exposed pulps from rats, dogs as well as minipigs; and this inflammation decreased over time (Asgary et al., 2008; Danesh et al., 2012; Dominguez et al., 2003; Li et al., 2018; Lin et al., 2017; Moazzami et al., 2014; Pedano et al., 2020; Queiroz et al., 2005; Tran et al., 2012; Trongkij et al., 2019). Apart from the trauma caused by mechanical pulp exposure, this initial inflammation may also be attributed to the high alkalinity of the materials upon setting.

Rat pulps represented a self-repairing capacity as reparative dentine bridge was formed in the absence of pulp-capping agent (Lin et al., 2017; Trongkij et al., 2019). In exposed pulps from rats, dogs and minipigs, ProRoot MTA generated the formation of reparative dentine; initially, the reparative dentine was incomplete and irregular; whilst at a later stage, it showed the morphology of tubular dentine, and the majority of the dentine bridge completely sealed the injury site and a well-organized odontoblast cell layer odontoblastic layer could be detected (Asgary et al., 2008; Danesh et al., 2012; Faraco & Holland, 2004; Guerrero-Gironés et al., 2021; Li et al., 2018; Lin et al., 2017; Queiroz et al., 2005; Trongkij et al., 2019). Similar reparative dentine formation was reported in exposed pulps capped with Biodentine, this also for rats, dogs and minipigs (Amin & Montaser, 2021; Pedano et al., 2020; Tran et al., 2012).

The materials tested most frequently for pulp capping are MTA and Biodentine. When antimicrobial characteristics are evaluated, there is usually no clear distinction as to what the clinical procedure specifically is. The antimicrobial activity of these materials placed in closed proximity to the pulp is important as is their interaction with the dentine. The testing undertaken used a range of bacteria with some not being so relevant for the clinical application particularly the enterococci, staphylococci and the pseudomonas. The agar diffusion method showed contrasting data, which could be due to the leaching activity as the test depends on the leaching in agar. Specifically, the agar diffusion method is not very relevant for testing hydraulic cements since the materials elute calcium hydroxide which give a halo that cannot be distinguished from the haloes produced as an integral part of the test. The direct contact test showed clearly that the hydraulic cements were less antimicrobial than calcium hydroxide and other pulpotomy agents, and although the live/dead assay was

not a very popular test, it also showed inadequate antimicrobial activity (Farrugia et al., 2018; Yang et al., 2014). The antimicrobial activity of hydraulic cements needs to be enhanced by antimicrobial agents such as quaternary ammonium salts (Yang et al., 2014) or cetrimide and chlorhexidine (Deveci et al., 2019). Thus, the current materials available clinically for use as pulp-capping agents do not seem to be effective antimicrobially indicating that further material development is necessary together with better methods for assessing the materials. Such methods of testing must include dentine as substrate due to the specific interaction for these materials with the dentine. The antimicrobial activity has been correlated to the calcium ion release profile for pulp-capping materials (Koutroulis et al., 2019a); thus, the sustained and tailor-made release of calcium can be a good pointer for future development of these material types leading to more predictable antimicrobial characteristics.

Regenerated dentine has a greater ability to protect the pulp against bacterial and physico-chemical insults than does any restorative or reparative material (Smith et al., 2000). Hence, VPT aims to preserve the pulp and the odontoblasts in an injured tooth (Duncan et al., 2019). Historically calcium hydroxide played an important role in the evolution of VPT, as it was the first material applied in direct pulp capping (Glass & Zander, 1949; Zander, 1939). In caries-free deep cavities, zinc oxide–eugenol was recommended due to its low pulpal toxicity and antibacterial properties (Murray et al., 2002). In indirect pulp capping, conventional and (the less biocompatible) resin-modified GIC were the materials of choice (Rosenberg et al., 2013). However, since the early 1990s, hydraulic calcium silicate cements have been available and they have been extensively used in VPT (Table 5). As described in the result section, the outcomes for the application of hydraulic cements in VPT in terms of clinical signs and symptoms, radiographic healing and survival are favourable and comparable or superior to previous materials. However, some limitations (mentioned here below) are present, implies future research is essential.

Eligibility criteria and study design remain important bias sources. It is known that periapical radiographs present an underestimation of the clinical reality (Goldman et al., 1972, 1974; Hashem et al., 2015). Furthermore, the carious lesion site (occlusal/interproximal), especially in primary teeth, may affect the success rate in pulp treatment (Cho et al., 2013; Kang et al., 2015a, 2017). More specifically, interproximal exposure may have an unfavourable prognosis, due to the fact that it is less accessible than an occlusal lesion, with respect to caries removal, application of biomaterials and sealing ability (Cho et al., 2013; Kang et al., 2015a, 2017). Several included articles have not taken these factors into account in their eligibility criteria (Carti et al., 2017; Elhamouly et al., 2021; Lourenço Neto et al., 2015). Additionally, the aetiological reason of the exposed pulp (caries, trauma or accidental mechanical) defines the microbiological load of the pulp. Hence, pooling several aetiological factors in one study (El-Meligy & Avery, 2006a, 2006b) or comparing the results of VPT studies with different aetiological factors may lead to false conclusions. Nevertheless, even if well-defined eligibility criteria were described in a few RCTs (Hashem et al., 2015, 2019; Kundzina et al., 2017), the exact depth of the carious lesion is generally determined during the treatment, which impedes proper randomization pre-operatively. Additionally, caries removal might be very subjective and practitioner-dependent if no additional caries detection tools and magnification are used (Macey et al., 2020; Steier et al., 2021).

Microbial leakage should be prevented during and after treatment, as it is not the role of hydraulic cements to seal the pulp during VPT. Nevertheless, not in all studies was rubber dam isolation used (Hilton et al., 2013; Koc Vural et al., 2017; Liu et al., 2011; Qudeimat et al., 2007) or the coronal restoration placed immediately (El-Meligy & Avery, 2006a, 2006b; Hashem et al., 2015, 2019; Jang et al., 2015; Katge & Patil, 2017; Leye Benoist et al., 2012; Song et al., 2015). Furthermore, no real consensus nor standardization exists regarding the coronal restoration nor the compatibility of the base liner/coronal restoration with the hydraulic cement (Table 5).

It is of utmost importance to respect the usage of the materials following the manufacturer's instructions, in order obtain the best physico-chemical properties of the applied material. Nevertheless, as described in the results section, in some of the studies, the manipulation of the materials is not described and in a few studies, the wrong attributes have been used. For instance, mixing MTA powder with sterile water and not the MTA-liquid that is provided by the manufacturer (Cuadros-Fernández et al., 2016; Doyle et al., 2010; El-Meligy & Avery, 2006a, 2006b; Jang et al., 2015; Leye Benoist et al., 2012; Song & Kim, 2012; Song et al., 2015; Tuna & Olmez, 2008). Furthermore, even if it is known amongst clinicians that the application of most biomaterials (MTA, Biodentine, CEM cement) inside a tooth is challenging, this issue is barely reported.

As regards outcome assessment, VPT is mostly performed in young children and the baseline and follow-up radiographs are generally periapical ones. Even if the ALARA principle should be respected (Farman, 2005), practitioners should keep in mind that assessment with periapical radiographs is subjective and lacks accuracy (Goldman et al., 1972, 1974; Hashem et al., 2015).

In several studies, the newly formed calcified bridge (that histologically is not dentine) underneath the material and its thickness are a measure for radiographic

success (Accorinte et al., 2009; Alsanouni & Bawazir, 2019; Cardoso-Silva et al., 2011; Holiel et al., 2021; Koc Vural et al., 2017; Leye Benoist et al., 2012; Mathur et al., 2016; Nowicka et al., 2013; Özgür et al., 2017). However, this calcified bridge is partially also due to the pulp's response to the irritation of the alkaline pH of the calcium hydroxide or hydraulic cement: Reparative hard tissue is produced after liquefaction necrosis of the pulp in direct contact with the material (Nair et al., 2008; Tronstad, 1974). Furthermore, pulp sensitivity tests reported in such deeply restored teeth are not reliable, as evoking sensibility in such teeth is difficult and might lead to false-negative results (Hashem et al., 2019; Leye Benoist et al., 2012).

Finally, one of the most reported adverse events regarding the intra-coronal application of hydraulic cements is discolouration due to MTA (Belobrov & Parashos, 2011). This has also been described in other studies and is mainly due to the interaction of bismuth oxide in ProRoot MTA with sodium hypochlorite (Camilleri, 2014) and with the collagen in the tooth structure (Marciano et al., 2014). Hence, materials containing bismuth oxide are not recommended to be used in the anterior region. Regarding pulpotomy in primary teeth: in all groups (without significant difference between the materials applied), internal and external root resorption occurred. Internal root resorption specifically could be ascribed to the osteoclastic activity of inflamed pulp, that in such cases could also not be controlled by the treatment nor the hydraulic cement applied (Celik et al., 2013). Furthermore, also pulp canal obliteration has been reported by a few studies concerning pulpotomy with biomaterials, but not as an adverse event (Alsanouni & Bawazir, 2019; Çelik et al., 2019). In fact, no real consensus exists to consider this type of excessive reparative hard tissue formation as success or failure. On the one hand, it can be considered as a sign of pulp vitality. However, on the other hand, pulp canal obliteration does not facilitate pulpectomy when required (Alsanouni & Bawazir, 2019).

Intra-radicular materials

Hydraulic root canal sealers and materials used for apical plugs are both intra-radicular materials. The latter are materials used in other clinical locations whilst the sealers. Can be either mixed with water (Type 4; Camilleri, 2020) or materials provided in a single syringe that require the environmental moisture for setting (Type 5; Camilleri, 2020). For all hydraulic cements, the environmental conditions are very important as this changes the material interactions and setting. Particularly for Type 5 materials, the presence of moisture is crucial as they may not set otherwise. Thus, for intra-radicular materials, the clinical protocol used is very important.

According to the specifications of International Organisation for Standardisation (ISO 6876:2012), the solubility of root canal sealers should not be above 3% of the initial mass after 24 h. This was achieved by most tested sealers in the hydraulic cement category. However, immersing sealers in solutions might not be the best model to simulate the intra-canal conditions. These sealers are sensitive to moisture and may exhibit high solubility if immersed before their final setting (Elyassi et al., 2019; Siboni et al., 2017). This is further complicated by the nature of the environment surrounding the sealer whilst setting. Lower solubility values were reported when the sealers were stored in phosphate-buffered saline due to calcium phosphate deposition (Prüllage et al., 2016; Urban et al., 2018). Using the same standard testing conditions but modifying the soaking solution was shown to drastically affect the solubility results (Kebudi Benezra et al., 2017).

Similar to hydraulic cements, sealers can release both calcium and hydroxyl ions after hydration. The alkaline and high calcium conditions may favour and encourage mineral deposition when exposed to the surrounding physiological environment, which may enhance the seal with time (Gandolfi et al., 2013b). These conditions may also act against the residual microorganisms that survived chemo-mechanical preparation (Arias-Moliz & Camilleri, 2016; Yoo et al., 2014); however, evidence is not yet conclusive about the superior antimicrobial properties of hydraulic sealers compared with others inside the root canals (Poggio et al., 2017; Šimundić Munitić et al., 2020; Wang et al., 2014). High pH may also neutralize the lactic acid produced by osteoclasts to prevent further dissolution of mineralized components (Lee et al., 2017).

Upon mixing, a gel phase of calcium silicate hydrate (CSH) forms on the surface of un-hydrated calcium silicate particles aggregating them together to form a porous mass with water-filled spaces in between (Atmeh & Watson, 2021). Pores vary in diameter ranging from few nanometres (gel pores) to few microns (capillary pores), or they may present as air voids if their diameter is above 10 μm (Aligizaki, 2005; Hearn et al., 2006). With an imaging resolution close to 9 μm, micro-CT might not be able to detect capillary and gel pores, which may underestimate the actual porosity (Bossa et al., 2015). Using water sorption for assessing solubility and porosity may not be accurate either. Considering the variable factors and conditions that may interfere with these measurements, such as the solution used, curing conditions and the accuracy of detecting mass changes (Kebudi Benezra et al., 2017; Siboni et al., 2017; Torres et al., 2019). As with cements, testing conditions such as the content and volume of the storage media would impact the solubility

values obtained for sealers. Storage in a phosphate-rich medium is associated with mineral deposition mediated by the ion exchange with surrounding environment This ion exchange is actually dictated by the ions leaching out of the sealer (and their concentration) and the nature of ions in the surrounding fluids (Kebudi Benezra et al., 2017).

From a clinical point of view, poor adaptation of sealers and formation of voids or open interconnected pores could provide access and refuge for microorganisms and passage for percolating fluids. However, no correlation has been found between root canal treatment failures and the type (rather than quality) of endodontic sealers used (Zavattini et al., 2020). On the contrary, the clinical relevance of sealers' penetration into the dentinal tubules remains also questionable. The depth of penetration is usually assessed using confocal laser scanning microscopy along with labelling sealers with a fluorophore. Beside the variation in methods used to quantify the penetration depth, such as measuring the maximum depth or penetration area, the staining technique can also contribute with the questionable reliability of this approach (Donnermeyer et al., 2021; Furtado et al., 2021). The use of dyes may also enhance the penetration since most dyes have an acidic pH (Camilleri & Pitt Ford, 2008).

The materials using the single component presentation that set, in contact with environmental liquids are less antimicrobial than materials mixed with water (Shin et al., 2018; Zancan et al., 2021). The antimicrobial activity can be enhanced with addition of active substances (Marashdeh et al., 2021). The irrigation protocol effects the properties of hydraulic cement sealers (Arias-Moliz & Camilleri, 2016; Kapralos et al., 2021; Zancan et al., 2021). Materials mixed with water (Type 4) are less sensitive to the irrigation protocol (Zancan et al., 2021). The use of EDTA rinse to remove the smear layer and open the dentinal tubules enhances the antimicrobial activity of BioRoot (Arias-Moliz & Camilleri, 2016) but has detrimental effects on the dentine microstructure which can be restored after application of BioRoot (Zancan et al., 2021) with the latter penetrating the dentinal tubules and having a killing effect even without the EDTA rinse. Chlorhexidine use is contraindicated as it does not enhance the antimicrobial action of BioRoot but deteriorates its physical characteristics (Kapralos et al., 2021).

Calcium silicate sealers generated cellular responses in a concentration-dependent manner (Giacomino et al., 2019; Tu et al., 2019). EndoSequence BC Sealer, CeraSeal (MetaBiomed) and ProRoot ES were cytocompatibility towards HPDLCs and IDG-SW3 (a murine osteoblast precursor cell line) and further promoted the mineralization and osteoblastic differentiation (Giacomino et al., 2019; López-García et al., 2020). ProRoot MTA, Biodentine, Retro MTA, iRoot FS all promoted the proliferation of SCAPs and further induced odontogenic/osteogenic differentiation (Liu et al., 2020; Wongwatanasanti et al., 2018), suggesting the potential to serve as a sealing material in root canal treatment or as an apical barrier in apexification. The effectiveness of ProRoot MTA as an apical barrier for revitalization has been confirmed in immature sheep teeth with open apices, increases in root length and dentine thickness as well as a reduction in the apical diameters with a narrow apical foramen were observed post-operatively after 6 months (Altaii et al., 2017). Due to the limited amount of studies available in current literature, the effectiveness of other calcium silicate cements as intra-radicular materials remains unclear.

Regarding the clinical articles reviewed, mainly the intensity of pain post-root canal treatment was assessed. Even if no difference was found between the different sealers, it is unlikely to be the case that only the type of sealer could have influenced this outcome. Patient- and treatment-related factors could have had an impact as well. In two studies (with iRoot SP and Totalfill BC; Graunaite et al., 2018; Tan et al., 2021), the warm vertical condensation technique was used, and in one study (with iRoot SP; Atav Ates et al., 2019), the obturator was heated beforehand (Table 5). Hence, the heat and pressure could have induced post-filling pain in the periapical region. Furthermore, practitioners should pay attention to the filling technique, as not every hydraulic cement sealer is resistant to heat. On the one hand, Totalfill BC sealer was reported to perform well at high temperatures (Hadis & Camilleri, 2020), but on the other hand, for iRoot SP sealer exposure to heat led to a significant reduction in setting time and flow (Qu et al., 2016). Water-based Type 4 sealers should not be heated as the raise in temperature results in significant changes in their properties (Camilleri, 2015).

The findings of the included studies for apexification were in accordance with the generally accepted shift in this treatment modality: There is enough evidence to support MTA apexification and too little evidence to support calcium hydroxide apexification (Bonte et al., 2015; Ree & Schwartz, 2017). Nevertheless, no consensus/standardization exists regarding the root canal filling of immature permanent teeth since significant inconsistency exists in the experimental set-up of laboratory-based studies in apexification and revitalization. Simulated immature root canals are filled with different hydraulic cements and on different depths with composite and/or different types of posts (Ali et al., 2019; Elnaghy & Elsaka, 2020; Mello et al., 2020; Seto et al., 2013). Hence, standardization in laboratory studies and high-quality clinical trials is needed to provide robust evidence to optimize the fracture resistance in treated immature permanent.

Extra-radicular materials

MTA was developed as an extra-radicular material (Torabinejad & White, 1993, 1995). Since then, a number of materials with different chemistries have been developed. The chemistry and hydration characteristics of these materials are different. MTA is a Type 1 cement; thus, it does not contain any additives and it is mixed with water. Thus, one cannot assume that a material with a different chemistry and hydration profile will be the same as MTA in practice. A distinct example of this is the use of Type 3 and 5 materials, which set in the presence of environmental moisture. Although MTA and in general hydraulic cements set in the presence of moisture, in the environment created at the root-end, there have been some concerns with failure of setting (Camilleri & Aznar Portoles, 2020) particularly for the Type 5 materials. There is also some laboratory research supporting the inability to set in the presence of tissue fluids and blood (Camilleri et al., 2013a; Nekoofar et al., 2010a, Nekoofar et al., 2010b. Furthermore, another concern with interaction of the material with blood is the reduction in antimicrobial properties (Farrugia et al., 2017) due to the formation of surface deposits that lead to the reduction in the surface pH thus reducing the killing effect of the material.

Applied as root-end filling materials, hydraulic calcium silicate cements are in direct contact with the cementum, periodontal ligament and alveolar bone tissues; however, all cellular studies used cement extracts whilst the information regarding the cellular responses in direct contact with cements is missing. ProRoot MTA, MTA Angelus, NeoMTA Plus, iRoot BP Plus and iRoot FS were biocompatible towards apical papilla cells, MC3T3-E1 cells (osteoblast cell line) or Saos-2 cells (a human osteoblast-like cell lineage; Lv et al., 2017; Sequeira et al., 2018; Tanomaru-Filho et al., 2017). Using ProRoot MTA as a root-end filling material in both healthy and inflamed dog teeth induced the new bone formation at the site of the resected apices (Economides et al., 2003; Tawil et al., 2009). MTA Angelus also generated new hard tissues in the periodontal space adjacent to material surface, indicating a bone healing (Wlivaara et al., 2012).

Animal studies assessing the application of calcium silicate cements in perforation repair showed that ProRoot MTA, NeoMTA Plus, MTA Angelus and EndoSequence RRM putty are all capable of generating new bone formation and inducing the healing of cementum and the periodontal ligament in dogs or rats (Abboud et al., 2021; Bakhtiar et al., 2017; Mahmood Talabani et al., 2020; Silva Neto et al., 2012). Even if no clinical studies regarding perforation repair were included in the current review, this does not imply that these favourable *in vivo* outcomes cannot be clinically achieved (Aminov et al., 2012).

The testing of hydraulic cements for extra-radicular applications is very limited with no microbiological assessments undertaken for perforation repair and only four papers discussing root-end filling. Only MTA was tested and showed adequate antimicrobial properties which were enhanced in the presence of dentine (Halkai et al., 2016; Prestegaard et al., 2014).

For the clinical articles assessing the application of MTA in root-end surgery, the favourable outcomes regarding periapical bone healing are in line with other studies (Tsesis et al., 2013). After all, MTA is known for its osseo- and cemento-inducing capacity (Torabinejad & Parirokh, 2010). However, it is unlikely that the patient's QoL in the early post-operative stage is importantly influenced by the type of root-end filling applied. Other factors such as pre-operative pain, lesion size and site, incision technique and operation time would probably have had a greater impact on this outcome (Von Arx et al., 2010; Del Fabbro et al., 2009). Here again, not every study reported the manipulation and application of the applied biomaterials (Chong & Pitt Ford, 2003, 2005; Lindeboom et al., 2005; Zhou et al., 2017) and no study reported the user-friendliness of these materials.

CLINICAL IMPLICATIONS

The main features of hydraulic calcium silicate cements are the formation of calcium hydroxide on hydration which leads to alkalinization of the environment with the associated implications of interaction with the clinical environment. In endodontic practice, the clinical environment varies with the intra-coronal materials being in contact with saliva when the materials are used as bulk filling materials in temporary restorations, in contact with other restorative materials and pulpal fluids. In intra-radicular applications, the various irrigating regimes, the heat applied during root canal filling also leads to an unstable environment. Extra-radicularly, the materials will be in contact with blood and tissue fluids. For all cases, there is always contact with dentine. The interaction of the material and its hydraulic nature is often not appreciated particularly since in Dentistry most of the materials are inert. Another complication to the understanding of the hydraulic cements is the role of additives making material comparison very difficult.

CONCLUSIONS

Hydraulic calcium silicate materials exhibit a wide scope of variation in relation to their physico-chemical properties. This can be attributed to the actual difference in

the chemistry and formulation of these cements, and to the variations in the testing conditions and standards used to evaluate them. Laboratory testing has its own constraints and has limited relevance to clinical performance, and further standardization is required in this context. These materials have their characteristic properties amongst other types of cements, such as the prolonged ion release which explains their instability. Colour stability is an issue in cements containing bismuth oxide. All hydraulic calcium-based cements require the addition of a radiopacifier for easy postoperative detection on radiographs.

The materials are biologiocally active as indicated by the various studies undertaken; however, the methodology used effects the results obtained clearly indicating caution with the interpretation of results and the clinical translation of the information. It is very difficult to differentiate between one material and another and making a selection based on the biological characteristics since the methodology affects the results obtained. The use of animal models also provides very little information as the reactions observed histologically have also been induced by the surgical procedures. The antimicrobial characteristics are not well investigated. The methods used are simple and with limited clinical significance. From the knowledge gained so far, the hydraulic cements do not provide any antimicrobial resistance and this is particularly challenging since they are being used in areas loaded with biofilm. Clinical protocols are not tailor-made for the materials thus the materials are being put at a disadvantage due to the poor understanding of their chemistry and environmental interactions.

Clinically, hydraulic silicate cements do perform well for all endodontic treatment modalities and are comparable or superior to conventional materials. For each specific endodontic treatment modality, the different hydraulic silicate cements applied, result in comparable (favourable) outcomes. The most prominent adverse event is discolouration due to bismuth oxide in MTA, which can be prevented by using MTA without bismuth oxide or another silicate cement for which no severe discolouration has been reported. Unfortunately, the limitations in clinical trials are mainly investigator and/or operator related: erroneous study designs, no preventive measures against microbial leakage during and after cement placement, incorrect cement manipulation and bias in outcome assessment. There is also a lack of consensus regarding intra-canal (for apexification) and coronal restoration of teeth root filled with hydraulic cements. Hence, high-quality clinical trials for and standardization in restoration of teeth treated with hydraulic cements are urgently needed.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

No ethical approval was required for this review.

AUTHOR CONTRIBUTION

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