Faculdade de Medicina da Universidade de Coimbra

Mestrado Integrado em Medicina Dentária

In Vitro Calcium Silicate-based Endodontic Sealers Antimicrobial Efficacy

A Review

Pedro Miguel da Silva Marques

Orientador: Professor Doutor Paulo J. Palma

Coorientadora: Doutora Patrícia Diogo

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Marques P[1], Diogo P[2], Palma PJ[3]

1) Aluno do Mestrado Integrado em Medicina Dentária da Faculdade de Medicina da Universidade de Coimbra

2) Assistente Convidada do Mestrado Integrado em Medicina Dentária da Faculdade de Medicina da Universidade de Coimbra

3) Professor Auxiliar Convidado do Mestrado Integrado em Medicina Dentária da Faculdade de Medicina da Universidade de Coimbra

Área da Medicina Dentária, FMUC, Coimbra, Portugal

Avenida Bissaya Barreto, Blocos de Celas

3000-075 Coimbra

Tel.: +351 239 249 151 | +351 239 249 152

Fax.: +351 239 402 910

Email do autor: pedromiguel.marques7@gmail.com

SUMMARY

I. ABSTRACT

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Introduction: The obturation of pulp canal space is performed using endodontic root canal sealers with ability to adhere core material to dentin walls and to fill irregularities, minimizing the microleakage risks. As such, root canal sealers must have antimicrobial properties that contribute to eliminate residual microorganisms. Bioceramic-based endodontic sealers (calcium silicate and calcium phosphate-based) presents high biocompatibility and bioactivity with cicatrization and new hard tissue formation. However, Calcium silicate based Endodontic Sealers (CsbES) antimicrobial efficacy is controversial.

Objectives: The present systematized review intends to evaluate CsbES antimicrobial efficacy shown in literature with the application of a new tool of risk of bias estimation at *in vitro* and *ex vivo* studies.

Methodology: A comprehensive automated search was performed in Pubmed, Cochrane, Wiley Online Library, Evidence Based Dentistry and Journal of Evidence-Based Dental Practice databases with the key-terms of indexation: "Antimicrobial Bioceramic Cement"; "Bioceramic Endodontic Sealer Efficacy"; "Calcium Silicate Sealers"; "MTA-Based Sealers"; "Hydraulic Cements"; "Root Canal Sealer"; "Calcium-silicate-based Endodontic Sealers Antimicrobial Efficacy" and "Calcium-silicate-based Sealers Cytotoxicity" limited to the period between January 1st of 2008 and April 30th, 2018. The selected studies were obtained and analysed according to the application of PRISMA flowchart and a new tool of risk of bias evaluation.

Results: Bibliographic research originated one thousand and forty-two titles and abstracts. After inclusion and exclusion criteria meticulous application, sixteen studies were examined. Endosequence BC™ (theoretically equivalent to iRoot SP®, iRoot BP®, TotalFill RRM® and TotalFill BC®) is classically compared to epoxy-resin based endodontic sealers and ProRoot MTA®. Endosequence BC™ exhibits lower antimicrobial efficacy and absence of *in vitro* cytotoxicity. However, this last parameter was only evaluated in four studies.

Conclusions: The CsbES nomenclature is diverse, however, there is a correspondence in data-sheet among the majority of CsbES components, which reveals consistency in several subtypes with commercial names differences. The major CsbES benefit relies on its biocompatibility and bioactivity with new hard tissue formation, as CsbES antimicrobial efficacy is low when compared with resin-epoxy based sealers.

Keywords: Calcium silicate sealers; bioceramic sealers; hydraulic sealers; MTA-based sealers; root canal sealer; antimicrobial efficacy; cytotoxicity

II. RESUMO

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Introdução: Na obturação do espaço canalar recorremos a cimentos endodônticos com capacidade de unir o material do núcleo à superfície dentinária e preencher as irregularidades, minimizando o risco de microinfiltração. Como tal, os cimentos devem possuir propriedades antimicrobianas que contribuam para a eliminação de microrganismos residuais. Aos cimentos biocerâmicos (à base de silicato de cálcio e de fosfato de cálcio) é lhes atribuída uma elevada biocompatibilidade e bioatividade, acompanhada de cicatrização com formação de novo tecido duro. Contudo, a eficácia antimicrobiana dos mesmos é discutível.

Objetivos: A presente revisão sistematizada da literatura pretende avaliar a eficácia de cimentos endodônticos à base de silicatos de cálcio na literatura com a aplicação de uma nova ferramenta para o cálculo do risco de viés em estudos *in vitro* e *ex vivo*, aproximando-se o mais possível na forma com uma revisão sistemática de estudos *in vivo*.

Metodologia: Foi realizada uma pesquisa bibliográfica na base de dados Pubmed, Cochrane, Wiley Online Library, Evidence Based Dentistry e Journal of Evidence-Based Dental Practice com as palavras-chave: “Antimicrobial Bioceramic Cement”; “Bioceramic Endodontic Sealer Efficacy”; “Calcium Silicate Sealers”; “MTA-Based Sealers”; “Hydraulic Cements”; “Root Canal Sealer”; “Calcium-silicate-based Endodontic Sealers Antimicrobial Efficacy” e “Calcium-silicate-based Sealers Cytotoxicity” combinadas com conetores boleanos e limitada entre 1 de Janeiro de 2008 e 30 de Abril de 2018. Os estudos selecionados derivam da aplicação de fluxograma e das normas PRISMA, contudo para a análise do risco de viés foi elaborada uma ferramenta exclusiva para a sua quantificação em estudos *in vitro* e *ex vivo*.

Resultados: A pesquisa bibliográfica originou mil quarenta e dois títulos e resumos, dos quais dezasseis foram analisados criteriosamente após a minuciosa aplicação dos critérios de inclusão e exclusão. O cimento Endosequence BC™ (teoricamente equivalente ao iRoot SP®, iRoot BP®; TotalFill RRM® e TotalFill BC®) é categoricamente comparado com cimentos de resina epóxica e ao ProRoot MTA® revelando menor

capacidade antimicrobiana e ausência de citotoxicidade *in vitro*. Contudo, este último parâmetro só foi analisado em quatro estudos.

Conclusões: Apesar da divergente nomenclatura, há correspondência na folha de dados entre os componentes maioritários dos diversos tipos de cimentos endodônticos de silicato de cálcio, o que revela similaridade entre os diversos subtipos de cimentos. A maior vantagem que corrobora a forte aplicação clínica dos cimentos à base de silicato de cálcio, deriva aparentemente do potencial biológico destes materiais (biocompatibilidade e bioatividade) na indução de formação de tecidos duros e não na eficácia antimicrobiana dos mesmos.

Palavras-chave: Cimentos endodônticos de silicato de cálcio; cimentos hidráulicos, cimentos biocerâmicos, eficácia antimicrobiana; citotoxicidade.

III. ABBREVIATIONS

ABT	Antibacterial broth test
ADT	Agar Diffusion Test
ATCC	American Type Culture Collection
BbES	Bioceramic-based Endodontic Sealers
BC	Bioceramic
BMP	Bone Morphogenetic Protein
BP	Bioceramic Putty
$Ca(OH)_2$	Calcium Hydroxide
Ca^{2+}	Calcium Ions
CEM	Calcium-Enriched Mixture
CFU	Colony forming units
CLSM	Confocal Laser Scanning Microscope
CpbES	Calcium phosphate-based Endodontic Sealers
CsbES	Calcium Silicate-based Endodontic Sealers
CSH	Calcium-Silicate Hydrates
d	day
DCT	Direct Contact Test
DDT	Disc Diffusion Test
ERbs	Epoxy Resin-based sealers
FGF	Fibroblast Growth Factor
FS	Fast Setting
FS-MTA	Fast-Set MTA
h	hour
H^+	Hydrogen
H_3O^+	Hydronium ion
HPO^-	Hydrogen Phosphate ions
Int Endod J	International Endodontic Journal
JOE	Journal of Endodontics

k	Cohen’s kappa coefficient
KCTC	Korean Collection for Type Cultures
log	Logarithm
MDCT	Modified Direct Contact Test
MeSH	Medical Subject Headings
min	minute
mL	mililiter
mm	milimeter
MNT	Micronucleus Formation Test
MRT	Membrane Restricted Test
MTA	Mineral Trioxide Aggregate
MTA-F	MTA Fillapex)
MTA-S	MTA-based sealer
NEC	New Endodontic Cement
OH^-	Hydroxide
PD	Diogo P. (reviewer)
PICO	Population, Intervention, Comparation and Outcome
PM	Marques P. (reviewer)
PP	Palma P. (senior investigator)
PRISMA	Preferred Reposition Items for a Systematic reviews and Meta-Analyses
RDSC	Radiopaque Dicalcium Silicate Cement
RRM	Root Repair Material
RRP	Root Repair Putty
s	second
Sd	Standard Deviation
SEM	Scanning Electron Microscopy
SiO^-	Silicon Oxide Ion
SP	Sealer Properties
VEGF	Vascular Endothelial Growth Factor
WMTA	White ProRoot® MTA

IV. INTRODUCTION

IV. INTRODUCTION

Nowadays it is recognized the crucial role of biofilms in the development and progress of pulpal and periapical disease as also in the endodontic treatment failure[1]. Furthermore, the endodontic treatment main goal is to eradicate biofilms from the infected root canal system and avoid recontamination; to prevent and, when required, to cure apical periodontitis and maintain or re-establish periapical tissue health[2–7].

Endodontic treatment procedures comprise the establishment of an aseptic environment acquired with biomechanical root canal preparation and root canal system three-dimensional filling to resist to potential microbial ingress and a coronal restoration to prevent microbial ingress[8,9]. Nevertheless, even when all therapeutic procedures are meticulously applied, in the long term, microorganisms can eventually invade the root canal via coronal access cavity, lateral canals and dentinal tubules[10]. Once the root canal system is invaded, microorganisms can propagate through the filled canal and interact with the host's periapical tissues. This interaction results in the development of post-treatment apical periodontitis, affecting negatively the final endodontic treatment outcome[11].

Biomechanical instrumentation and antimicrobial irrigation with sodium hypochlorite solution reduce near 50% of canals microorganisms-free, however the remaining canals contain small numbers of recoverable bacteria[12]. When the biomechanical preparation is combined with an antimicrobial dressing applied to the clean canal for a suitable interval of time before root filling, microorganisms, mostly bacteria, may be consistently eliminated from the canal[13]. Nevertheless, when endodontic treatment is performed in one visit, with no inter-appointment antimicrobial dressing, residual microorganisms may be present in the canal at the time of root filling. Only two studies evaluated the effect of infection at the time of root filling on the endodontic treatment prognosis and they have shown that the success rate of endodontic treatment is approximately 10-26% lower for teeth which yield a positive culture before root canal filling than for teeth which yield a negative culture[14,15].

Further studies have not been capable to indicate significant healing differences among teeth filled with positive or negative cultures from the root canal[16], as well as between treatments executed in one or two appointments[16,17]. Nonetheless, it is largely recognized that primary apical periodontitis healing depends on effective root canal system biofilms eradication[18]. Subsequently to this, it is required to settle the root canal instrumentation and irrigation with a properly root canal sealer as the root canal filling

aim is to reduce the microbial load left within the root canal and connecting with the periradicular tissues below the threshold for clinical and radiographic success[19].

ROOT CANAL SEALERS

The root canal filling involves the use of a root canal sealer along with a solid core material, such as gutta-percha, to provide an adequate seal[20]. Gutta-percha, a polyisoprene trans-isomer, has been the most used core material in Endodontic field as a solid and inert core filling material[21] since its introduction in 1867 by Bowmans's. Although gutta-percha is not the epitome filling material for root canals, it has satisfied most of the root filling criteria as a semi-ideal material. Gutta-percha *per si* (by its lack of adhesiveness to dentin canal wall) is inadequate to fill and seal completely the root canal system as it is necessary to fulfill the irregularities and minor discrepancies among gutta-percha, the core and the root canal wall[19].

The sealer primary role is to obliterate irregularities and minor discrepancies between the root canal wall and the core material and must have cohesive strength to hold the filling material jointly[22]. An endodontic sealer must have the ability to obturate the lateral canals, act as a lubricant, have radiopacity, have microbial control and enhances the ability for an impervious seal[23].

Subsequently, several types of root canal sealers are used with each one having its own qualities and disadvantages even in the smear layer presence. Classically, smear layer removal was recommended as it supposedly meliorate directly the root canal filling features[24] and reduce coronal leakage[25,26]. Although, smear layer presence or absence have significant effect on the apical seal[27]; recently has been demonstrated that smear layer removal does not improve the root canal filling[28–30]. In 2003, Saleh *et al.* performed a scanning electron microscopy (SEM) study revealing that with several types of endodontic sealers (zinc oxide eugenol based, calcium-hydroxide based, glass-ionomer based, resin-based and silicone-based) the sealer penetration into the dentinal tubules was not directly associated with higher bond strength when smear layer was removed. The sealer micromechanical retention and penetration tags inside the tubules is not an important factor that affects the sealer adhesion as endodontic sealer tubular penetration is undoubtedly dependent on the sealer chemical and physical properties[28].

Several endodontic sealers have been used over the last fifty years[19] and are conventionally collected centered on their prime constituent or chemical structure as described in **Table I.**

Table I. Root canal sealers distribution based on prime constituent or structure and some examples excluding MTA-based sealers and calcium-silicate based sealers.

ENDODONTIC SEALER TYPE	BRAND NAME	
Zinc oxide-based	Proco-Sol, Grossman's sealer, Pulp Canal Sealer, TubliSeal; Endomethasone; Medicated Canal Sealer	
Gutta percha-based	Kloropercha, Eucapercha	
Dentin adhesive materials	Cyanoacrylates, dentin bonding agents, polycarboxylates	
Calcium hydroxide-based	SealApex; Life; Apexit, Vitapex	
Calcium phosphate-based	BioSeal, Capseal I and II	
Resin-based	Epoxy resin	AH-26/AH-26 silver free; AH-Plus; Top Seal, 2-Seal
	Diketone	EndoRez
	Methacrylate resin	**1st Generation (G):** Hydron; **2nd G:** EndoREZ, RealSeal; **3rd G:** Epiphany, Fibrefill; **4th G:** MetaSeal SE, RealSeal SE; SmartSeal
Silicone-based	RoekoSeal Automix ; Gutttaflow; Lee Endofill	
Glass ionomer-based	Ketac-Endo	

Moreover, there are former forms to classify endodontic sealers as an important case from American Dental Association (specification No.57) who allocates endodontic filling sealers in three types: type I (class 1. metallic and class 2. polymeric); type II (class 1. powder and liquid nonpolymerizing; class 2. paste and paste nonpolymerizing and class 3. polymer resin system); finally type III (class 1. powder and liquid nonpolymerizing; class 2. paste and paste nonpolymerizing; class 3. metal amalgams and class 4. polymers)[31].

Supplementary classifications might be constructed based on the addition of therapeutic compounds to sealers that supports cementogenesis and dentinogenesis (as calcium hydroxide-based) and/or suppression of post-operative pain with resilient disinfectants and antiphlogistic (paraformaldehyde-based sealers). Finally, there are also classifications based on endodontic sealers permeability and longevity. For the first, there are highly permeable sealers (calcium-phosphate and calcium-hydroxide based sealers) and moderately permeable sealers (glass-ionomer based sealers and polycarboxylate based sealers). For the last one, longevity, categorization is based on resorbable pastes (iodoform and calcium-hydroxide based sealers) and partially resorbable, the rest of endodontic sealers[23].

Root canal sealers (even if they not extruded beyond the apical foramen) are constantly in direct contact with periodontal ligament or periapical bone over extensive periods of time and may release toxic elements, irritating periapical tissues and affecting the root canal final outcome[32].

Successively, an endodontic sealer must have several biological and physical characteristics previously described by Grossman in 1976[33] labelled in **Table II.**

Table II. Classic Grossman's eleven properties listed and pre-requirements for an ideal root filling sealer[33].

	GROSSMAN PROPERTIES	DESCRIPTION
1	EASY TO HANDLE	Easely introduced into the root canal system to provide good adhesion and sheer removed from the canal (if necessary).
2	HERMETIC SEAL	To seal the canal completely (laterally and apically).
3	RADIOPAQUE	To the clinician visualize and control the obturation quality on the radiograph.
4	POWDER PARTICLES FINE	The powder particles should be impervious to moisture.
5	DIMENSIONAL STABILITY	It should not shrink upon setting.
6	NO DISCOLORATION	It must not stain/discolour the tooth.
7	BACTERICIDAL/BACTERIOSTATIC ANTIMICROBIAL/ANTIBIOFILM	It should be bacteriostatic/antibiofilm to impossiblitate or at least not encourage the microrsganisms re-growth.
8	ADEQUATE SETTING TIME	It should set slowly.
9	INSOLUBLE	INSOLUBLE in tissue fluids.
10	SOLUBLE	SOLUBLE in common solvents if it is necessary to remove the root filling.
11	BIOCOMPATIBLE NOR MUTAGENIC OR CARCINOGENIC	It must NOT IRRITATE periapical tissue, it must be TOLERATED by periapical tissue.

BIOCERAMIC-BASED ENDODONTIC SEALERS

Bioceramic-based Endodontic Sealers (BbES) are ceramic sealers based on Mineral Trioxide Aggregate (MTA) introduced in 1993 by Lee, Monsef and Torabinejad[34] and contain calcium silicate and/or calcium phosphate as their main composition[30]. Moreover, MTA is also known to interact with dentine to promote intertubular calcium and silicone incorporation[35]; intrafibrillar apatite deposition[36] and tag-like structure formation in the presence of phosphate-buffered saline[37].

BbES may be catalogued in two sub-groups, one named as Calcium silicate-based Endodontic Sealers (CsbES), and the other as Calcium phosphate-based Endodontic Sealers (CpbES), **Figure 1.** BbES are characterized with recognized biocompatibility with human tissue and are widely used in musculoskeletal surrounding tissue repair and replacement to encourage the hard and durable tissues formation and/or reparation[30].

It is important to mention that in CpbES group, calcium phosphate (PO_4) is the most important inorganic constituent of biological hard tissues. CpbES are easily soluble in acid and insoluble in alkaline conditions and the mineral phase is carbonated hydroxyapatite as well as monocalcium phosphate monohydrate, calcium phosphate monobasic, dicalcium phosphate dihydrate, dicalcium phosphate anhydrous, octacalcium phosphate, amorphous calcium phosphate, and hydroxyapatite[38].

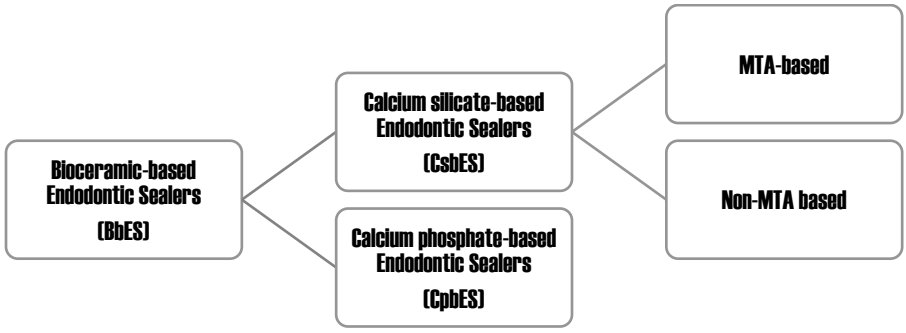

Figure 1. Bioceramic-based Endodontic Sealers distribution with focus on CsbES as CpbES are not are not subject of evaluation in this study.

CpbES further details and descriptions were not subject of evaluation in this review.

Debelian and Trope classified BbES as bioinert, bioactive, biodegradable, soluble or resorbable[39], **Table III**.

Table III. BbES classification accordingly to Debelian and Trope[39].

DEBELIAN AND TROPE BbES PROPERTIES		DESCRIPTION
1	BIOINERT	Non-interactive beside biologic systems.
2	BIOACTIVE	Durable in tissues that may undergo interfacial interactions with surrounding tissue.
3	BIODEGRADABLE, SOLUBLE OR RESORBABLE	It is replace, substitute or incorporated into host tissue.

BbES are chemical and dimensional stable (slightly expansion), non-corrosive[19]; not sensitive to moisture and blood contamination and as a consequence, are not technique sensitive as BbES are hydrophilic (high affinity for water)[32,39,40] and preform pollination through water. BbES when unset has antimicrobial properties; after set develops hard consistency allowing full compaction (with pH above 12, occurs the hydration reaction), and when fully set, BbES ensure a long-term seal, getting insoluble over time[19,39]. It is expected that in clinical practice, alkaline conditions may be lower, (pH under 12) due to dentine buffer ability to create the idyllic conditions for BbES biological effect[41] as well dentin is widely recognized as a reservoir of growth factors who reinforce the hard tissue reparation[42] accompanied by the secretion of angiogenic and osteogenic growth factors representing a prerequisite for bone regeneration, such as bone morphogenetic protein (BMP-2), vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF-2)[43].

CALCIUM-SILICATE BASED ENDODONTIC SEALERS

CsbES, **Table IV** had calcium silicate, particularly hydrated di- and tricalcium silicate (C_3S) powder as well as the major sealer component and up to 70% of Portland cement in their composition[44], also include alumina, zirconia, bioactive glass, glass ceramics, hydroxyapatite and calcium phosphates with recognized biocompatibility and bioactivity[38,43].

Table IV. CsbES classification based on chemistry accordingly to Dutta and Saunders 2014[44].

Cassification based	BRAND NAME
1ST GENERATION	Grey and White MTA
2nd GENERATION	MTA Angelus, MTA Brancos, MTA Bio
3rd GENERATION	ENDO COM, Pro-Root ENDo, iRootSP, Endosequence BC, MasrtPaste Bio, MTA Obtura, Tech Biosealer Endo; Bioaggregate, Biodentine, Tech Biosealer, Aureoseal, Ortho MTA, MTA Plus, Generax A and B; Capasio, Ceramicrete-D
4tH GENERATION	Calcium phosphate/Calcium silicate/Bismutite cement, NRC (with HEMA incorporation), MTA with 4-META/MMA-TBB Light-cured sealers as TheraCal LC

CsbES had good flow properties, particularly when delivered at a relatively rapid rate[45], biocompatible, nontoxic, non-shrinking and chemically stable within the biological environment[46–48]. The principal inconvenient of these materials relies at the difficulty of removing them from the root canal in case of retreatment or post-space preparation[49].

CsbES are hydraulic sealers as they demand the water present for setting; water setting[44,50,51] and are composed of five physicochemical phases[52] that contribute to the manifestation of *in vitro* bioactivity with the final formation of a nanoporous gel of Calcium-Silicate Hydrates phases (CSH phases), a soluble fraction of calcium hydroxide, $Ca(OH)_2$ or Portlandite and calcium aluminate hydrate phases resulting in apatite, described in **Table V.**

CsbES set in humid and wet environments, such as water and blood. When in direct contact with organic tissue fluids, CsbES release calcium hydroxide which can interact with phosphates present in tissue fluids to form hydroxyapatite (precipitation reaction) during the setting process[53], creating a bond between dentin and the root filling material[54]. Once the apatite nuclei are formed, it spontaneously grows by consuming the calcium and phosphates from physiological fluids. Calcium ions released from sealers to host tissue also accelerates apatite formation, because calcium ions increase the ionic activity product of apatite of fluids and this property is responsible for the tissue-inductive properties of CsbES sealers[19].

Table V. Resume of the five sequential stages of events that contribute to the manifestation of *in vitro* bioactivity of hydraulic CsbES after immersion in simulated body fluid.

CsbES phases		DESCRIPTION
1	HYDRATION – ions exchange	Ion exchange occurs following hydration of the calcium silicate particles, with rapid exchange of calcium ions (Ca^{2+}) with hydrogen (H^{+}) or hydronium ion (H_3O^{+}) from the aqueous mixing solution to form a solid-liquid interface. Reaction of Ca^{+} ions with hydroxide (OH^{-}) derived from water results in the formation of calcium hydroxide (portlandite) that creates a highly alkaline ecosystem.
2	Setting	Formation of calcium silicate hydrate, the main binding phase in a set sealer matrix. Cation exchange increases the hydroxyl concentration of the solution and the surfaces of the calcium silicate particles are attacked by hydroxide in solution, resulting in hydrolysis of silicon oxide ion (SiO^{-}) group in an alkaline environment.
3	Binding of calcium hydrate with calcium ions	Consists of a charged surface and an equal but opposite charge in the solution, it is called an electric double layer over which other substances may deposit under proper conditions.
4	Precipitation of Amorphous Calcium Phosphat (ACP)	When the set calcium-silicate sealer is immersed in a phosphate-containing solution of hydrolyzed hydrogen phosphate ions (HPO^{-}), electrostatic interaction occurs between the HPO^{-} and Ca^{2+} on the calcium-silicate hydrate surface.
5	Cabonated apatite formation	Nucleation and transformation of amorphous calcium phosphate into carbonated apatite. In the presence of a nucleation-inducing calcium-silicate hydrates (CSH) surface, the ACP undergoes phase transformation over time into carbonated apatite.

CsbES have been available in endodontics for the past thirty years and claimed as sealers with properties similar to MTA without its drawbacks and all CsbES have a common property to produce spontaneously apatite layer when in contact with phosphate from physiological fluids, encouraging the growth of reparative tissues [55] with high osteo-conductivity[56].

There are several branded CsbES already available on Dentistry market and others are still experimental, requiring further laboratory and clinical testing to ascertain their efficacy. Some, classified according to their major constituents, are identified in **Table A** (MTA-based) and **Table B** (Non-MTA based) (consult attachment chapter of this thesis).

V. AIM

V. AIM

This systematized literature review concerning CsbES *in vitro* studies main goal is to quantify the antimicrobial outcomes of this particular biocompatible endodontic sealers as well as its cytotoxic outcomes.

VI. MATERIALS AND METHODS

VI. MATERIALS AND METHODS

For this systematized literature review of CsbES *in vitro* and *ex vivo* studies, despite being a review centred on basic investigation, authors adapted the PICO (Population, Intervention, Comparation and Outcome) framework model for clinical questions[57] **Table VI**, and used a PRISMA approach (Preferred Reposition Items for a Systematic reviews and Meta-Analyses)[58] adapted for pre-clinical investigation.

Table VI. PICO question parameters adapted to *in vitro* and *ex vivo* studies.

PICO parameters	PICO definition with detailed description
Patient, **P**opulation or Problem	Specimens for *in vitro* and/or *ex vivo* investigation
Intervention, Prognostic factor or Exposure	CsbES antimicrobial efficacy…
Comparison or Intervention (if appropriate)	… *versus* conventional endodontic sealers
Outcome researchers would like to measure or achieve	Antimicrobial outcomes (and cytotoxic data) of CsbES

Using the National Library of Medicine (http://www.ncbi.nlm.nih.gov/PubMed), Cochrane Oral Health Group, Wiley Online Library, Evidence Based Dentistry and Journal of Evidence-Based Dental Practice data bases, a literature search was performed with a personal computer of articles published from January 2008 to April 2018 in English language.

Initially, the aim was to investigate the terms and key-words ('Antimicrobial Bioceramic Cement' OR 'Bioceramic Endodontic Sealer Efficacy') AND ('Calcium-Silicate Sealers' OR 'MTA-based sealers' OR 'Hydraulic Cements') AND ('Root Canal Sealer') AND ('Calcium-Silicate based Endodontic Sealers antimicrobial efficacy') AND ('Calcium-Silicate sealers cytotoxicity').

Briefly, authors used PubMed to identify Medical Subject Headings (MeSH) terms corresponding to each term. Though, MeSH terms use is not common to all articles, making this search method unachievable. Hand searching of reference lists of original and reviewed articles that were found to be relevant was also performed. Data sources also included contact with experts when it was considered appropriate. Two reviewers (PD and PM) screened all articles titles and abstracts retrieved from electronic search independently and in duplicate. A second review of all content was performed and in a third phase, the relevant full text of all studies was obtained, included if related to the subject of the present review and independently examined by two reviewers (PD and PM) based on the defined exclusion criteria. Disagreement regarding inclusion/exclusion of full papers among reviewers was solved via debate, although in specific cases of

disagreement an opinion from a senior investigator (PP) was required. Because of the limited number of relevant *in vitro* publications, it was decided to include both *in vitro* and *ex vivo* experimental studies of antimicrobial efficacy and cytotoxic outcomes in order to acquire the broadest possible spectrum of subject information. Entirely detailed information is described in a four-phase PRISMA flow diagram, **Figure 2.**

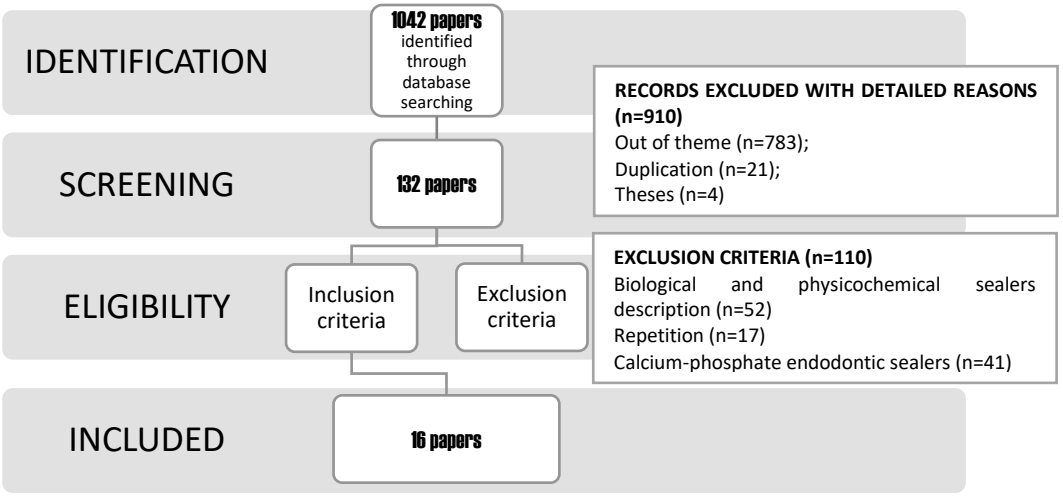

Figure 2. PRISMA information flow through the phases of the present systematized review.

The admissibility criteria were a) complete available articles published in English language; b) original *in vitro* and *ex vivo* investigation; c) endodontic sealers antimicrobial studies; d) endodontic sealers cytotoxic outcomes; e) bioceramic based-endodontic sealers; f) *in vitro* and *ex vivo* MTA-based endodontic sealers and g) *in vitro* and *ex vivo* calcium-silicate based endodontic sealers. The exclusion criteria were a) unpublished data; b) conference papers; c) letters to editor; d) *in vivo* studies (clinical and animals investigations); e) revision papers and f) papers due to calcium phosphate-based endodontic sealers

DATA EXTRACTION AND ANALYSIS

The studies that achieved the inclusion criteria (16 articles) were analyzed in a PRISMA form[58] and the quality of each *in vitro* and *ex vivo* study was assessed using a new risk of bias assessment tool described to a new optimal approach to address basic research studies (consult attachment chapter of this thesis).

VII. RESULTS

VII. RESULTS

The literature search provided 1042 potentially relevant titles and abstracts. At the first evaluation phase, 910 publications were rejected based on several detailed reasons as investigation out of theme (783), papers duplication (21), theses (4) and human studies with no emphasis on calcium-silicate based endodontic sealers (102). At the second phase, the full text of the remaining 132 publications were retrieved for more detailed evaluation. All 132 papers after the application of clear inclusion and exclusion criteria, result in 16 papers: 14 performed only at *in vitro* conditions and 2 with *in vitro* and *ex vivo* approach. The 16 studies included in this systematized review are plentifully described in **table VII**. From all, 11 studies are from Scimago Journal & Country Rank (scimagojr.com) first quartile and 3 papers from second, revealing the high impact factor of the bulk of publications included in the systematized review. The risk of bias was calculated accordingly to a new assessment tool independently by two investigators (PD and PP). The Cohen's kappa (k) coefficient first calibration outcome was 0,846% (p<0.001) and the procedure was repeated after one week with the final value of 0,962% (p<0.001).

From all, the majority of investigators analyzed the CsbES antimicrobial efficacy in planktonic suspensions with variable culture growth: 2 hours (h)[59], overnight[60–63]; 24h[64–66]; 48h[67–69]; although, two studies do not identify the incubation period used[70,71].

Three studies calculated the CsbES antimicrobial efficacy at biofilms and the difference among them were in terms of biofilms maturation periods: 24h[66], 2[72] and 3 weeks[73] as all used monospecies biofilms. It is not possible to quantify the biofilm antimicrobial elimination with colony forming units (CFU), as a consequence, only Wang *et al.* used an appropriate methodology (LIVE/DEAD BacLight Bacterial Viability stain with Confocal Laser Scanning Microscope (CLSM) to quantify the final antimicrobial outcomes in biofilms substrate[73].

Bacterias are the microorganism type most studied in this field with *E. faecallis* American Type Culture Collection, ATCC 29212 as the most prevalent strain, followed by VP3-181[74] a clinical strain isolated from persistent apical periodontitis[60,73]. The *E. faecallis* specie is described in 56,3% (9) of papers, followed by *Streptococcus mutans* in 31,3% (5)[59,62,66,68,70] with the strain ATCC 25175 prevalent in 18,8% (3) papers[59,68,70] and two studies used a fungus *C. albicans* ATCC 10231[63] and the same yeast combined with bacteria[59].

To quantify the CsbES antimicrobial efficacy, the most used assays were Agar Diffusion Test (ADT) applied in 43,8% (7) studies[59,63–65,67–69]; and Direct Contact Test (DCT) in 37,5% (6) investigations[61,63,65–67,69] and in 12,5% (2) was observed a modification, accordingly to the designation of Modified Direct Contact Test (MDCT)[60,66].

In terms of chronological events, SEM[75], CLSM[73] and Membrane Restricted Test (MRT)[66] were also applied to overpass the widely limitations of the classical tests for antimicrobial studies (ADT and DCT).

In terms of antimicrobial efficacy times periods evaluation, it varies from minutes (2-60min)[60,71] or hours (h) (1-24h)[59,69]; to days (d) (2-30d)[70,73] as well as the units applied. However, the most studied time is 24h[59,61,64–66,68–70,75] and 7d[60,63,66,68,71,73]. Furthermore, in some studies, there are fulfil description in terms of setting times moments. If it is quantified before[67], under[61] and/or after setting[60,66,67,71] which compromises the currently comparation among outcomes.

The CsbES most studied was EndoSequence BC™[61,69,73,75] and it is typically compared with Epoxy Resin-based sealers (ERbs)[64,66,72,75] as well as Pro-Root MTA®[59,64,65,68,70]. It is important to mention that from all data sheet avaiable in dentistry market checked for this review, EndoSequence BC™ is equivalent to iRoot SP®, iRoot BP®; TotalFill RRM® and TotalFill BC®. Only the brand name suffers modification according to the manufacturer country (consult attachment chapter of this thesis). Resuming, from all information reunited in this review, CsbES antimicrobial efficacy is lower when compared to ERbs.

CsbES cytotoxic outcomes were also an issue considered in this review, accomplished in 25% (4) of studies[65,69,70,75] as an antimicrobial efficacy outcomes complement. Candeiro *et al*. evaluated CsbES genotoxicity outcomes. From the four papers, Endosequence BC™ was tested twice[69,75] and none of the CsbES tested (Fast-Set MTA and radiopaque dicalcium silicate cement, also) had cytotoxic outcomes. Cytotoxic evaluation was performed in mouse[70] and human fibroblasts cell lines[69,75] as well as human osteoblast-like cells[65] estimated with AlamarBlue[65,75] and MTT assay[69].

Table VII. *In vitro* and *ex vivo* studies features compilation.

YEAR	AUTHOR JOURNAL	SPECIES	BIOFILMS *OR* PLANKTONIC SUSPENSION ?	SEALERS BRAND NAME	ANTIMICROBIAL EXPERIMENTAL METHODS OUTCOMES UNITS	EVALUATION PERIODS REPETITION NUMBER	ANTIMICROBIAL OUTCOMES	CYTOTOXIC OUTCOMES	CELL LINES EXPERIMENTAL METHODS
2018	**Kapralos *et al.*** [66] Journal of Endodontics (JOE)	*Enterococcus faecalis* (ATCC 19434); *S. epidermidis* (ATCC 35984) *S. aureus Newman*; *S. mutans* (ATCC700610)	Planktonic suspension (overnight) and monospecie biofilms (24h)	**TotalFill BC®** AH Plus™ (epoxy resin-based sealer); RoekoSeal (positive control) and GuttaFlow 2 (both are silicon-based sealers).	For planktonic bacteria: Modified Direct Contact Test assay (MDCT) Antibacterial assay on Biofilm: Direct Contact Test (DCT) and Membrane Restricted Test (MRT) Colony forming units Mean log (CFU/mL)	Two periods: 24 hours (h), 7 days (d) Experiments were conducted in triplicate and with 3 parallels for each material investigated.	TotalFill BC sealer showed marked antibacterial effect on planktonic bacteria up to 7 days after setting. TotalFill BC sealer had lower antibacterial activity against biofilms of *S. aureus* and *E. faecalis* compared with AH Plus.	NOT EVALUATED	NOT APPLIED
2017	**Usman *et al.*** [71] Journal of International Dental and Medical Research	*E. faecalis* from a non-vital-teeth with periapical disease	Planktonic suspension (Incubation period not specified)	**IRoot SP** **MTA Fillapex®**	DCT. Means, standard deviation (SD) and significant *p*-values. CFU/mL	Four periods: 2 minutes (min) (fresh), 4h (initial setting), 1d and 7d. Experiments were conducted in triplicate.	IRoot SP and MTA Fillapex have good antibacterial effect at fresh, initial setting and day 1. At 7 days, MTA Fillapex is better than IRoot SP.	NOT EVALUATED	NOT APPLIED
2017	**Shin *et al.*** [70] BioMed Research International Journal	*S. mutans* (ATCC 25175) *E. faecalis* (ATCC 19433) *F. nucleatum* (ATCC 49256) *P. intermedia* (ATCC 49046) *P. gingivalis* (ATCC 33277)	Planktonic suspension (Incubation period not specified)	**Fast-Set MTA (FS-MTA)** **Grey Pro-Root MTA**	Kirby-Bauer Disk-Diffusion Method Inhibition zones size at millimetres (mm).	Antimicrobial and cytotoxicity outcomes at two periods: 24 and 48h Antimicrobial approach: 4 samples for each material.	There was no difference in antimicrobial effect between FS-MTA and Pro-Root MTA.	FS-MTA has no cytotoxic properties and is comparable to Pro-Root MTA.	L929 mouse fibroblast cells (NCTC clone 929, ATCC CCL1, Manassas, VA) Agar Overlay Method for Citotoxicity with Neutral Red dye
2016	**Candeiro *et al.*** [69] International Endodontic Journal (Int Endod J)	*E. faecalis* (ATCC 29212)	Planktonic suspension (48h)	**EndoSequence BC™** AH Plus™	Antibacterial effectiveness: Inhibition zone in agar diffusion test (ADT) and colony forming units for DCT. Means and standard deviation (SD). CFU/mL	Antimicrobial effectiveness was evaluated after 1, 24, 72 and 168h. Cell viabilities were measured at 1, 3, 5 and 7 days. ADT: n=10 DCT: n=6 Cytotoxicity outcomes: n=3	EndoSequence® BC™ had similar antibacterial effect against *E. faecalis* as AH Plus.	EndoSequence BC™ cytotoxicity and genotoxicity is better than AH Plus.	Immortalized human gingival fibroblast cell (FMM1 cell lineage) CYTOTOXIC OUTCOMES: MTT assay GENOTOXIC OUTCOMES: Micronucleus Formation Test (MNT)

Table VII. *In vitro* and *ex vivo* studies features compilation (continuation).

YEAR	AUTHOR JOURNAL	SPECIES	BIOFILMS *OR* PLANKTONIC SUSPENSION ?	SEALERS BRAND NAME	ANTIMICROBIAL EXPERIMENTAL METHODS OUTCOMES UNITS	EVALUATION PERIODS REPETITION NUMBER	ANTIMICROBIAL OUTCOMES	CYTOTOXIC OUTCOMES	CELL LINES EXPERIMENTAL METHODS
2015	**Kim *et al.*[68]** Archives of Oral Biology	*S. mutans (*ATCC 25175) *E. faecalis* (ATCC 4082) *P. gingivalis* (ATCC 33277) *L. rhamnosus* (KCTC 3237) *L. paracasei* (KCTC 3165)	Planktonic suspension (48h)	**MTA-Angelus** **Endocem MTA** **White ProRoot MTA**	Disc diffusion test (DDT) Antibacterial broth test (ABT) Means and standard deviation (SD). CFU/mL	DDT: *S. mutans, E. faecalis, L. rhamnosus* and *L. paracasei:* 3 days; *P. gingivalis*: 7 days ABT: *P. gingivalis*: 2 days; *S. mutans, E. faecalis, L. rhamnosus* and *L. paracasei:* day For each strain: 8 plates (n=32)	MTA-Angelus and Endocem MTA were the most effective, respectively.	NOT EVALUATED	NOT APPLIED
2014	**Wang *et al.*[73]** JOE	*E. faecalis (*VP3-181)	Monospecie biofilms with 3 weeks in single-rooted human teeth extracted for orthodontic reasons	**EndoSequence BC™** AH Plus™; Pulp Canal Sealer™ (zinc oxide-eugenol sealer)	LIVE/DEAD BacLight Bacterial Viability stain (SYTO 9 and propidium iodide) with Confocal Laser Scanning Microscope (CLSM)	Three times: 1, 7 and 30 days Dentin 30 semicylindrical halves (n=60) were randomly divided into 5 groups with 6 specimens in each group.	BC Sealer had superior Antibacterial effects compared with Pulp Canal Sealer™.	NOT EVALUATED	NOT APPLIED
2014	**Wu *et al.*[65]** Int Endod J	*E. faecalis* (ATCC 29212)	Planktonic suspension (24h)	**Radiopaque dicalcium silicate cement (RDSC)** **White ProRoot MTA**	DCT ADT Means and SD. Bacteriostasis ratio (%) Inhibition zones size at millimetres (mm).	Four times (DCT): 5, 10, 30 and 60 min Three times (ADT): 6, 12 and 24h Results obtained in triplicate.	RDSC and White ProRoot MTA possessed similar antimicrobial activity and no significant differences were found at all culture time-points.	RDSC has significantly cytotoxicity than White ProRoot MTA.	MG63 human osteoblast-like cells (BCRC 60279, Hsinchu, Taiwan) L929 mouse fibroblast cells (BCBR 60279, Hsinchu, Taiwan) AlamarBlue reagent
2013	**Faria-Júnior *et al.*[72]** Int Endod J	*E. faecalis* (ATCC 29212)	Monospecie biofilms with 2 weeks in sectioned blocks from bovine central incisors with closed apex.	**MTA Fillapex (MTA-F)** **MTA-based sealer (MTA-S)** AH Plus™; Sealer 26™ (epoxy resin-based sealer); Epiphany SE (methacrylate resin-based sealer); Sealapex™ (calcium-hydroxide based sealer); Activ GP (glass ionomer-based sealer)	Root dentine blocks containing biofilm. Mean log CFU/mL Final outcome is a mean of the four specimens in each group.	Three times: 5, 10, 15h Results obtained in triplicate.	MTA-F was associated with the best outcome in a reduction in the number of bacteria in biofilms as well as Sealapex™.	NOT EVALUATED	NOT APPLIED

Table VII. *In vitro* and *ex vivo* studies features compilation (continuation).

YEAR	AUTHOR / JOURNAL	SPECIES	BIOFILMS *OR* PLANKTONIC SUSPENSION ?	SEALERS BRAND NAME	ANTIMICROBIAL EXPERIMENTAL METHODS / OUTCOMES UNITS	EVALUATION PERIODS / REPETITION NUMBER	ANTIMICROBIAL OUTCOMES	CYTOTOXIC OUTCOMES	CELL LINES / EXPERIMENTAL METHODS
2013	**Özcan *et al.*[63]** / Acta Odontologica Scandinavica	*Candida albicans* (ATCC 10231)	Planktonic suspension (overnight)	**iRoot® SP MTA Fillapex,** GuttaFlow®; AH Plus Jet (a resin based-sealer as control)	DCT and ADT / Mean log CFU/mL	Three periods: 20min; 1 and 7 days / Experiments were conducted in triplicate.	iRoot SP and MTA Fillapex were equally effective in inhibiting fungal growth, but both were less effective than AH Plus Jet, when freshly mixed.	NOT EVALUATED	NOT APPLIED
2013	**Heyder *et al.*[64]** / Dental Materials	*E. faecalis* (DSMZ 20376) *F. nucleatum* (DSMZ20482); *P. gingivalis* (DSMZ20709)	Planktonic monoculture suspension (24h)	**ProRoot MTA®** AH Plus™; Hermetic® (zinc oxide-base); RoekoSeal (Polydimethyl siloxane base); Sealapex™ (Salicylate base) Apexit Plus (calcium-hydroxide base) 2Seal (epoxy resin-base); EndoRez® (methacrylate resin-base); Calxyl Red and Gangraena Merz®(Calcium-hydroxide-base temporary)	ADT was applied to all samples. The best sealers in ADT were analysed with more detail in the DCT. / Inhibition zones size at mm. Optical density (OD): 0-1 CFU/mL	ADT: Freshly mixed and set state. DCT: seven periods: 0, 2, 4, 6, 8, 12, 24h CFU: 24h / ADT: Ten test series with freshly mixed and six test series with set sealer. DCT: Experiments were conducted in triplicate.	ProRoot MTA® only in the freshly mixed state showed a slight antibacterial effect on *F. nucleatum* and *P. gingivalis*, whereas this sealer did not suppress *E. faecalis.*	NOT EVALUATED	NOT APPLIED
2012	**Mohammadi *et al.*[62]** / Int Endod J	*S. aureus* *S. mutans* (strains not specified)	Planktonic suspension (overnight)	**White ProRoot MTA® (WMTA)** **CPM sealer®** AH 26®	ADT / Inhibition zones size at mm.	Two periods: 24h and 7d / Experiments were conducted in triplicate.	WMTA and CPM sealer had similar antibacterial effects against *S. aureus* in both periods, but CPM sealer was better than WMTA against *S .mutans.*	NOT EVALUATED	NOT APPLIED
2011	**Lovato and Sedgley[61]** / JOE	*E. faecalis* (n=10 strains) Strains previously isolates from infected root canals. GS1, GS2, GS4, GS6, GS7, GS9, GS10, GS18, GS25, GS33	Planktonic suspension (overnigth)	**EndoSequence Root Repair®** Premixed putty (ESP) and Syringeable paste (ESS) **WMTA**	DCT: Mean and SD / log CFU/mL	Two periods: 30min and 24h / Experiments were conducted in duplicate.	WMTA an EndoSequence Root Repair (ESP and ESS) demonstrated similar antimicrobial efficacy during their setting against 10 clinical strains of *E. faecalis.*	NOT EVALUATED	NOT APPLIED

Table VII. *In vitro* and *ex vivo* studies features compilation (continuation).

YEAR	AUTHOR JOURNAL	SPECIES	BIOFILMS *OR* PLANKTONIC SUSPENSION ?	SEALERS BRAND NAME	ANTIMICROBIAL EXPERIMENTAL METHODS OUTCOMES UNITS	EVALUATION PERIODS REPETITION NUMBER	ANTIMICROBIAL OUTCOMES	CYTOTOXIC OUTCOMES	CELL LINES EXPERIMENTAL METHODS
2011	**Morgental *et al.***[67] Int Endod J	*E. faecalis* (ATCC 29212)	Planktonic suspension (48h)	**Endo CPM Sealer, MTA Fillapex** White MTA Angelus and Endofill (both as references for comparation).	ADT (before setting): Means and SD DCT (after setting) ADT: Inhibition zones size at mm. DCT: log CFU/mL	ADT: 48 h DCT (four periods): 1, 6, 15 e 60min ADT: Ten replica plates DCT: Experiments were conducted in triplicate.	MTA Fillapex had antibacterial effect before setting similar to the positive control (Endofill) and Endo CPM Sealer did not have antibacterial activity before and after setting.	NOT EVALUATED	NOT APPLIED
2011	**Wilershausen *et al.***[75] Head & Face Medicine	*E. faecalis (*DSM 20478) *Parvimonas micra (*ATCC 33270)	Planktonic suspension *E. faecalis* (24h) *Parvimonas micra* (48h)	**Endosequence BC™** GuttaFlow® (silicon-based sealer); Pulp Canal Sealer EWT™ (zinc oxide-eugenol sealer); AH Plus Jet® (epoxy resin-based sealer).	Scanning Electron Microscopy (SEM) NOT APPLIED	Visual analysis of the scanning electron micrographs of the root canal sealers incubated for 24 and 48h (bacteria organized in micro-colonies).	No antibacterial effect of EndoSequence BC could be detected by SEM.	EndoSequence BC showed the best biocompatible outcome.	Human Periodontal Ligament Fibroblasts (Clonetics® HPdLF Lonza, Switzerland) CELL VIABILITY ASSAYS: AlamarBlue reagent; Toxilight® BioAssay Kit; Fluorescent dyes analysed by SEM: Phallacidin and LIVE/DEAD® Viability/Citotoxicity Kit (Calcein-AM/ethidium homodimer II stain)
2009	**Zhang *et al.***[60] JOE	*E. faecalis* (VP3-181)	Planktonic suspension (overnight)	**iRoot SP®**; AH Plus™; Apexit Plus; Tubli Seal™ (zinc oxide-eugenol sealer); Sealapex™ Epiphany SE®; EndoRez®	Modified DCT Log CFU/mL	Five periods: 0, 2, 5, 20 and 60 min Antimicrobial approach at five periods of fresh, 1d, 3d and 7d sealers. Experiments were conducted in triplicate.	Fresh iRoot SP eradicated all bacteria within 2 minutes of contact. After 1 day of setting, all bacteria were killed within 60min.	NOT EVALUATED	NOT APPLIED
2009	**Zarrabi *et al.***[59] Journal of Oral Science	*E. faecalis* (ATCC 29212) *E. coli* (ATCC 33780) *S. mutans* (ATCC 25175) *C. albicans* (ATCC 10231) *A. viscosus* (ATCC 15987)	Planktonic suspension (2h)	**ProRoot MTA; NEC** (New Endodontic Cement); **Portland Cement**	ADT: Means and SD Inhibition zones size at mm.	Three times: 24, 48 e 72 h Experiments were conducted in triplicate.	All sealers have antimicrobial activity with the pattern: NEC> ProRoot MTA> Portland. No sealer showed antimicrobial efficacy against *E. faecalis.*	NOT EVALUATED	NOT APPLIED

VIII. DISCUSSION

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In the present systematized literature review of CsbES, from 16 studies, 11 were from scimagojr first quartile revealing the high impact factor of publications included. Authors followed the PRISMA guidelines for systematic reviews, but do not agree with the risk of bias resolution, as the presumption of the risk of bias of experimental investigation is similar to the clinical surveys[76]. From a detailed analysis with a new risk of bias approach by two operators calibrated previously, 68% papers included had low risk of bias; medium risk of bias was observed in 12.5% (2) and high risk of bias was detected in 18,7% (3). None of the *in vitro* studies had very low risk of bias accordingly to the new approach to endorse the present systematized *in vitro* investigation. As the final value of Cohen's kappa (k) coefficient is high (0,962% $p<0.001$), it has a strong correlation between operators. Also, authors affirmed that this systematized literature review was well-succeed been a pilot study to analyse the new risk of bias tool.

The interpretation of all studies and subsequently the risk of bias of *in vitro* experiments focuses at the non-correct approach according to the substrate, methodology and assays selected to evaluate it. Subsequently, attending the obvious limitations of *in vitro* studies (with no external validity), clinical inferences must not be drawn, but standard experimental approaches are needed to compare final outcomes and relate research.

In the CsbES antimicrobial efficacy quantification, similar approach must be used as when new materials are introduced in dentistry market, more than one method should be employed to evaluate the sealer performance and 43,75% (7) investigations used more than one approach[63–69].

CsbES antimicrobial efficacy quantification was mainly achieved with classical methodology, the Agar Diffusion Test (ADT) the legitimate test used in the past decade, currently expropriated of its lack of reliability[77,78] and the Direct Contact Test (DCT)[60]. The ADT was applied in 43,8% (7) studies[59,62–65,67,69], however, as this technique presented several limitations, in 1996, Weiss *et al*. described the DCT to overcome ADT restrictions specifically to antibacterial activity of endodontic sealers[79]. DCT reflects better the proper antimicrobial potential of various sealers in standardized settings[60]. However, DCT has its own limitations in predicting CsbES antimicrobial efficacy because in clinical conditions, the sealer is not directly applied to microorganisms. Theoretically, endodontic sealers contact straight to the dentinal wall and microorganisms that might still remain inside the dentinal tubules in a biofilm form. As a consequence, several

essential features such as root canal microanatomy[80–82], endodontic sealer chemistry, microorganisms (bacteria or fungus), dentinal inhibitors[83,84] and biofilm formation[85] were not included in experimental assays cited above.

In this systematized review, only three studies quantify CsbES antimicrobial activity using monospecies biofilms[66,72,73] and only two used a mineral substrate as bovine dentin[72] and human dentin[73]. Kapralos *et al.* in the most recent investigation, for antimicrobial quantification in biofilms, used DCT and a Membrane Restricted Test (MRT) in which authors concluded that TotalFill BC® sealer has antimicrobial effect on planktonic microorganisms, but lower antimicrobial efficacy against 24h biofilms[66], when compared with an ERbs as AHPlus (Dentsply International Inc, York, PA).

In 2004, Saleh *et al.* designs an approach to investigate the antimicrobial efficacy of endodontic sealers in experimental infected dentinal tubules[28]. Even using 3-weeks biofilms, authors evaluate the antimicrobial outcomes with CFU and this is not reliable to quantify the biofilm removal and the same occurs in 2013[72] and recently in 2018[66]. Therefore, there is a strong lack of knowledgment, namely displaying antimicrobial biofilmes outcomes in CFU (means or logarithm)[86].

In 2011, Ma *et al*. elaborate a non-invasive model to study the dentin disinfection using CLSM[87] and this is the procedure presently is the most precise and reliable combined with a fluorescent staining that offers the possibility to directly investigate biofilms in a quantitative and qualitative approach[88]. Besides, this approach must be complemented with a LIVE/DEAD™ BacLight™ Bacterial Viability Kit for microscopy, only used in one study[73].

CsbES most studied was Endosequence BC™, although it is equivalent to iRoot SP®, iRoot BP®; TotalFill RRM® and TotalFill BC®[39] and the species most prevalent at *in vitro* studies was *Enterococcus faecalis* as it is commonly detected in secondary or refractory endodontic infections[60,73,74].

Kim *et al.* compared three different MTA-based sealers (MTA-Angelus, Endocem MTA, White ProRoot MTA (WMTA) and concluded that MTA-Angelus and Endocem MTA were the most effective at five different planktonic suspension species with 48h[68].

Heyder *et al.* conclude that freshly mixed state ProRoot MTA showed a minor antibacterial effect on 24h planktonic monocultures suspension of *F. nucleatum* and *P. gingivalis*, with no suppress effect over *E. faecalis*[64].

Mohammadi and collaborators compared WMTA with CPM sealer against overnight suspensions of *S. aureus* and *S. mutans* (with no strains specifications) and both had parallel antibacterial effects against *S. aureus*, but CPM sealer was better against *S. mutans*[62].

iRoot SP was evaluated by three teams[60,63,71]. Zhang *et al.* evaluate the iRoot SP antimicrobial efficacy in overnight planktonic suspension of a resistant strain of *E. faecalis*[60]. Ozcan group tested in *Candida albicans* overnight planktonic suspension[63] and Usman team in a planktonic suspension of *E. faecalis* from non-vital teeth with periapical disease[71]. All three groups showed that freshly iRoot SP had good antimicrobial properties, particularly after 2 min of direct contact[60]. Therefore, in terms of species, MTA Fillapex was better than iRoot SP in bacteria substrate[71] and iRoot SP with MTA Fillapex were equally effective in inhibiting yeasts growth[63]. MTA Fillapex has also been associated with good outcomes in a reduction in the number of bacteria in biofilms, as well as Sealapex™[72].

Endosequence BC™ exhibited antimicrobial efficacy in all studies where it was tested[65,69,70,75], although no antimicrobial effect was observed by scanning electron microscopy (SEM) at discs of equal size prepared from the endodontic sealers[75].

It is important to mention that this systematized review fail in answering the question of which CsbES is the best option as no study had in the experimental phase several CsbES been tested and compared among them. Even, Endosequence BC™ is the most prevalent without knowing if others CsbES could behave better than it in the same pre-clinical conditions.

The CsbES cytotoxicity outcomes were performed as a complement of antimicrobial evaluations in 25% (4) of studies[65,69,70,75] and all were realized according to ISO 10993–5 specifications[89].

In summary, in this systematized review, CsbES had significant lower antimicrobial efficacy and no cytotoxic outcomes, when compared to other endodontic sealers types, particularly, with epoxy resin-based[63,66]. Still, CsbES has prodigious biocompatibility properties, by encouraging the reparative tissues growth[55] with osteo-conductivity[56] promoting the apatite layer formulation when in contact with phosphate from physiological fluids from the host.

IX. CONCLUSION

IX. CONCLUSION

Calcium silicate-based Endodontic Sealers (CsbES) have been avaiable in Endodontics for the past thirty years and are used with well-defined protocols in clinical research with consistent outcomes as CsbES are widely known for the induction of new bone formation particularly because of spontaneously apatite layer formation when in direct contact with phosphates from physiological fluids encouraged by hydrated di- and tricalcium silicate as well as hydroxyapatite and calcium phosphates with recognized biocompatibility and bioactivity. Information about CsbES antimicrobial efficacy is limited. And for this reason, the present revision was elaborated.

According to the sixteen papers reviewed, fourteen were exclusively done with *in vitro* conditions and the antimicrobial efficacy is consistently obtained over planktonic suspensions with variable culture growth. The CsbES most studied was Endosequence BC™ (theoretically equivalent to iRoot SP®, iRoot BP®; TotalFill RRM® and TotalFill BC®) that exhibited antimicrobial efficacy in all studies where it was tested. However, it is important to clarify that it is not possible to endorse Endosequence BC™ as the best CsbES. In this systematized review none information is given of which CsbES is the best option among this family, because CsbES are not compared amid them, instead CsbES are compared with others endodontic sealers types and the literature concerning this as no information about that.

Endosequence BC™ is the sealer most tested, without knowledge if others CsbES behave better in the same experimental conditions. Subsequently, CsbES has prodigious biocompatibility properties and no cytotoxic outcomes were obtained when compared to other endodontic sealers types (particularly, those with epoxy resin-based). This parameter was only evaluated in four studies with highlight for Endosequence BC™ tested twice over human fibroblasts cell lines.

In the near future, multispecies biofilms as substrates; suitable endodontic sealers antimicrobial efficacy approaches as the counterpart parameters analysis are mandatory to allow adequate comparation among studies outcomes.

In conclusion, with the limitations of this revision, the avaiable studies level of evidence to determine the CsbES antimicrobial efficacy is very low and no clinical information should be obtained because *in vitro* clinical data has no external validity. As a consequence, further research with adequate methodology is required, including *in vivo* standardized experimental models to finally compare several types of CsbES among them.

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XII. ATTACHMENTS

Table A. Examples of Calcium silicate-based Endodontic Sealers (CsbES) MTA-based and components.

SEALER TYPES	BRAND NAME	MARKET INTRODUCTION	COMPONENTS	SETTING TIME	MANUFACTURER
MTA-based sealer	**ProRoot® MTA (Grey and White)**	2000	**(GREY) Powder:** Tricalcium silicate, dicalcium silicate, bismuth oxide, tricalcium aluminate, calcium sulphate dihydrate (gypsum) and calcium aluminoferrite. **Liquid:** distilled water. **(WHITE) Powder:** Tricalcium silicate, dicalcium silicate, bismuth oxide, tricalcium aluminate, calcium sulphate dihydrate (gypsum). **Liquid:** distilled water.	70-74min (initial) 210-320min (final)	Dentsply Tulsa Dental Specialties, Johnson City, TN, USA
	ProRoot®ES Endo Root Canal Sealer	2016	**Powder:** Fine and hydrophilic MTA based particles. **Liquid:** ProRoot® ES Endo Root Canal Sealer gel.	12h (final)	
	BioRoot™ RCS	2014	**Powder:** Tricalcium silicate, zirconium oxide (opacifier) and povidone. **Liquid:** Aqueous solution of calcium chloride and polycarboxylate.	< 4h	Septodont, Saint-Maur-des-Fosses Cedex, France
	Biodentine®	2008	**Powder:** Tricalcium silicate, dicalcium silicate, calcium carbonate as filler, zirconium oxide (radiopacity), calcium oxide and iron oxide (shade). **Liquid:** calcium chloride (accelerator), a hydrosoluble polymer (water reducing agent) and water.	6-45min	
	Angelus-MTA®	2001	**Powder:** Tricalcium silicate, dicalcium silicate, tricalcium aluminate, tricalcium aluminate, bismuth oxide, calcium oxide, aluminium oxide, silicon dioxide. **Liquid:** distilled water.	6-11min (initial) 130-230min (final)	Angelus Indústria de Produtos Odontológicos, S/A, Londrina, PR, Brazil
	Bio-MTA	not found	Portland cement and bismuth oxide.	11min (initial) 23.22 (final)	
	MTA-Fillapex	Braga *et al.* 2014	A dual paste material. **Base paste:** containing calcium silicate in a disalicylate resin, calcium tungstate, silica, salicylate resin and natural resin and, bismuth trioxide. **Catalyst Paste:** Diluent Resin, MTA, nanoparticulated silica and pigments.	19.3min	
	MTA Repair HP	not found	**Powder:** Tricalcium silicate, Dicalcium silicate, Tricalcium aluminate, Calcium oxide, Calcium Tungstate. **Liquid:** Water and Plasticizer.	15min	
	MicroMega-MTA™	2011	Tricalcium silicate, dicalcium silicate, tricalcium aluminate, bismuth oxide, calcium sulphate dehydrate and magnesium oxide.	20min (manufacturer) 120-150min (reports)	MicroMega, Besancon, France
	OrthoMTA®	2011	Tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, free calcium oxide and bismuth oxide.	321.9-326.1min	BioMTA, Seoul, Republic of Korea

Table A. Examples of Calcium silicate-based Endodontic Sealers (CsbES) MTA-based and components (continuation).

SEALER TYPES	BRAND NAME	MARKET INTRODUCTION	COMPONENTS	SETTING TIME	MANUFACTURER
MTA-based sealer	**RetroMTA®**	2011	**Powder:** Calcium carbonate, silicon oxide, aluminium oxide and hydraulic calcium zirconia complex. **Liquid:** water.	150-180s (initial) 360min (final)	
	Aureoseal®	not found	**Powder:** Portland cement, bismuth oxide, setting-time controllers, plastifying agents and radiopaque substances. **Liquid:** distilled water.	No report	Giovanni Ogna and Figli, Muggi o, Milano, Italy
	EndoSeal MTA	2017	Calcium silicates, calcium aluminates, calcium aluminoferrite, calcium sulphates, radiopacifier and a thickening agent.	12.31min	Maruchi, Wonju, Korea
	EndoCem MTA	Maruchi 2012	Calcium oxide, aluminium oxide, water, carbon dioxide, bismuth oxide, silicon dioxide, magnesium oxide, iron oxide, sulfur trioxide and titanium dioxide. **EndoCem MTA** derived from pozzolan-based cement.		
	EndoCem Zr		Calcium oxide, aluminium oxide, silicon dioxide, magnesium oxide, ferrous oxide and zirconium oxide.	4.5-15min	
	Endo CPM®	not found	MTA with silicon dioxide, calcium carbonate, bismuth trioxide, barium sulfate, propylene glycol alginate, sodium citrate, calcium chloride and active ingredients.	6–15 min (initial) 22-27min (final)	EGEO SRL, Buenos Aires, Argentina
	MTA-S Experimental sealer	**Guerreiro-Tanomaru *et al.* 2011** Developed by the authors at the Discipline of Endodontics	White Portland Cement (80%) and 20% of bismuth oxide, zirconium oxide, calcium chloride, resinous and water-soluble polymers.	535-982min	Endo Araraquara Dental School UNESP, São Paulo, Brazil
	MTA Plus®	2011	**(GREY)** Tricalcium silicate, dicalcium silicate, bismuth oxide, tricalcium aluminium oxide, calcium sulphate and brownmillerite. **(WHITE)** Tricalcium silicate, dicalcium silicate, silicon dioxide, tricalcium silicate, aluminium oxide, calcium sulfate.	120-136min	Avalon Biomed Inc, Bradenton, FL
	NeoMTA Plus®	2015	Tricalcium silicate, dicalcium silicate, tantalite, calcium sulphate and silica.	50-60min	
	BioAggregate® DiaRoot	2007	**Powder:** Tricalcium silicate, dicalcium silicate, calcium phosphate monobasic, amorphous silicon oxide and tantalum pentoxides. **Liquid:** deionized water.	240min	Innovative BioCeramix, Vancouver, BC, Canada
	TheraCal LC®	2010	Portland cement type III (45%), calcium oxide, strontium glass, fumed silica (5% of hydrophilic thickening agent), barium sulphate, barium zirconate and metacrylic resin (45% of containing Bis-GMA and PEGDMA).	0.3min (light cure technology)	Bisco Inc., Schaumburg, IL, USA
	Portland cement	--	The main composition of MTA and Portland cement are very similar. Tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetra calcium aluminoferrite, lime, silica, alumina, iron oxide, magnesia, sulfur trioxide, soda and potach (information avaiable online).	**Grey** 15.26-70min (initial) 32.65-172.6min (final) **White** 37.84-42.16min (initial) 131.3-138.56min (final)	All over the world

Table B. Example of CsbES Non-MTA based and components.

SEALER TYPES	BRAND NAME	MARKET INTRODUCTION	COMPONENTS	SETTING TIME	MANUFACTURER
Non-MTA based	**TotalFill BC®**	2003	Is a pre-mixed bioceramic single paste sealer with calcium silicates, zirconium oxide, calcium phosphate monobasic, calcium hydroxide, filler and thickening agents.	4h	KFG Dentaire, La-Chaux-de-Fonds, Switzerland
	EndoBinder	2007	Calcium oxide and aluminum oxide.	60min	Binderware, São Carlos, SP, Brazil
	CEM Cement	2008	**Powder:** Calcium oxide, phosphorus pentoxide, silicon dioxide, calcium phosphate, calcium silicate, calcium sulphate, calcium carbonate, calcium hydroxide and calcium chloride, aluminium trioxide, magnesium oxide, sulfur trioxide, phosphorus pentoxide, sodium oxide and chloride. **Liquid:** water-based solution.	50min	BioniqueDent, Tehran, Iran
	EndoSequence BC™ RRM and **RRP**	**EndoSequence BC** 2008 **EndoSequence RRM** and **RRP** 2009	Is a pre-mixed bioceramic single paste sealer with calcium silicates, zirconium oxide, calcium phosphate monobasic, calcium hydroxide, tantalum pentoxide, filler and thickening agents. **iRoot BP**, **BP Plus** and **EndoSequence BC** have the same formula.	58.6-63.6min (initial) 198-218min (final)	Brasseler USA, Savannah, GA, USA
	iRoot: four forms. SP, **FS**, **BP** and **BP Plus**	2009	**iRoot SP**: Zirconium oxide, calcium silicates, calcium phosphate, calcium hydroxide, filler and thickening agents **iRoot FS:** Calcium silicates, zirconium oxide, tantalum oxide and calcium phosphate monobasic **iRoot BP**: Tricalcium silicate, dicalcium silicate, zirconium oxide, tantalum pentoxide, calcium sulfate, filler and thickening agents. **iRoot BP**, **BP Plus** and **EndoSequence BC** have the same formula.	60min	Innovative BioCeramix Inc., Vancouver, Canada
	Tech Biosealer (Four forms: **Capping, Root End**, **Apex** and **Endo**)	2010	**Powder:** Mixture of white CEM, calcium sulphate, calcium chloride, bismuth oxide and montmorillonite. **Liquid:** Dulbecco's Phosphate Buffered Saline Modified (DPBS)	55-77min	Isasan, Como, Italy
	Quick-Set	Patent register finished in 2015	**Monocalcium aluminosilicate cement** that contains bismuth oxide (as a radiopacifier) and hydroxyapatite.	12min	Avalon Biomed Inc, Bradenton, FL

Legend:

Calcium-Enriched Mixture (CEM); Bioceramic Putty (BP); Bioceramic (BC); Fast Setting (FS); Root Repair Material (RRM); Root Repair Putty (RRP); Sealer Properties (SP)

Table C. CsbES MTA-based manufacturer information.

SEALER TYPES	BRAND NAME	MANUFACTURER INFORMATION	MANUFACTURER
MTA-based sealer	**ProRoot® MTA (Grey and White)**	Root repair material in both original gray and tooth-colored formulas provide predictable results and healing response that experts know and trust. Made of fine hydrophilic particles that set in the presence of water, seals off pathways between the root canal system and surrounding tissues, significantly reducing bacterial migration. Its excellent compatibility with the dentinal wall allows for a predictable clinical healing response. And its water-based chemistry permits normal setting in the presence of moisture. For added convenience, each packet comes with a premeasured unit dose of water for expedient preparation. Once the material is mixed, it quickly reaches a working consistency for efficient application. **Setting time of 165min (2h45).**	Dentsply Tulsa Dental Specialties, Johnson City, TN, USA
	BioRoot™ RCS	Hydrophilic sealer that continues the sealing process in the presence of moisture, adhesion to dentin and gutta-percha. Pure mineral formulation (not stain teeth), resin-free (made from pure calcium silicate and is monomer-free ensuring zero shrinkage), great flowability, used with cold single cone or cold lateral condensation. Radiopacity for clear imagens on radiographs (>5mm). **Setting time less than 240min (4h).**	Septodont, Saint-Maur-des-Fosses Cedex, France
	Biodentine®	The power is mixed with liquid in capsule triturator for 30seconds, physical process of crystal growth within dentine tubules leading to a micromechanical anchor which ensuring long lasting seal. Ion exchanges between the cement and dental tissues (adhesive system better than MTA) and the lower porosity leads do higher mechanical strength. Release hydroxyl and calcium ions. **Setting time of 6min.**	
	Angelus-MTA®	Hydrophilic high alkalinity sealer capable of calcium ions release, low solubility, induce periradicular cement neo-formation and oxide aggregate. **Setting time of 15min.**	Angelus Indústria de Produtos Odontológicos, S/A, Londrina, PR, Brazil
	Bio-MTA	Bio-MTA is substantially equivalent to Retro-MTA. **Setting time is 2min30s.**	
	MTA Fillapex	**Setting time is 150min.**	
	MTA Repair HP	Reparative restorative cement with high plasticity, low solubility, bismuth free, less particle size, high alkalinity with calcium ions release. Composed of mineral oxides in the form of fine hydrophilic particles. Allows for use in wet medium without change of its properties. New formula whose particle size after hydration allows for easy manipulation and insertion into the dental cavity. Addition of scheelite (radiopacifier) which does not cause teeth discoloration. **Setting time is 15min.**	
	MicroMega MTA™	Quick and automatic mixing. Homogenous consistency. Good radiopacity. Induce formation of a protective waterproof layer, resistant to bacterial infiltration. Excellent adhesion to the dentine. Optimal results, even in humid conditions. **Setting time is 20 minutes.**	MicroMega, Besancon, France
	OrthoMTA®	**Setting time is** (5h30).	BioMTA, Seoul, Republic of Korea
	RetroMTA®	Hydraulic hydrophilic bioceramic material, Portland cement is not used as a raw material. Contains hydraulic calcium zirconia complex as a contrast media. Has no discoloration even in the blood contamination, contains no heavy metals. **Setting time is 2min30s.**	

Table C. CsbES MTA-based manufacturer information (continuation).

SEALER TYPES	BRAND NAME	MANUFACTURER INFORMATION	MANUFACTURER
MTA-based sealer	**Aureoseal®**	The material is made of hydrophilic microparticles that contain modified mineral oxides. The powder should be mixed only with water. The material does not have such side-effects as irritative effects on the periodont and low adhesion in the wet environment specific for cements based on zinc oxide and eugenol. **The working time is 3-5 min** with the temperature 20°C, however **complete hardening occurs 28 days after.**	Giovanni Ogna and Figli, Muggi o, Milano, Italy
	EndoSeal MTA	A paste-type root canal sealer based on pozzolan cement that has excellent physical and biological properties of MTA. It is premixed and pre-loaded in a syringe that allows direct application of the sealer into the root canal without requiring powder/liquid mixing. The product has outstanding flowability and maneuverability. It is eugenol-free and will not impede adhesion inside the root canal. **Setting time is 12.31min.**	Maruchi, Wonju, Korea
	EndoCem MTA	EndoCem MTA is a fast-setting (up to four minutes) pozzolan-based. Exhibits high efficacy ad high manipulability (consistent level of bond strength regardless of external factors). Super sealing properties. Strong antibacterial effect and excellent biocompatibility. **Setting time up to 4 minutes.**	
	EndoCem Zr	EndoCem Zr is a white, fast-setting time, pozzolan-based MTA with minimal discoloration and calcification. Tooth color formula for enhanced esthetics. Adequate consistency/ Mechanical property. Excellent radiopacity. Super sealing properties. Strong antibacterial effect and excellent biocompatibility. **Setting time up to 4 minutes.**	
	Endo CPM®	Root-end filling material with powder and liquid. Treating internal root resorption. Apical plug during apexification. Pulp-caping material. Repair of root perforation during root canal therapy. **NOTE: Setting time not mentioned.**	EGEO SRL, Buenos Aires, Argentina
	MTA-S Experimental sealer	MTAS-sealer evaluated in this study exhibits the capacity to release calcium and hydroxyl ions as well as to prolong the setting time, which are favorable properties. **Initial setting 9h and final setting ~16h**	Endo Araraquara Dental School UNESP, São Paulo, Brazil
	MTA Plus®	It is a tricalcium silicate-based dental cement, slightly more radiopaque than (white) MTA. Finer powder mixes smoothly and it is easy to dispense. The powder and gel may be mixed from 1:1 to 3:1 depending on whether you want the syrup-like consistency of a sealer, or the high viscosity needed for pulpotomies or root-end filling. The unique gel enables more stable placement, washout resistance and faster clinical setting. **Setting Time is inferior to 1h at 37°C**, when thickly mixed with gel. E	Avalon Biomed Inc, Bradenton, FL
	NeoMTA Plus®	It is a powder and gel system consisting of an extremely fine, inorganic powder of tricalcium and dicalcium silicate. Power mixes more smoothly, is easier to dispense and the unique gel enables more stable placement, washout resistance and faster clinical setting. The powder and gel may be mixed from 1:1 to 4:1 depending on whether you want the syrup-like consistency of a sealer, or the high viscosity needed for pulpotomies or root-end filling. This material is both bioactive and radiopaque. **Setting Time is ~20min at 37°C**, when thickly mixed with gel.	
	BioAggregate®	It is is a biocompatible pure white powder composed of ceramic nano-particles. Upon mixing with BioA Liquid, the hydrophilic powder promotes cementogenesis and forms a hermetic seal inside the root canal. **The working time is at least 5 minutes and takes 28 days to form a hard impermeable solid.** If additional working time is required, simply cover the mixture with a moist gauze sponge while unattended to prevent dehydrate or add some BioA liquid to the mixture to achieve better workability.	Innovative BioCeramix, Vancouver, BC, Canada
	TheraCal LC®	TheraCal LC® is a light-cured, resin-modified calcium silicate. Its unique apatite stimulating ability makes it ideal for direct and indirect pulp capping and as a protective base/liner. The light-cured set permits immediate placement and condensation of the restorative material. Its proprietary formulation allows for a command set with a light curing unit while maintaining ease of placement due to thixotropic properties. The proprietary hydrophilic resin formulation creates a stable and durable liner.	Bisco Inc., Schaumburg, IL, USA

Table D. CsbES Non-MTA-based manufacturer information.

SEALER TYPES	BRAND NAME	MANUFACTURER INFORMATION	MANUFACTURER
Non-MTA based	**TotalFill BC®**	TotalFill® is a pre-mixed bioceramic obturation material. It is dispensed using a syringe in cases of root canal obturation and with either a syringe or as a putty when doing root repair and retrograde fillings. This highly radiopaque and hydrophilic sealer, TotalFill® BC Sealer, forms hydroxyapatite upon setting and chemically bonds to both dentine and to our bioceramic points (TotalFill® BC Points). BC Sealer is anti-bacterial during setting due to its highly alkaline pH and unlike traditional sealers, BC Sealer exhibits absolutely zero shrinkage! Setting time of 4h for TotalFill BC and 20 min for the Fast Set Putty. However, in very dry root canals, the setting time can be more than 10 hours. **Setting time is 4 hours.**	KFG Dentaire, La-Chaux-de-Fonds, Switzerland
	EndoBinder	A new calcium aluminate-based cement called EndoBinder was developed by the Federal University of São Carlos (UFSCar-Brazil, patent number PI0704502-6) to preserve the properties and clinical applications of MTA without its negative features. **NOTE: Setting time not mentioned.**	Binderware, São Carlos, SP, Brazil
	CEM Cement	Calcium compound hydrophilic tooth colored alkaline cement with setting time and film thickness lower than MTA and higher antibacterial effect. Releases calcium hydroxide during and after setting. **Setting time of 50min.**	BioniqueDent, Tehran, Iran
	EndoSequence BC™ (≈ iRoot SP, BP; TotalFill RRM/BC)	EndoSequence BC Sealer™ utilizes the moisture naturally present in the dentinal tubules to initiate its setting reaction. This highly radiopaque and hydrophilic sealer forms hydroxyapatite upon setting and chemically bonds to both dentin and to bioceramic points (EndoSequence® BC Points™). BC Sealer is anti-bacterial during setting due to its highly alkaline pH and unlike traditional sealers, BC Sealer exhibits absolutely zero shrinkage. However, in very dry root canals, the setting time can be more than 10 hours. **Setting time of 4 hours.**	Brasseler USA, Savannah, GA, USA
	iRoot®	**iRoot SP Injectable Root Canal Sealer (iRoot SP):** A premixed ready-to-use injectable white hydraulic (hydrophilic) cement paste of aluminum-free based on a calcium silicate composition for permanent root canal filling and sealing applications for safe and effective sealing of root canals. **iRoot® BP Injectable Root Canal Repair Filling Material (iRoot BP)**: Ready-to-use white hydraulic premixed injectable paste insoluble, radiopaque and aluminum-free material based on a calcium silicate composition, which requires the presence of water to set and harden. **iRoot BP Plus**: Ready-to-use white hydraulic premixed putty in a preloaded jar. **iRoot FS:** Ready-to-use white hydraulic premixed putty with fast setting properties. **NOTE: Setting time of 150min.**	Innovative BioCeramix Inc., Vancouver, Canada
	Tech Biosealer	Mixture of white CEM with high antibacterial activity resulting from its ability to release calcium ions during the setting. In contact with biological fluids, calcium ions are transformed into calcium phosphate crystals that stratify the on the cement surface, creating an alkaline environment and seal of porosity due to the formation of apatite deposits. **NOTE: Setting time not mentioned.**	Isasan, Como, Italy
	Quick-Set	A calcium aluminosilicate bioactive cement with acid-resistant and fast-setting. **Setting time of 9minutes** (derived from Capasio powder with no cationic surfactant).	Avalon Biomed Inc, Bradenton, FL

XIII.

INDEX

XIV.

LIST OF FIGURES

LIST OF TABLES