Faculdade de Medicina da Universidade de Coimbra

Mestrado Integrado em Medicina Dentária

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A Systematic Review

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Abbreviations

BC	BioCeramic
BP	Bioceramic Putty
Cavit™ G	Cavit™ Gray
CCK-8	Cell Counting Kit-8
CONSORT	Consolidated Standards of Reporting Trials
DMSO	Dimethyl sulfoxide
ERRM	Endosequence® BC Root Repair Material™
ES	Endodontic Sealer
EWT	Extended Working Time
FS	Fast Setting
G	Gray
IRM®	Intermediate Restorative Material
ISO	International Organization for Standardization
MC3T3-E1	Mouse osteoblast-like cell line
MG63	Human osteoblast-like cell line
MTA	Mineral Trioxide Aggregate
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide
PCS	Kerr's Pulp Canal Sealer™
PICO	Population, Intervention, Comparison and Outcome

PMMA	Polymethyl methacrylate
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
®	Registered
RCS	Root Canal Sealer
ROS 17/12.8	Rat osteossarcoma 17/12.8 cell line
RPC-C2A	Rat clonal dental pulp cell line
SE	Self-Etch
SP	Sealing Paste
SuperEBA™	Super ethoxybenzoic acid
SYRCLE	SYstematic Review Centre for Laboratory animal Experimentation
™	Trademark
UDMA	Urethane dimethacrylate
USA	United States of America
V79	Chinese hamster fibroblasts
XTT	2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide
WST	Water Soluble Tetrazolium Salt
ZnO	Zinc Oxide
ZOE	Zinc Oxide-Eugenol

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Resumo

Introdução e Objetivos: A utilização de cimentos endodônticos de selagem canalar é fundamental na prática clínica. No entanto, vários estudos têm evidenciado que o contacto destes materiais com os tecidos periapicais pode determinar uma resposta citotóxica com efeitos negativos no processo de cicatrização. Neste contexto, o nosso objetivo foi realizar uma revisão sistemática da literatura acerca da citotoxicidade e biocompatibilidade dos cimentos endodônticos de selagem canalar, a qual inclui os diversos tipos de cimentos comercialmente disponíveis e evidência baseada em estudos *in vitro* e *in vivo*.

Métodos: Esta revisão sistemática foi realizada de acordo com as normas PRISMA (*Preferred Reporting Items for Systematic Reviews and Meta-Analyses*), utilizando as seguintes bases de dados: *PubMed*, *Cochrane Library*, *ClinicalTrials.gov*, *Science Direct*, *Web of Science Core Collection*. Foram incluídos estudos que avaliaram a citotoxicidade (avaliada como viabilidade/ proliferação celular) e a biocompatibilidade (avaliada como resposta tecidular) de cimentos endodônticos de selagem canalar. O risco de viés dos estudos *in vitro* foi avaliado com as normas *CONSORT* modificadas e dos estudos *in vivo* com a ferramenta de risco de viés *SYRCLE*.

Resultados: A pesquisa inicial originou um total de 1382 estudos, dos quais foram incluídos 72 *in vitro* e 25 *in vivo*. Em geral, os estudos sugerem que os cimentos endodônticos de selagem canalar induzem efeitos tóxicos ligeiros a graves a nível celular e tecidular. Os cimentos biocerâmicos parecem exibir um menor potencial tóxico *in vitro*. Vários fatores parecem influenciar a biocompatibilidade, nomeadamente a condição de presa do material e o tempo e tipo de exposição.

Conclusões: A evidência disponível demonstra que os cimentos endodônticos de selagem canalar exibem um potencial tóxico variável, embora a heterogeneidade entre os estudos incluídos nesta revisão sistemática não permita concluir qual o tipo de cimento que apresenta melhor biocompatibilidade. Desta forma, são essenciais futuras investigações no sentido de uma melhor compreensão dos efeitos biológicos dos cimentos endodônticos de selagem canalar.

Palavras-chave: "*Endodontia*", "*Cimentos de selagem canalar*", "*Citotoxicidade*", "*Biocompatibilidade*", "*Revisão sistemática*".

Abstract

Background and Aims: The use of root canal sealers is crucial in clinical practice. However, several studies have reported that the contact between these materials and the periapical tissues may determine a toxic response which may hinder tissue healing. In this context, our aim was to perform a systematic review of the literature on the cytotoxicity and biocompatibility of root canal sealers which encompasses the several types of sealers that are commercially available and both *in vitro* and *in vivo* evidence.

Methods: This systematic review was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, using the following databases: PubMed, Cochrane Library, ClinicalTrials.gov, Science Direct, Web of Science Core Collection. Studies that evaluated the cytotoxicity (assessed as cell viability/proliferation) and the biocompatibility (assessed as tissue response) of root canal sealers were included. The risk of bias of *in vitro* studies was assessed with the modified CONSORT guidelines and of *in vivo* studies with the SYRCLE's risk of bias tool.

Results: The initial search retrieved a total of 1382 studies, from which 72 *in vitro* and 25 *in vivo* studies were included. In general, studies suggest that root canal sealers elicit mild to severe toxic effects at cellular and tissue level. Bioceramic sealers seem to exhibit a lower toxic potential *in vitro*. Several factors may influence biocompatibility, e.g. material setting condition and time and type of exposure.

Conclusions: The available evidence shows that root canal sealers exhibit variable toxic potential, although the heterogeneity among studies included in this systematic review does not allow to conclude which type of sealer presents higher biocompatibility. Thus, further research is crucial to achieve a better understanding of the biological effects of root canal sealers.

Keywords: "*Endodontics*", "*Root Canal Filling Materials*", "*Cell Death*", "*Biocompatibility*", "*Systematic Review*".

1. INTRODUCTION

Root canal therapy encompasses the sequence of procedures with the aim to treat the infected root of a tooth, thus resulting in the resolution of the infectious process and in the prevention of microbial invasion in the intervened tooth.[1] Usually, root canal therapy is performed by an orthograde method, which initiates with the removal of the infected pulp, proceeds to the shaping of the root canal, its cleaning and decontamination and culminates with the obturation (Fig. 1B-C). In some cases, however, periradicular surgery also known as retrograde filling, which involves root-end preparation and root-end filling or retrobturation (Fig. 1D-E), may be indicated. These include the presence of persistent periradicular pathology, which did not respond to conventional orthograde treatment, or when this orthograde technique is not viable, e.g. teeth with fiber post, which would be more prone to root perforation or fracture.[2,3]

The usage of endodontic sealers to perform root canal filling in obturation procedures is an established mainstay in Endodontics and plays a key role in the success of treatment [4]. Therefore, these materials should exhibit a set of characteristics that allow successful root canal filling with resolution of periapical inflammatory and/or infectious processes and prevent further microbial contamination.[4] In this context, Grossman previously listed the properties of an ideal sealer: (a) exhibits tackiness when mixed to provide good adhesion with the canal wall, (b) establishes a hermetic seal, (c) is radiopaque, so that it can be observed through radiographic observation, (d) is a very fine powder which can be easily mixed with liquid, (e) does not shrink on setting, (f) does not stain tooth structure, (g) is bacteriostatic (or at least does not promote bacterial growth), (h) displays a slow setting, (i) is insoluble in host tissue fluids, (j) is biocompatible, i.e. without irritant potential to periradicular tissue, and (k) is soluble in common solvent, allowing for removal when necessary.[4,5]

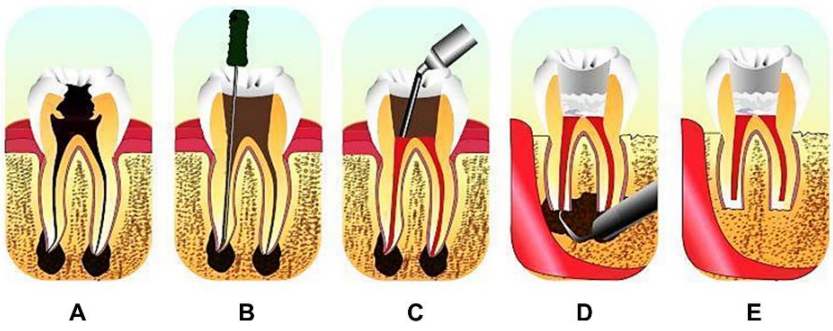

Figure 1 Workflow of root canal therapy of an infected tooth (A) by orthograde filling (B-C) and retrograde filling (D-E). Reproduced from Ma *et al*.[3]

Over the years, scientific and technological advances have allowed the improvement of equipment and materials used in several areas, particularly in Endodontics, and the development of better materials that are available to professionals thus allowing better results.[6] However, no sealer has yet fulfilled the entire set of Grossman's criteria.[4]

In fact, a number of materials have been developed, which may be categorized according to their chemical composition and structure, into the following classes: zinc oxide-eugenol-based, resin-based, glass ionomer-based, silicone-based, calcium hydroxide-based and bioceramic (i.e. calcium silicate-based, mineral trioxide aggregate (MTA)-based and calcium phosphate-based) sealers. The physical, chemical and biological properties have been previously reviewed.[7,8]

In regard to retrograde filling, several materials have been used over the years, including amalgam, Intermediate Restoration Material (IRM®, Caulk-Dentsply, Milford, USA) and super ethoxybenzoic acid (SuperEBA™, Bosworth Company, Skokie, USA).[3] More recently, the development of MTA has opened new perspectives in endodontic surgery, despite some known limitations namely the long setting time and discoloration potential.[9–12]

As mentioned above, biocompatibility is one of the main properties of root canal sealers, as these materials become in direct contact with periradicular tissues.[4] This biocompatibility corresponds to the ability to achieve an appropriate host response in a specific application, i.e. when in contact with the tissue fails to trigger an adverse reaction.[8,13,14] However, all sealers tend to exhibit a certain degree of toxicity especially when in freshly mixed state, even though it tends to decrease with setting.[4,15] Therefore, extrusion of sealer into periradicular tissues should be avoided.[4]

Most studies evaluate such biocompatibility through *in vitro* assessment of cytotoxicity with cell models.[16] Furthermore, multiple *in vivo* studies which assess tissue response have also been published. However, the multiplicity of methods and conditions that have been tested in previous studies make it difficult to get an overview of the subject as well as its interpretation. Such integration of concepts and results may be achieved through the systematic review of the literature. In fact, previous systematic reviews have focused on calcium silicate-based sealers and their comparison with conventional materials.[17–19]

In this context, we aimed to perform a systematic review of the literature on the cytotoxicity and biocompatibility of root canal sealers which encompasses all types of sealers and both *in vitro* and *in vivo* studies. Furthermore, we also aimed at understanding how the material set condition and concentration and the type and time of exposure influence the cytotoxicity and biocompatibility of these materials.

2. METHODS

This systematic review was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[20] and was registered in PROSPERO with the ID 140445. Considering the non-clinical nature of this systematic review, the PICO (Population, Intervention, Comparison and Outcome) research question was adapted from the PICO framework[21] (Table 1) and was formulated as follows: How do root canal sealers (individually or by type) perform in terms of cytotoxicity and biocompatibility in experimental cell and animal models?

Table 1 PICO strategy used for assessment of scientific literature.

Parameter	Assessment
Population (P)	*In vitro*: cell models *In vivo*: animal models of tissue inflammatory reaction
Intervention (I)	*In vitro*: sealer specimens or sealer extracts *In vivo*: sealer implants (subcutaneous, alveolar socket or intraosseous) or root filling procedures
Comparison (C)	Other root canal sealers or non-exposed control groups
Outcome (O)	*In vitro*: cytotoxicity (measured as cell viability or proliferation) *In vivo*: biocompatibility (measured as tissue response to the material)

2.1. Search Strategy and Study Selection

The electronic search was performed in several databases, specifically Medline via PubMed (www.ncbi.nlm.nih.gov/pubmed), Cochrane Library (www.cochranelibrary.com), ClinicalTrials.gov (clinicaltrials.gov), Science Direct (www.sciencedirect.com) and Web of Science Core Collection (webofknowledge.com/WOS). Date limit was set from 2000 to 2019, as the last search was performed in June 11, 2019. The following language filters were applied: English, Portuguese and Spanish. The search equations used for each electronic database were detailed in Table 2.

Articles were initially screened based on the title and abstract according to the scope (i.e. articles that do not report the cytotoxicity and/or biocompatibility of endodontic sealers

for root canal filling) and publication type (i.e. reviews, comments, letters or abstracts). Furthermore, a hand search of the reference lists of relevant studies was also performed. Reference management was performed with Mendeley© v1.19.4 (Mendeley Ltd).

In the eligibility assessment phase, this systematic review was split into two main sections based on the population and the outcomes: (a) one referring exclusively to *in vitro* models of cytotoxicity assessment and (b) one referring exclusively to *in vivo* animal models of biocompatibility assessment. Two independent reviewers critically assessed eligibility of studies for inclusion. A third reviewer was consulted in case of uncertainty or discrepancies regarding eligibility, and a decision by consensus was made.

Table 2 Search strategy for each of the databases.

Database	Search equation
Medline (via PubMed)	((“Root Canal Filling Materials”[Mesh] OR root canal sealer OR root canal filling OR root canal obturation OR “Epoxy Resins”[Mesh] OR “Zinc Oxide-Eugenol Cement”[Mesh] OR “Glass Ionomer Cements”[Mesh] OR “Calcium Hydroxide”[Mesh] OR “mineral trioxide aggregate”[Supplementary Concept] OR “endocem”[Supplementary Concept] OR bioceramic sealer OR “Dental cements”[Mesh]) AND “Endodontics”[Mesh]) AND (“Toxicity Tests”[Mesh] OR “Materials Testing”[Mesh] OR “Cell Death”[Mesh] OR “Cell Survival”[Mesh] OR cytotoxicity)
Science Direct	((“Root Canal Filling Materials” OR "root canal sealer" OR "root canal obturation") AND "Endodontics") AND (“Toxicity Tests" OR “Materials Testing” OR “Cell Death” OR “Cell Survival” OR "cytotoxicity")
Cochrane Library	(MeSH descriptor: [Root Canal Filling Materials] AND MeSH descriptor: [Endodontics]) AND (MeSH descriptor: [Materials Testing] OR MeSH descriptor: [Cell survival])
Web of Science Core Collection	TS=(root canal filling materials* OR root canal sealer* OR root canal obturation) AND TS=(endodontics) AND TS=(toxicity tests* OR materials testing* OR cell death* OR cell survival* OR cytotoxicity)
ClinicalTrials.gov	“Root Canal Obturation” (Limit: Status – Completed).

For the *in vitro* section, *in vitro* studies that evaluated the cytotoxicity, by assessing cell viability/proliferation of root canal sealers were included, and the following exclusion criteria were considered: (i) studies whose cytotoxicity assessment method is not clear or incompletely described or that do not evaluate or only evaluate qualitatively the cytotoxicity of endodontic sealers for root canal filling; (ii) studies that do not evaluate cytotoxicity through methods specific for cell viability/proliferation evaluation; (iii) studies that only report other biological properties (e.g. antimicrobial effect), physicochemical properties (e.g. bond strength, radiopacity, pH, solubility, setting or working time, dimensional change, flow or calcium release) or clinical outcomes (e.g. apical leakage or adaptation, sealing ability); (iv) studies that report the cytotoxic effects of experimental sealers not commercially available,

modified commercially-available root canal sealers, modified sealer components or dental materials used as pulp capping materials and others (e.g. adhesive systems); and (v) studies other than *in vitro*, e.g. *in vivo* or *in silico*.

For the *in vivo* section, *in vivo* animal studies that have evaluated the biocompatibility of root canal sealers through the assessment of tissue reaction after subcutaneous, intraosseous, alveolar socket or root canal implantation were included. For this section, the following exclusion criteria were considered: (i) studies that do not report the biocompatibility of endodontic sealers for root canal filling according to the methods described in the inclusion criteria; (ii) studies that only report other biological properties or clinical outcomes; (iii) studies that report the biocompatibility of experimental sealers not commercially available, modified commercially-available root canal sealers or dental materials used as pulp capping materials; and (iv) studies other than *in vivo*, e.g. *in vitro*.

Studies with missing data were excluded.

2.2. Data Collection

The following descriptive and quantitative information was extracted from each of the eligible studies for both sections, i.e. *in vitro* and *in vivo*: authors and year of publication, tested sealer(s) and controls, sample size, sealer material condition (i.e. fresh or set), the setting time if set materials were used, method of sealer preparation (i.e. if in accordance to manufacturer's instructions), results and main conclusions. Relatively to *in vitro* studies, the following information was also extracted: method (i.e. direct or indirect contact with sealer specimens or extracts), extraction time and extracts concentration if extracts were obtained, cell model and exposure time, cell viability/proliferation assay. In regard to *in vivo* studies, the following information was also extracted: method of biocompatibility assessment (i.e. subcutaneous, alveolar, intraosseous or root canal implantation), teeth used for root canal filling if this method was used, animal model, exposure time and method of histologic analysis (including staining method and outcomes measured).

2.3. Risk of Bias

The methodologic quality of eligible studies was checked by assessing the risk of bias of individual studies. The modified Consolidated Standards of Reporting Trials (CONSORT)

guidelines[22] were used for *in vitro* studies, by assessing several items as presented in Table 3. For the *in vivo* studies, the SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE) risk of bias tool[23] was used, which includes several items listed in Table 4.

Table 3 Item assessment according to the modified CONSORT checklist.[22]

Section/topic	Items	Description
Abstract	1	Structured abstract
Introduction	2a	Scientific background and rationale
	2b	Specific objectives and/or hypotheses
Methods	3	Intervention of each group
	4	Outcomes definition
	5	Sample size determination
	6	Randomization: sequence generation
	7	Allocation concealment mechanism
	8	Implementation (who)
	9	Blinding (who and how)
	10	Statistical methods used to compare groups
Results	11	Outcomes and estimation: results for each group, estimated effect size and precision (e.g. 95% confidence interval)
Discussion	12	Limitations
Other information	13	Funding
	14	Protocol (if available)

Table 4 Item assessment according to the SYRCLE's risk of bias tool.[23]

Type of bias	Items	Domain
Selection	1	Allocation sequence generation
	2	Baseline characteristics
	3	Allocation concealment
Performance	4	Random housing
	5	Caregiver and/or researcher blinding
Detection	6	Random outcome assessment
	7	Outcome assessor blinding
Attrition	8	Incomplete outcome data
Reporting	9	Selective outcome reporting
Other	10	Other sources

3. RESULTS

The full process of article retrieving, screening and eligibility assessment is presented in Fig. 2. As can be seen, the initial search retrieved a total of 1382 studies, from which 188 were excluded after removal of duplicates. A total of 1194 studies were screened based on the title and abstract, from which 1027 were excluded, resulting in 167 full-text studies that were considered potentially eligible for inclusion, which included 135 *in vitro* studies, 30 *in vivo* studies and 2 studies with both *in vitro* and *in vivo* testing. A total of 77 studies (65 *in vitro*, 11 *in vivo* and 1 both *in vitro* and *in vivo*) was excluded because they did not meet the inclusion criteria. Studies that did not specify the material condition, i.e. freshly mixed or set, were excluded. After reviewing the full texts, 6 *in vivo* and 1 both *in vitro* and *in vivo* studies were added to the analysis by hand searching. Finally, 70 *in vitro*[24–93], 25 *in vivo*[94–118] and 2 both *in vitro* and *in vivo* studies[119,120] were included in this review. The studies with both *in vitro* and *in vivo* methodologies were included only for the *in vitro* data, as the *in vivo* methodology did not meet inclusion criteria. In Table 5, we listed the several root canal sealers for orthograde and retrograde filling, the respective manufacturers and the included articles in which they were studied.

As can be seen, the most studied sealers *in vitro* were: AH 26®, AH Plus™, EndoREZ®, Endosequence BC™, Epiphany®, MTA Fillapex®, Kerr's Pulp Canal Sealer™ (PCS), ProRoot® MTA and Sealapex™. Other materials included: Amalgam[55,81,87], Castor Oil Polymer (Poliquil, Brazil)[61], Cavit™ Gray or G (3M ESPE, Seefeld, Germany)[45], CYMED 8410 (NANO, Kaohsiung, Taiwan)[68], Diaket™ (3M ESPE, Seefeld, Germany)[68], Endosequence® BC Root Repair Material™ (ERRM, Brasseler, Savannah, USA)[45,51], Geristore® (DenMat® Corporation, Santa Maria, USA)[66,81], IRM® [35,45], Retroplast (Retroplast Trading, Dybesøvej, Denmark)[66] and SuperEBA™.[30,66,81,87]

Regarding *in vivo* studies, AH Plus™, EndoREZ®, Epiphany® and ProRoot® MTA were the most studied. Other materials were also studied, such as the high-copper amalgams Oralloy (Coltène AG, Altstatten, Switzerland)[108] and Sinaalloy (Faghihi, Iran)[102], the calcium-hydroxide and polyethylene-glycol-based paste Calen® (S.S.White Artigos Dentários Ltda., Rio de Janeiro, Brazil)[114], the calcium phosphate-based sealers Capseal I and II[105], the zinc oxide-eugenol-based sealer Fillcanal (DG Ligas Odontológicas Ltda, Rio de Janeiro, Brazil)[110], Intrafill (Dentsply Ind. e Com. Ltda., Rio de Janeiro, Brazil)[96], IRM® [109] and SuperEBA™.[109]

3.1. *In Vitro* Cytotoxicity

The characteristics of the included studies in respect to *in vitro* cytotoxicity of root canal sealers is presented in Table 6. From the 72 studies, 18 used a direct contact testing method with sealers prepared either as fresh sample, disc, layer or cylindrical specimens[27,31,32,38,55,56,58,63,65,74–77,79,81,83,89,93], as others used root models.[26,40,60,82,84] In terms of material setting condition, 22 studies evaluated root canal sealers in a fresh or freshly mixed state, 16 in a set condition with 24h of incubation, 17 in both freshly mixed and set conditions and 17 in a set condition with other or multiple times of incubation.

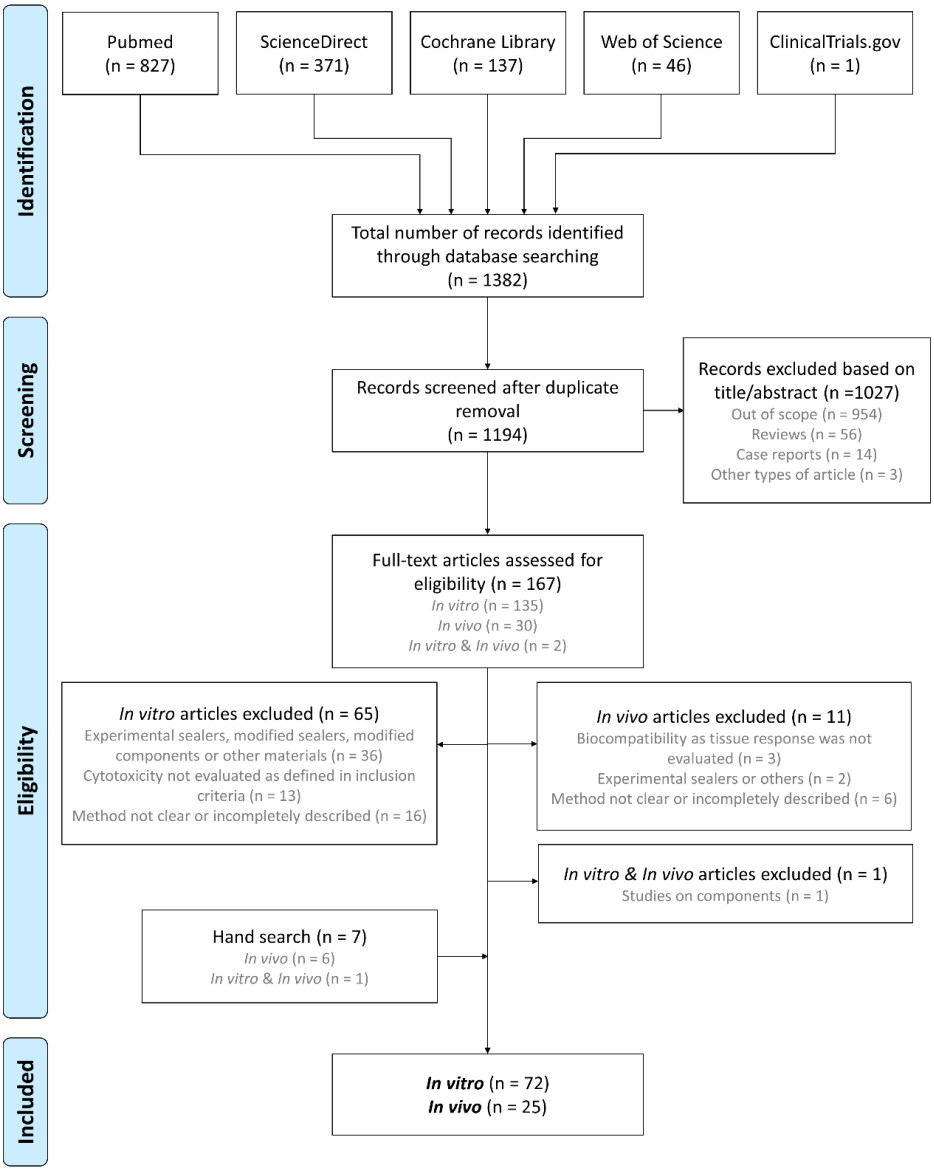

Figure 2 Flow diagram of identification of studies for inclusion in this systematic review according to PRISMA guidelines.

Table 5 Root canal sealers used in studies with *in vitro* and *in vivo* methodologies included in this systematic review.

Type	Sealer	Manufacturer	*In vitro*	*In vivo*
ZnO-eugenol	PCS	Kerr, Romulus, USA	25–27,54,56,59,65,78,79,83	105,116,117
	PCS EWT	Kerr, Romulus, USA	49	–
	N2®	Indrag-Agsa, Losone, Switzerland	53,62,67,70,72,84,86	–
	Endofill	Produits Dentaires, Vevey Switzerland	43,64,83,119	112
	Canals	Showa Pharmaceutical Co., Tokyo, Japan	53,62,67	–
	Endométhasone	Septodont, Saint-Maur-des-Fossés, France	84,86	99,106
	Roth's Sealer	Roth International, Chicago, USA	36,42	–
	Grossman's	Sultan Chemists, Englewood, USA	75	–
	Zinc Oxide-Eugenol (ZOE)	Produits Dentaires, Vevey Switzerland	40,88	114
	Tubli-Seal™	Kerr, Romulus, USA	58	–
	Tubli-Seal Xpress™	Kerr, Romulus, USA	48	–
	Cortisomol™	Pierre Rolland, Merignac, France	82	–
Resin (epoxy)	AH Plus™	Dentsply DeTrey Gmbh, Konstanz, Germany	24,28,33,34,36,39,41,42,49,52,54,58,61,64,73,74,76,77,80,82,84–86,88–90,93,119	94,96,97,111,115
	AH 26®	Dentsply DeTrey Gmbh, Konstanz, Germany	50,51,53,62,67,70–72,85,88,89	110
	AH Plus Jet®	Dentsply DeTrey Gmbh, Konstanz, Germany	31,37,44,48,65	–
	Acroseal	Septodont, Saint-Maur-des-Fossés, France	61,93	–
	SimpliSeal®	Discuss Dental LLC, Calver City, USA	47,119	–
	TopSeal®	Dentsply DeTrey Gmbh, Konstanz, Germany	79	–
	Sealer Plus	MK Life, Porto Alegre, Brazil	119	–
	ThermaSeal®	Dentsply/Maillefer, Konstanz, Germany	75	–
	ThermaSeal® Plus	Dentsply/Maillefer, Konstanz, Germany	42	–
Resin (methacrylate)	EndoREZ®	Ultradent, South Jordan, USA	28,34,44,56,58,74,79	99,107,117,118
	Epiphany®	Pentron, Wallingford, USA	59,61,63,73–76	96,98,100,112,116
	Epiphany® SE	Pentron, Wallingford, USA	43,59	–
	RealSeal™	SybronEndo, Orange, USA	33,44,56,57	117
	RealSeal SE™	SybronEndo, Orange, USA	42,56	–

	RealSeal XT	SybronEndo, Orange, USA	31	95
	MetaSEAL™	Parkell, Inc., Farmington, USA	56,65	–
Glass ionomer	Ketac™ Endo	3M ESPE, St. Paul, USA	84,86	–
	Ketac™ Fil Plus	3M ESPE, St. Paul, USA	66	–
	Activ GP™	Brasseler, Savannah, USA	57	–
	Endion®	VOCO, Cuxhaven, Germany	68	–
Silicone	GuttaFlow®	Roeko/Coltène/Whaledent, Langenau, Germany	28,42,48,76	–
	GuttaFlow®2	Roeko/Coltène/Whaledent, Langenau, Germany	33,80	111
	GuttaFlow® Bioseal	Roeko/Coltène/Whaledent, Langenau, Germany	80	111
	RoekoSeal	Roeko/Coltène/Whaledent, Langenau, Germany	34,79,84	96
	RoekoSeal Automix	Roeko/Coltène/Whaledent, Langenau, Germany	74,77,78	97
Calcium hydroxide	Sealapex™	Kerr, Romulus, USA	32,42,58,70,72,75,78,82	103
	Apexit®	Ivoclar Vivadent, Schaan, Liechtenstein	28,84,86	–
	Sealapex Xpress™	SybronEndo, Orange, USA	–	95
	Sealer 26	Dentsply/Maillefer, Konstanz, Germany	64	103,110
Bioceramic	ProRoot® MTA	Dentsply Tulsa Dental, Tulsa, USA	30,33,35,38,40,45,51,55,60,66,68,81,91,92	101,102,104,113
	ProRoot® ES	Dentsply Tulsa Dental, Tulsa, USA	36	–
	MTA Fillapex®	Angelus, Londrina, Brazil	24,29,32,37,41,43,47,80,93	94
	Endosequence BC™	Brasseler, Savannah, USA	24,29,36,38,48,49	–
	iRoot® SP	Innovative BioCeramix Inc., Vancouver, Canada	32,37,46,52,91	–
	iRoot® BP Plus	Innovative BioCeramix Inc., Vancouver, Canada	30,40,92	–
	iRoot® FS	Innovative BioCeramix Inc., Vancouver, Canada	30,92	–
	BioRoot™ RCS	Septodont, Saint-Maur-des-Fossés, France	25–27,47	–
	MTA Angelus®	Angelus, Londrina, Brazil	38,41,120	–
	BioAggregate®	Innovative BioCeramix Inc., Vancouver, Canada	46,60	–
	Endoseal® MTA	Maruchi, Seoul, Korea	69	–
	MTA High plasticity	Angelus, Londrina, Brazil	120	120
	Endocem	Maruchi, Seoul, Korea	35	–
	Sankin apatite root sealer	Sankin Kogyo, Tokyo, Japan	32	105

Abbreviations: BC, BioCeramic; BP, Bioceramic Putty; ES, Endodontic Sealer; EWT, Extended Working Time; FS, Fast Setting; GP, Gutta-percha; MTA, Mineral Trioxide Aggregate; PCS, Kerr's Pulp Canal Sealer™; RCS, Root Canal Sealer; SE, Self-Etch; SP, Sealing Paste; ZnO, Zinc Oxide.

Concerning the cell models used for cell viability assessment, several studies used cultures of human cells, namely: dental follicle-derived mesenchymal stem cells[93], tooth germ-derived stem cells[37], gingival fibroblasts[28,29,33,42,44,45,75,88], osteoblasts[38,40,43,53,62,67,93], periodontal ligament cells[25,26,32,38,55,63,68,69,80,81,84,86,87], human osteoblast-like cells (MG63)[30,35,52] and cervical carcinoma cells or HeLa cells.[73,77] Other cell lines were also used, as can be seen in Table 6, e.g. L929 mouse fibroblasts, mouse osteoblast-like cells (MC3T3-E1), RAW 264.7 mouse macrophages, Chinese hamster fibroblasts (V79), rat osteosarcoma (ROS) 17/12.8 cells, Balb/c fibroblasts and rat clonal dental pulp cells (RPC-C2A).

Regarding the type of cell viability assay, most of the studies used assays that measure metabolic activity, specifically: 36 studies used the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay, 3 used the 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assay, 4 used the Alamar blue® assay, 3 used the Cell Counting Kit-8 (CCK-8/WST-8) assay, 2 used the Water Soluble Tetrazolium Salt-1 (WST-1) assay, 1 used the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. Other methods included the Trypan blue dye exclusion assay (5 studies), the Neutral Red uptake assay (2 studies), the ATP-based luminescence assay (1 study), the Sulforhodamine B assay (1 study), the Live/Dead Viability assay by flow cytometry (1 study), the crystal violet assay (3 studies), the propidium iodide fluorescence assay (1 study), the Hoechst 33258 fluorescence assay (1 study), the Millipore filter assay (1 study), fluorescent cell attachment with proprietary green fluorescent dye (1 study), the Nigrosin dye assay (1 study) and the lactate dehydrogenase-leakage assay (1 study). Also, 4 studies used multiple methods to assess cell viability.

3.1.1. Cytotoxicity of root canal sealers

In general, the tested root canal sealers exhibited cytotoxicity (Table 7).

The most studied sealer was the epoxy resin-based sealer AH Plus, which was reported as cytotoxic in most of the studies in which it was tested. However, one study[82] reported as noncytotoxic, one[88] reported a cytotoxic effect only in early phase and one[90] reported as cytotoxic when eluted in dimethyl sulfoxide (DMSO) but noncytotoxic when eluted in sodium chloride.

Table 6 General characteristics of included studies in regard to *in vitro* cytotoxicity.

Year	Author(s)	Groups	N	Material condition (setting time)	Method	Cell model	Assay
2019	Lee *et al.*[24]	AH Plus™, MTA Fillapex®, Endosequence BC™, Medium (control)	N≥2 per group (triplicate)	Set (24h)	Indirect contact testing with extract (sealer disc)	MC3T3-E1	WST-1
	Jeanneau *et al.*[25]	BioRoot™ RCS, PCS, Medium (control)	N=3 per group (triplicate)	Freshly mixed	Indirect contact testing with extract (specimen)	hPDLFs	MTT
	Giacomino *et al.*[36]	Roth´s Sealer, AH Plus™, Endosequence BC™, ProRoot® ES, No cells (control), Medium (control)	N=6-12 per group	Freshly mixed	Indirect contact testing with extract (specimen)	IDG-SW3	ATP-based Luminescence
2018	Vouzara *et al.*[47]	SimpliSeal®, MTA Fillapex®, BioRoot™ RCS, Medium (control)	N≥2 per group (6 replicate)	Set (48h)	Indirect contact testing with extract (specimen)	NIH/3T3	Sulforhodamine B
2017	Arun *et al.*[58]	Tubli-Seal™, AH Plus™, Sealapex™, EndoREZ®, Medium (control) [groups with pachymic acid]	N=3 per group	Freshly mixed	Direct contact testing with sealer	L929	MTT
	Collado-González *et al.*[69]	BioRoot™ BCS, Endoseal®, Nano-ceramic Sealer (NCS), Medium (control)	N=1 per group (5 replicate)	Set (48h)	Indirect contact testing with extract (sealer disc)	hPDLSCs	MTT
	Collado-González *et al.*[80]	GuttaFlow® Bioseal, GuttaFlow®2, MTA Fillapex®, AH Plus™, Medium (control)	N≥2 per group (5 replicate)	Set (48h)	Indirect contact testing with extract (sealer disc)	hPDLSCs	MTT
	Cintra *et al.*[120]	MTA High plasticity (HP), MTA Angelus®, Medium (control)	N=1 (triplicate)	Set (6h)	Indirect contact testing with extract (sealer disc)	L929	Alamar blue®
	Zhu *et al.*[91]	iRoot® SP, MTA, Medium (control)	n/s	Set (24h)	Indirect contact testing with extract (sealer disc)	RAW 264.7 macrophages	MTT
	Cintra *et al.*[119]	Sealer Plus, AH Plus™, Endofill, SimpliSeal®, Medium (control)	N=1 (triplicate)	Set (6h)	Indirect contact testing with extract (sealer disc)	L929	MTT
	Lv *et al.*[92]	iRoot® FS, iRoot® BP Plus, ProRoot® MTA, Medium (control)	N=3	Set (7d)	Indirect contact testing with extract (sealer disc)	MC3T3-E1	CCK-8/WST-8
2016	Suciu *et al.*[93]	MTA Fillapex®, AH Plus™, Acroseal, Plastic surface (control)	N=1 (triplicate)	Set (24h)	Direct contact testing with sealer	hOCs & DF-MSCs	Alamar blue®

Year	Author(s)	Groups	N	Material condition (setting time)	Method	Cell model	Assay
2015	Camps *et al.*[26]	BioRoot™ RCS, PCS, Medium (control)	N=30 (N=3/ group)	Set (24h)	Indirect contact testing with extract (root model)	hPDLCs	MTT
	Dimitrova-Nakov *et al.*[27]	BioRoot™ RCS, PCS, Untreated cells (controls)	N≥3 (triplicate)	Set (24h)	Direct contact testing with sealer disc	A4 mouse pulp SCs	Trypan Blue Dye Exclusion
	Konjhodzic-Prcic *et al.*[28]	GuttaFlow®, AH Plus™, Apexit®, EndoREZ®, Control (n/s)	N=60 (total)	Set (immediately after, 24h, 48h, 7d)	Indirect contact testing with extract (sealer disc)	hGFs	WST-1
	Zhou *et al.*[29]	Endosequence BC™, MTA Fillapex®, Medium (control)	N=1 (triplicate)	Freshly mixed & Set (3x specified time)	Indirect contact testing with extract (sealer disc)	hGFs	Live/Dead Viability (Flow cytometry)
2014	Jiang *et al.*[30]	iRoot® BP Plus, iRoot® FS, ProRoot® MTA, SuperEBA™, Medium (control)	n/s	Set (7d)	Indirect contact testing with extract (sealer disc)	L929 & MG63	MTT
	Cotti *et al.*[31]	RealSeal XT, AH Plus Jet®, Untreated (control)	N=3 per group	Fresh	Direct contact testing with sealer	L929	MTT & Neutral Red
	Chang *et al.*[32]	Sealapex™, Apatite Root Sealer, MTA Fillapex®, iRoot® SP, Medium with & without O.S. (control)	N=3 (4 wells/ condition)	Set (24h)	Direct contact testing with sealer disc (with O.S.)	hPDLCs	MTT
	Mandal *et al.*[33]	GuttaFlow®2, ProRoot® MTA, AH Plus™, RealSeal™, Medium (control)	N=1 (5 replicate)	Fresh & Set (72h)	Indirect contact testing with extract (sealer disc)	hGFs	CCK-8/WST-8
	Camargo *et al.*[34]	AH Plus™, EndoREZ®, RoekoSeal, Medium (control)	N=3 (4 wells/ condition)	Freshly mixed & Set (12h, 24h)	Indirect contact testing with extract (sealer layer)	V79	MTT
2013	Choi *et al.*[35]	ProRoot® MTA, Endocem, IRM®, Medium (control)	n/s	Set (24h)	Indirect contact testing with extract (sealer disc)	MG63	MTT
	Güven *et al.*[37]	MTA Fillapex®, iRoot® SP, AH Plus Jet®, Control (n/s)	N=6 per group	Set (24h)	Indirect contact testing with extract (sealer disc - insert)	hTGSCs	MTS
	Willershausen *et al.*[38]	MTA Angelus® (gray & white), ProRoot® MTA, Endosequence BC™, Untreated (control)	N=6 per group	Set (24h)	Direct contact testing with sealer disc	hPDLFs & hOCs	Alamar blue®

Year	Author(s)	Groups	N	Material condition (setting time)	Method	Cell model	Assay
	Kim *et al.*[39]	AH Plus™	N≥3 per group	Freshly mixed	Indirect contact testing with extract (sealer cylinder)	MC3T3-E1	MTT
2012	De-Deus *et al.*[40]	iRoot® BP Plus, ProRoot® MTA, Medium (negative control), ZOE cement (positive control)	N=2	Fresh (after root-end filling)	Indirect contact testing with extract (root model)	hOCs	XTT, Neutral Red, Crystal violet dye
	Bin *et al.*[41]	MTA Angelus®, MTA Fillapex®, AH Plus™, Untreated (control)	N=3 (4 replicate/ group)	Set (12h, 48h, 72h)	Indirect contact testing with extract (specimen)	V79	MTT
	Scelza *et al.*[42]	RealSeal SE™, AH Plus™, GuttaFlow®, Sealapex™, Roth 801, ThermaSeal® Plus, Medium (control)	N=2 (triplicate)	Freshly mixed	Indirect contact testing with extract (sealer fragments)	hGFs	MTT
	Salles *et al.*[43]	MTA Fillapex®, Epiphany® SE, Endofill, Untreated (control)	N=3 (duplicate)	Set (24h)	Indirect contact testing with extract (sealer disc - insert)	Saos-2	MTT
	Landuyt *et al.*[44]	AH Plus Jet®, EndoREZ®, RealSeal™, Calcicur (control), Medium (negative control), 1% Triton X-100 (positive control)	N=4 per group	Freshly mixed	Indirect contact testing with extract (specimen)	hGFs	XTT
2011	Ma *et al.*[45]	ERRM Putty, ERRM Paste, ProRoot® MTA (g), IRM® (control), Cavit™ G (control), Medium (control)	n/s	Fresh (2d) & Set (7d)	Indirect contact testing with extract (sealer disc)	hGFs	MTT
	Mukhtar-Fayyad[46]	BioAggregate®, iRoot® SP, Medium (control)	N≥2 per group	Set (3x specified time)	Indirect contact testing with extract (sealer disc)	hMRC-5 fibroblasts	MTT
	Zoufan *et al.*[48]	GuttaFlow®, Endosequence BC™, AH Plus™ Jet, TubliSeal Xpress™, Untreated (control)	N=3 per group	Freshly mixed & Set (72h)	Indirect contact testing with extract (sealer specimen)	L929	MTT
	Loushine *et al.*[49]	Endosequence BC™, AH Plus™, PCS EWT (positive control), Teflon (negative control)	N=1 (6 replicate)	Set (72h AH Plus and 240h others)	Indirect contact testing with extract (sealer disc-insert)	MC3T3-E1	MTT
2010	Yu *et al.*[50]	AH 26®, Control (n/s)	N≥3 per group	Freshly mixed	Indirect contact testing with extract (sealer cylinder)	MC3T3-E1	MTT
	AlAnezi *et al.*[51]	ERRM, ProRoot® MTA, AH 26® (positive control), Control (n/s)	N=3	Freshly mixed & Set (72h)	Indirect contact testing with extract (sealer specimen)	L929	MTT

Year	Author(s)	Groups	N	Material condition (setting time)	Method	Cell model	Assay
	Zhang *et al.*[52]	iRoot® SP, AH Plus™, Medium (control)	N≥2 (6 replicate)	Set (24h)	Indirect contact testing with extract (sealer disc)	MG63	MTT
	Huang *et al.*[53]	AH 26®, Canals, N2®, Untreated (control)	N=3 per group	Set (24h)	Indirect contact testing with extract (sealer disc)	U2OS	Alamar blue®
	Bryan *et al.*[54]	Experimental sealer (calcium silicate-based), AH Plus™, PCS, Teflon (negative control)	n/s	Set (24h)	Indirect contact testing with extract (sealer disc - insert)	MC3T3-E1	MTT
	Badr[55]	PMMA, MTA, amalgam, Control (n/s)	N=1 (6 replicate)	Freshly mixed & Set (24h)	Direct contact testing with sealer disc	hPDLCs	Trypan Blue Dye Exclusion
2009	Ames *et al.*[56]	EndoREZ®, RealSeal™, MetaSEAL™, RealSeal SE™, PCS (positive control), Teflon (negative control)	n/s	Set (72h)	Direct contact testing with sealer disc	ROS 17/12.8	MTT
	Donadio *et al.*[57]	Activ GP™, RealSeal™, AH 26®, Kerr Sealer, Untreated (control)	N=3	Freshly mixed & Set (72h)	Indirect contact testing with extract (sealer disc)	L929	MTT
	Gambarini *et al.*[59]	Epiphany® SE, Epiphany®, PCS, Untreated (control)	N=1 (6 replicate)	Set (24h)	Indirect contact testing with extract (sealer cylinder)	Mouse 3T3 fibroblasts	Neutral Red
	De-Deus *et al.*[60]	BioAggregate®, ProRoot® MTA, Empty root canal (control)	N=2	Fresh (after root-end filling)	Indirect contact testing with extract (root model)	hMCs	XTT, Neutral Red & Crystal violet dye
	Camargo *et al.*[61]	AH Plus™, Epiphany®, Acroseal, Castor Oil Polymer sealer, Untreated (control)	N=4 (4 replicate)	Set (6h)	Indirect contact testing with extract (sealer disc)	V79	Crystal violet dye
	Huang *et al.*[62]	AH 26®, Canals, N2®, Untreated (control)	N=3	Set (24h)	Indirect contact testing with extract (sealer disc)	U2OS	Propidium iodide
2008	Heitman *et al.*[63]	Epiphany®, Untreated (control)	N=1 (triplicate)	Freshly mixed	Direct contact testing with fresh sealer	hPDLFs	Crystal violet dye
	Valois *et al.*[64]	AH Plus™, Endofill, Sealer 26, Medium from empty molds (control)	N=2 (6 replicate)	Freshly mixed	Indirect contact testing with extract (sealer disc)	Mouse 3T3 fibroblasts	MTT

Year	Author(s)	Groups	N	Material condition (setting time)	Method	Cell model	Assay
	Pinna *et al.*[65]	MetaSEAL™, AH Plus Jet®, PCS, PMMA (positive control), Teflon (negative control)	n/s	Set (72h)	Direct contact testing with sealer disc	ROS 17/12.8	MTT
	Al-Sa´eed *et al.*[66]	Retroplast, Geristore®, Ketac™ Fil, SuperEBA™, ProRoot® MTA, Medium (control)	N=1 (10 replicate)	Set (specified time)	Indirect contact testing with extract (sealer disc)	Balb/c 3T3 fibroblasts	MTT
	Huang *et al.*[67]	AH 26®, Canals, N2®, Untreated (control)	N≥3 (triplicate)	Freshly mixed	Indirect contact testing with extract (sealer disc)	U2OS	Hoechst 33258 fluorescence
2007	Gorduysus *et al.*[68]	ProRoot® MTA, Diaket™, Endion®, CYMED 8410, Untreated (control)	N=7	Set (24h)	Indirect contact testing with extract (sealer specimen)	hPDLFs	MTT & Trypan Blue
	Lee *et al.*[70]	N2®, Sealapex™, AH 26®, Control (n/s)	N=1 (triplicate)	Freshly mixed	Indirect contact testing with extract (sealer sample)	RAW 264.7 macrophages	CCK-8/WST-8
	Lee *et al.*[71]	AH 26®, UDMA, Control (n/s)	N=1 (triplicate)	Freshly mixed	Indirect contact testing with extract (sealer sample)	RPC-C2A	MTT
	Lee *et al.*[72]	N2®, Sealapex™, AH 26®, Control (n/s)	N=1 (triplicate)	Freshly mixed	Indirect contact testing with extract (sealer sample)	MC3T3-E1	MTT
	Merdad *et al.*[73]	Epiphany®, AH Plus™, Filters with cells and no sealer and Filters no cells and with sealer (controls)	N=3	Freshly mixed & Set (24h, 48h)	Indirect contact testing with extract (sealer specimen)	HeLa	Millipore filter assay
	Lodiene *et al.*[74]	AH Plus™, EndoREZ®, RoekoSeal Automix, Epiphany®, Medium (control)	N=6-9	Fresh & Set (24h or light-curing)	Direct contact (sample) & Indirect contact (extract)	L929	MTT
2006	Key *et al.*[75]	Epiphany®, Resilon, GP, Grossman, Thermaseal®, Sealapex™. Isotonic saline and 10% formaldehyde (controls)	N=1 (triplicate)	Fresh (1h) & Set (24h)	Direct contact testing with sealer	hGFs	Trypan Blue Dye Exclusion
	Bouillaguet *et al.*[76]	AH Plus™, Epiphany®, GuttaFlow®, Teflon (control)	N=4	Set (overnight)	Direct contact testing with sealer disc	Balb/c 3T3 fibroblasts	MTT
2005	Miletic *et al.*[77]	Roekoseal Automix, AH Plus™, Control (n/s)	N=2 per group	Set (1h, 1d, 2d, 7d, 1m)	Direct contact testing with sealer	HeLa & L929	Nigrosin Dye

Year	Author(s)	Groups	N	Material condition (setting time)	Method	Cell model	Assay
2004	Al-Awadhi *et al.*[78]	Sealapex™, PCS, Roekoseal Automix, Medium (control)	n/s	Freshly mixed	Indirect contact testing with extract (sealer sample)	Embryonic rat osteoblasts	Trypan Blue Dye Exclusion
	Bouillaguet *et al.*[79]	PCS, RoekoSeal, TopSeal®, EndoREZ®, Teflon (control)	N=4	Fresh (after setting) & Set (24h)	Direct contact testing with sealer	Balb/c 3T3 fibroblasts	MTT
	Bonson *et al.*[81]	ProRoot® MTA, Geristore® (HICR), SuperEBA™, Bone, Amalgam, Plastic	N=2-4 (triplicate)	Freshly mixed & Set (24h)	Direct contact testing with sealer particles	hPDLFs & hGFs	Fluorescent cell attachment
2003	Camps *et al.*[82]	AH Plus™, Cortisomol™, Sealapex™, Medium (control)	N=10 per group	Freshly mixed & Set (24h)	Indirect contact with extract (sample & root model)	L929	MTT
	Mendes *et al.*[83]	PCS, Endofill, Medium (control)	N=3 (duplicate)	Freshly mixed	Direct contact testing with sealer fragments	Balb/c macrophages	Trypan Blue Dye Exclusion
2002	Schwarze *et al.*[84]	AH Plus™, Apexit®, Endométhasone, Ketac™ Endo, N2®, RoekoSeal, Gutta-percha, Medium (control)	N=3 (6 replicate)	Fresh (after root-end filling)	Indirect contact testing with extract (root model)	3T3 fibroblast & hPDLFs	XTT
	Huang *et al.*[85]	AH 26®, AH Plus™, Medium and DMSO (controls)	N=5 per group	Freshly mixed	Indirect contact testing with extract (sealer sample)	Rat cerebral astrocytes	MTT
	Schwarze *et al.*[86]	N2®, Endométhasone, Apexit®, AH Plus™, Ketac™ Endo, Untreated (control)	N=3 (5 replicate)	Freshly & Set (1h, 5h, 24h)	Indirect contact testing with extract (sealer sample)	3T3 fibroblast & hPDLFs	XTT
2000	Keiser *et al.*[87]	MTA, SuperEBA™, Amalgam, Medium (control)	N=1 (5 wells/ extract)	Freshly mixed & Set (24h)	Indirect contact testing with extract (sealer sample)	hPDLFs	MTT
	Azar *et al.*[88]	AH 26®, AH Plus™, ZOE, Distilled water (positive control)	N=4-8	Freshly mixed	Indirect contact testing with extract (sealer disc)	hGFs	Neutral Red
	Huang *et al.*[89]	AH 26®, AH Plus™, Medium (control)	N=3	Freshly mixed	Direct contact testing with DMSO-immersed sealer	Rat hepatocytes	LDH-leakage
	Schweikl *et al.*[90]	AH Plus™, Control (n/s)	N≥3 (8 replicate)	Freshly mixed & Set (24h)	Indirect contact testing with extract (sealer specimen)	V79B lung fibroblasts	Crystal violet dye

N represents the number of independent experiments. Setting time defined in hours (h), days (d) or months (m).
Cell lines: DF-MSCs, dental follicle-derived adult mesenchymal stem cells; HeLa, human cervical carcinoma cells; hGFs, human gingival fibroblasts; hMCs, human mesenchymal cells; hMRC-5, human fibroblasts; hOCs, human osteoblastic cells; hPDLCs, human periodontal ligament cells; hPDLFs, human periodontal ligament fibroblasts; hPDLSCs, human periodontal ligament stem cells; hTGSCs, human tooth germ stem cells; IDG-SW3, murine osteoblast-precursor cells; L929, mouse fibroblasts; MG63, human osteoblast-like cells; MC3T3-E1, mouse osteoblast-like cells; NIH/3T3, mouse fibroblasts; RAW 264.7, mouse macrophages; ROS 17/12.8, rat osteosarcoma cells; Saos-2, human osteoblast-like cells; U2OS, human osteoblasts; V79, Chinese hamster fibroblasts.
Abbreviations: GP, gutta-percha; HP, high plasticity; LDH, lactate dehydrogenase; n/s, non-specified; O.S., osteogenic supplementation (with ascorbic acid, β-glycerophosphate and dexamethasone); SCs, stem cells.

Similarly, PCS showed cytotoxicity in all the studies, except one.[83] Also, the formaldehyde-releasing epoxy resin-based sealer AH 26® and the zinc oxide-eugenol-based sealer N2® showed cytotoxic effects in all the studies. Moreover, methacrylate resin-based and silicone-based sealers, all showed cytotoxic effects.

In terms of sealer type, several studies reported no cytotoxic effect from bioceramic sealers, e.g. BioRoot™ RCS, ProRoot® MTA.[25,27,29,32,40,41,51,52,60,66,68,69,91,92,120] However, a cytotoxic effect has also been reported in comparison with other materials, either similar – compared to epoxy resin-based[29,36,47,80,93] or calcium hydroxide-based[32] sealers – or lower – compared to zinc oxide-eugenol-based[25,26,36,40,43,48], epoxy resin-based[24,33,36,37,47,48,93], methacrylate resin-based[33,43] or other materials.[30,35,45,55,81,87] Some studies reported a higher cytotoxic effect of MTA Fillapex® compared with epoxy resin-based sealers in set material condition.[29,37,41,93] Although one study[49] showed a higher cytotoxicity of Endosequence BC™ compared to epoxy resin-based sealers in set material conditions (although lower than PCS), one study[38] showed a lower cytotoxicity of this sealer compared with MTA-based materials. In respect to other materials, no cytotoxic effect was reported for the silicone-based sealer GuttaFlow® Bioseal[80] and the glass ionomer-based sealer Ketac™ Fil Plus and two root-end filling materials (i.e. Retroplast, Geristore®).[66] Also, Mendes *et al.*[83] reported no cytotoxic effect for zinc oxide-eugenol-based sealer Endofill, although other studies showed a cytotoxicity.[43,64,119] In addition, the cytotoxicity of urethane dimethacrylate (UDMA) and polymethyl methacrylate (PMMA) has also been reported by Lee *et al.*[71] and Pinna *et al.*[65], respectively. Furthermore, one study[82] showed no cytotoxic effect for the calcium hydroxide-based sealer Sealapex in set material condition. However, other studies showed lower[58,70,72], similar[32] and higher[32,82] cytotoxicity compared to other sealers. One study[75] showed opposing cytotoxic potential according to the setting condition, as Sealapex exhibited a lower cell toxicity in fresh material conditions (1h after mixing) compared to set material conditions (24h after preparation).

Generally, the results from the included studies suggested that bioceramic sealers may exhibit a lower cytotoxic potential compared to other types of root canal sealer.

Table 7 Summary of parameters and results collected from included *in vitro* studies.

Year	Author(s)	Groups	Extraction time	Extract concentration	Cell exposure time	Cytotoxic potential
2019	Lee *et al.*[24]	AH Plus™, MTA Fillapex®, Endosequence BC™, Medium (control)	7d	1, 1:5, 1:10, 1:50, 1:100	1d	Endosequence BC™ < MTA Fillapex® < AH Plus™
	Jeanneau *et al.*[25]	BioRoot™ RCS, PCS, Medium (control)	1d	0.2 mg/mL	3d, 6d, 9d	BioRoot™ RCS (nontoxic) < PCS
	Giacomino *et al.*[36]	Roth´s Sealer, AH Plus™, Endosequence BC™, ProRoot® ES, No cells (control), Medium (control)	3d	Several dilutions	7d	Endosequence BC™ < ProRoot® ES < Roth´s, AH Plus™
2018	Vouzara *et al.*[47]	SimpliSeal®, MTA Fillapex®, BioRoot™ RCS, Medium (control)	1d, 1w	1:1, 1:2	1d, 3d	BioRoot™ RCS < MTA Fillapex®, SimpliSeal®
2017	Arun *et al.*[58]	Tubli-Seal™, AH Plus™, Sealapex™, EndoREZ®, Medium (control) [groups with pachymic acid]	-	-	1d	Sealapex™ < AH Plus™ < Tubli-Seal™ < EndoREZ®
	Collado-González *et al.*[69]	BioRoot™ BCS, Endoseal®, Nano-ceramic Sealer (NCS), Medium (control)	1d	1:1, 1:2, 1:4	1d, 2d, 3d	BioRoot™ RCS (biocompatible) < NCS < Endoseal®
	Collado-González *et al.*[80]	GuttaFlow® Bioseal, GuttaFlow®2, MTA Fillapex®, AH Plus™, Medium (control)	1d	Undiluted, 1:2, 1:4	1d, 2d, 3d, 7d	GuttaFlow® Bioseal (nontoxic) < GuttaFlow®2, AH Plus™, MTA Fillapex®
	Cintra *et al.*[120]	MTA High Plasticity, MTA Angelus®, Medium (control)	3d	1:50	6h, 1d, 2d, 3d	MTA High Plasticity (nontoxic) < MTA Angelus®
	Zhu *et al.*[91]	iRoot® SP, MTA, Medium (control)	1d	Undiluted	1d, 2d	iRoot® SP, MTA (nontoxic)
	Cintra *et al.*[119]	Sealer Plus, AH Plus™, Endofill, SimpliSeal®, Medium (control)	3d	Undiluted, 1:2, 1:4	6h, 1d, 2d, 3d	Sealer Plus < SimpliSeal® < AH Plus™, Endofill
	Lv *et al.*[92]	iRoot® FS, iRoot® BP Plus, ProRoot® MTA, Medium (control)	3d	Undiluted, 1:2, 1:4	1d, 2d, 3d	iRoot® FS, iRoot® BP Plus, ProRoot® MTA (nontoxic)
2016	Suciu *et al.*[93]	MTA Fillapex®, AH Plus™, Acroseal, Plastic surface (control)	-	-	2d, 5d, 9d, 14d	hOCs: Acroseal, MTA Fillapex® < AH Plus™. DF-MSCs: Acroseal < AH Plus™ < MTA Fillapex®

Year	Author(s)	Groups	Extraction time	Extract concentration	Cell exposure time	Cytotoxic potential
2015	Camps *et al.*[26]	BioRoot™ RCS, PCS, Medium (control)	1d	Undiluted	2d, 5d, 7d	BioRoot™ RCS < PCS
	Dimitrova-Nakov *et al.*[27]	BioRoot™ RCS, PCS, Untreated cells (controls)	-	-	7d, 10d	BioRoot™ RCS (nontoxic) < PCS
	Konjhodzic-Prcic *et al.*[28]	GuttaFlow®, AH Plus™, Apexit®, EndoREZ®, Control (n/s)	1d	Undiluted	1d	All slightly cytotoxic
	Zhou *et al.*[29]	Endosequence BC™, MTA Fillapex®, Medium (control)	Fresh: 1d. Set: 1d, 1w, 2w, 3w, 4w	1:2, 1:8, 1:32, 1:128	3d	Endosequence BC™ (nontoxic). Fresh: MTA Fillapex® < AH Plus™. Set: AH Plus™ < MTA Fillapex®
2014	Jiang *et al.*[30]	iRoot® BP Plus, iRoot® FS, ProRoot® MTA, SuperEBA™, Medium (control)	1d, 3d, 7d, 14d	100%, 50%, 25%	1d	iRoot® BP Plus, iRoot® FS, ProRoot® MTA < SuperEBA™
	Cotti *et al.*[31]	RealSeal XT, AH Plus Jet®, Untreated (control)	-	-	1h, 1d, 2d, 3d	RealSeal XT < AH Plus Jet®
	Chang *et al.*[32]	Sealapex™, Apatite Root Sealer, MTA Fillapex®, iRoot® SP, Medium with & without O.S. (control)	-	-	3d, 7d, 14d	MTA Fillapex® (nontoxic) < Sealapex™, Apatite Root Sealer, iRoot® SP
	Mandal *et al.*[33]	GuttaFlow®2, ProRoot® MTA, AH Plus™, RealSeal™, Medium (control)	1d, 3d	0.5, 1, 1.5 cm^2/mL	1d	GuttaFlow®2 (nontoxic), ProRoot® MTA < AH Plus™, RealSeal™
	Camargo *et al.*[34]	AH Plus™, EndoREZ®, RoekoSeal, Medium (control)	1d	1:1, 1:2, 1:4, 1:8, 1:16, 1:32	1d	RoekoSeal < AH Plus™ < EndoREZ®
2013	Choi *et al.*[35]	ProRoot® MTA, Endocem, IRM®, Medium (control)	3d	Undiluted	12h, 1d, 2d, 3d	ProRoot® MTA, Endocem < IRM®
	Güven *et al.*[37]	MTA Fillapex®, iRoot® SP, AH Plus Jet®, Control (n/s)	-	-	1d, 3d, 7d, 14d	iRoot® SP < AH Plus™ < MTA Fillapex®
	Willershausen *et al.*[38]	MTA Angelus® (gray & white), ProRoot® MTA, Endosequence BC™, Untreated (control)	-	-	6h, 1d, 2d, 3d, 4d	Endosequence BC™ < MTA-based materials

Year	Author(s)	Groups	Extraction time	Extract concentration	Cell exposure time	Cytotoxic potential
	Kim *et al.*[39]	AH Plus™	1d	30%	1d	AH Plus™ was cytotoxic
2012	De-Deus *et al.*[40]	iRoot® BP Plus, ProRoot® MTA, Medium (negative control), ZOE (positive control)	1d, 2d	Undiluted	1d	ProRoot® MTA (nontoxic) < iRoot BP Plus < ZOE
	Bin *et al.*[41]	MTA Angelus®, MTA Fillapex®, AH Plus™, Untreated (control)	1d	1:1, 1:2, 1:4, 1:8, 1:16, 1:32	1d	MTA Angelus® (nontoxic) < AH Plus™ < MTA Fillapex®
	Scelza *et al.*[42]	RealSeal SE™, AH Plus™, GuttaFlow®, Sealapex™, Roth 801, ThermaSeal® Plus, Medium (control)	1d, 7d, 14d, 21d, 28d	Undiluted	1d	GuttaFlow® < AH Plus™ < ThermaSeal® Plus < Roth 801 < RealSeal™ < Sealapex™
	Salles *et al.*[43]	MTA Fillapex®, Epiphany® SE, Endofill, Untreated (control)	-	-	1d, 2d, 3d, 7d	MTA Fillapex® (toxic only for 3d) < Epiphany® SE, Endofill
	Landuyt *et al.*[44]	AH Plus Jet®, EndoREZ®, RealSeal™, Calcicur (control), Medium (negative control), 1% Triton X-100 (positive	1d	1:1, 1:3, 1:10, 1:30, 1:100, 1:300	1d	EndoREZ® < RealSeal™ < AH Plus Jet®
2011	Ma *et al.*[45]	ERRM Putty, ERRM Paste, ProRoot® MTA, IRM® (control), Cavit™ G (control), Medium (control)	1d	Undiluted, 1:1, 1:2, 1:4, 1:8	1d, 3d, 7d	ERRM, ProRoot® MTA < IRM®, Cavit™ G (related to setting and exposure times)
	Mukhtar-Fayyad[46]	BioAggregate®, iRoot® SP, Medium (control)	5d	Undiluted, 1:2, 1:10, 1:50, 1:100	1d, 3d, 7d	iRoot® SP < BioAggregate® (concentration-dependent)
	Zoufan *et al.*[48]	GuttaFlow®, Endosequence BC™, AH Plus™ Jet, TubliSeal Xpress™, Untreated (control)	1d, 3d	Eluates (300, 600 and 1000 µL)	1d	GuttaFlow®, Endosequence BC™ less toxic. F[1]: Tubli-Seal Xpress™ < AH Plus™. S[1]: AH Plus™ < Tubli-Seal Xpress™
	Loushine *et al.*[49]	Endosequence BC™, AH Plus™, PCS EWT (positive control), Teflon (negative control)	-	-	1d/week (for 6 weeks)	AH Plus™ < Endosequence BC™ < PCS
2010	Yu *et al.*[50]	AH 26®, Control (n/s)	1d, 3d, 5d, 7d	30%	1d, 2d	AH Plus™ was cytotoxic (extraction time-dependent)
	AlAnezi *et al.*[51]	ERRM, ProRoot® MTA, AH 26® (positive control), Control (n/s)	1d, 3d	Eluates (300, 600 and 1000 µL)	1d	ERRM, MTA (nontoxic) < AH 26® (both conditions)

Year	Author(s)	Groups	Extraction time	Extract concentration	Cell exposure time	Cytotoxic potential
	Zhang *et al.*[52]	iRoot® SP, AH Plus™, Medium (control)	1d	1:1, 1:2, 1:4	1d	iRoot® SP (nontoxic) < AH Plus
	Huang *et al.*[53]	AH 26®, Canals, N2®, Untreated (control)	1d	1:2, 1:4, 1:8	1d	Canals < N2® < AH 26® (concentration-dependent)
	Bryan *et al.*[54]	Experimental sealer (calcium silicate-based), AH Plus™, PCS, Teflon (negative control)	-	-	3d/week (for 5 weeks)	Experimental sealer < AH Plus™ < PCS (concentration-dependent)
	Badr[55]	PMMA, MTA, amalgam, Control (n/s)	-	-	2d	MTA, Bone cement < Amalgam (even more toxic in freshly conditions)
2009	Ames *et al.*[56]	EndoREZ®, RealSeal™, MetaSEAL™, RealSeal SE™, PCS (positive control), Teflon (negative control)	-	-	3d/week (for 5 weeks)	RealSeal SE™, MetaSEAL™ (both ↓ with time) < EndoREZ®, RealSeal™, PCS
	Donadio *et al.*[57]	Activ GP™, RealSeal™, AH 26®, Kerr Sealer, Untreated (control)	1d, 3d	Eluates (200, 400, 800 and 1200 µL)	1d	F[1]: Kerr < RealSeal™, Activ GP™ < AH 26® S[1]: AH 26®, Kerr < Activ GP™ < RealSeal™
	Gambarini *et al.*[59]	Epiphany® SE, Epiphany®, PCS, Untreated (control)	1d	Undiluted	1d	Epiphany®, Epiphany® SE, PCS
	De-Deus *et al.*[60]	BioAggregate®, ProRoot® MTA, Empty root canal (control)	1d, 2d, 3d	Undiluted	1d	ProRoot® MTA, BioAggregate® (nontoxic)
	Camargo *et al.*[61]	AH Plus™, Epiphany®, Acroseal, Castor Oil Polymer sealer, Untreated (control)	1d	1:1, 1:2, 1:4, 1:8, 1:16, 1:32	1d	Castor Oil Polymer << AH Plus™, Epiphany® < Acroseal
	Huang *et al.*[62]	AH 26®, Canals, N2®, Untreated (control)	1d	1:2, 1:4, 1:8	2d	Canals < AH 26®, N2® (concentration-dependent)
2008	Heitman *et al.*[63]	Epiphany®, Untreated (control)	-	25, 50, 100, 200, 400, 800 µg/mL	1d, 3d, 7d	Epiphany® was cytotoxic (concentration- and exposure time-dependent)
	Valois *et al.*[64]	AH Plus™, Endofill, Sealer 26, Medium from empty molds (control)	1d	20%, 10%, 5%	1d	All cytotoxic (concentration-dependent)

Year	Author(s)	Groups	Extraction time	Extract concentration	Cell exposure time	Cytotoxic potential
	Pinna *et al.*[65]	MetaSEAL™, AH Plus Jet®, PCS, PMMA (positive control), Teflon (negative control)	-	-	3d/week (for 5 weeks)	AH Plus Jet®, PMMA < MetaSEAL™ < PCS (time-dependent, except for PCS)
	Al-Sa´eed *et al.*[66]	Retroplast, Geristore®, Ketac™ Fil, SuperEBA™, ProRoot® MTA, Medium (control)	1d, 2d, 3d	Undiluted	1d	Retroplast, Geristore®, Ketac™ Fil and ProRoot® MTA (nontoxic); SuperEBA™ (cytotoxic)
	Huang *et al.*[67]	AH 26®, Canals, N2®, Untreated (control)	1d	1:2, 1:4, 1:8	2d	Canals < AH 26® < N2® (concentration-dependent)
2007	Gorduysus *et al.*[68]	ProRoot® MTA, Diaket™, Endion®, CYMED 8410, Untreated (control)	1d	Undiluted	1d, 2d, 3d	ProRoot® MTA (nontoxic) < Diaket™, Endion, CYMED 8410
	Lee *et al.*[70]	N2®, Sealapex™, AH 26®, Control (n/s)	1d	Dilution factor: 10 – 80	1d	Sealapex™ < AH 26® < N2® (concentration-dependent)
	Lee *et al.*[71]	AH 26®, UDMA, Control (n/s)	1d	5mg/mL and dilutions	1d	Cytotoxicity was concentration-dependent (prevented by NAC)
	Lee *et al.*[72]	N2®, Sealapex™, AH 26®, Control (n/s)	1d	Dilution factors: 6-18, 1-7, 5-100	1d	Sealapex™ < N2® < AH 26® (concentration-dependent)
	Merdad *et al.*[73]	Epiphany®, AH Plus™, Filters with cells and no sealer and Filters no cells and with sealer (controls)	-	-	2h	Epiphany® < AH Plus™
	Lodiene *et al.*[74]	AH Plus™, EndoREZ®, RoekoSeal Automix, Epiphany®, Medium (control)	1d (set)	Undiluted	2h	EndoREZ® < AH Plus™, RoekoSeal < Epiphany®
2006	Key *et al.*[75]	Epiphany®, Resilon, GP, Grossman, Thermaseal®, Sealapex™; Isotonic saline and 10% formaldehyde (controls)	-	-	1h, 1d	F[1]: Sealapex™ < others. S[1]: Thermaseal®, Epiphany® < others.
	Bouillaguet *et al.*[76]	AH Plus™, Epiphany®, GuttaFlow®, Teflon (control)	-	-	1d, 3d	GuttaFlow® < AH Plus™ < Epiphany® (exposure time-dependent)
2005	Miletic *et al.*[77]	Roekoseal Automix, AH Plus™, Control (n/s)	-	-	5d	RoekoSeal < AH Plus™ (setting time-dependent for AH Plus™)

Year	Author(s)	Groups	Extraction time	Extract concentration	Cell exposure time	Cytotoxic potential
2004	Al-Awadhi *et al.*[78]	Sealapex™, PCS, Roekoseal Automix, Medium (control)	1d	190mm²/1mL,50 or 300µL (b, ED50)	(a) 1d (b) 1d, 3d	(a) RoekoSeal, Sealapex™ < PCS (b) RoekoSeal < PCS, Sealapex™
	Bouillaguet *et al.*[79]	PCS, RoekoSeal, TopSeal®, EndoREZ®, Teflon (control)	-	-	1d 1d, 7d	RoekoSeal < PCS, TopSeal®, EndoREZ® (both fresh and set)
	Bonson *et al.*[81]	ProRoot® MTA, Geristore® (HICR), SuperEBA™, Bone, Amalgam, Plastic	-	-	1d, 3d, 5d, 7d, 9d, 11d, 13d	ProRoot® MTA, Geristore®, Bone < SuperEBA™, Amalgam
2003	Camps *et al.*[82]	AH Plus™, Cortisomol™, Sealapex™, Medium (control)	1d, 2d, 30d	Undiluted	1d	(a)AH Plus™ < Cortisomol™ < Sealapex™ (b)Sealapex™ < AH Plus™ < Cortisomol™
	Mendes *et al.*[83]	PCS, Endofill, Medium (control)	-	-	2h, 1d, 2d	PCS, Endofill (nontoxic)
2002	Schwarze *et al.*[84]	AH Plus™, Apexit®, Endométhasone, Ketac™ Endo, N2®, RoekoSeal, Gutta-percha, Medium (control)	24h, 1w-52w	Undiluted	1d	Pronounced cytotoxicity only by N2®
	Huang *et al.*[85]	AH 26®, AH Plus™, Medium and DMSO (controls)	1d	0.10, 0.08, 0.04, 0.02, 0.01 mg/mL	1d	Both cytotoxic (concentration-dependent)
	Schwarze *et al.*[86]	N2®, Endométhasone, Apexit®, AH Plus™, Ketac™ Endo, Untreated (control)	1d	Undiluted	1d	Apexit® < AH Plus™ < Ketac™ Endo < Endométhasone < N2®
2000	Keiser *et al.*[87]	MTA, SuperEBA™, Amalgam, Medium (control)	1d	1:1 and dilutions	1d	F[1]: MTA (lowest), Amalgam (highest) S[1]: MTA (lowest), SuperEBA™ (highest)
	Azar *et al.*[88]	AH 26®, AH Plus™, ZOE, Distilled water (positive control)	1h, 4h, 8h, 1d, 2d, 5d, 1-5w	Undiluted	22h	AH Plus™ only toxic in early phase (4h). AH 26® toxic for 1w and ZOE for 5w.
	Huang *et al.*[89]	AH 26®, AH Plus™, Medium (control)	-	-	(a) 1d (b) 4h, 10h, 1d	AH Plus™ < AH 26®
	Schweikl *et al.*[90]	AH Plus™, Control (n/s)	1d	Diluted	1d	Sealer eluted in DMSO was toxic. Sealer eluted in sodium chloride was nontoxic.

Extraction time and cell exposure time were defined as hours (h), days (d) or weeks (w).
[1] Material setting condition defined as fresh (F) or set (S).
Abbreviations: DF-MSCs, dental follicle-derived adult mesenchymal stem cells; G, Gray; GP, gutta-percha; hOCs, human osteoblastic cells; n/s, non-specified; NAC, N-acetyl-L-cysteine; O.S., osteogenic supplementation (with ascorbic acid, β-glycerophosphate and dexamethasone).

3.1.2. Influence of condition and time of material setting on cytotoxicity

To understand how the material set condition influences cytotoxicity, we focused on studies that used both set conditions, i.e. freshly mixed and set. Comparing AH Plus™ and MTA Fillapex®, Zhou *et al.*[29] showed that AH Plus™ was more toxic in freshly mixed conditions but less toxic after setting. This decrease in cytotoxicity with setting has also been confirmed by other authors.[34,48] Similarly to AH Plus™, Donadio *et al.*[57] showed that AH 26® was considerably more cytotoxic in freshly mixed conditions compared to set conditions (72h after preparation). Also, Badr[55] and Keiser *et al.*[87] showed a higher cytotoxicity of amalgam in freshly mixed conditions.

3.1.3. Influence of sealer concentration on cytotoxicity

In order to evaluate the influence exerted by the amount of sealer on cytotoxicity, we focused on studies that used an indirect contact testing methodology with several concentrations of sealer extract. In fact, a concentration-dependency of the cytotoxic effect was demonstrated for Activ GP™ [57], AH Plus™ [29,34,36,41,44,64,85], AH 26® [57,62,67,70–72,85], BioAggregate® and iRoot® SP[46], Canals[62,67], Endofill[64], EndoREZ® [34,44], Endosequence BC™ [24,36], Epiphany® [63], MTA Fillapex® [24,29,41], N2® [62,67,70,72], ProRoot® ES[36], RealSeal™ [44,57], RoekoSeal[34], Roth's Sealer[36], Sealapex™ [70,72] and Sealer 26.[64] Lee *et al.*[71] also showed a concentration-dependent cytotoxicity for UDMA.

3.1.4. Influence of exposure time to sealer on cytotoxicity

To evaluate the influence of the time of exposure, we considered only studies which tested more than one cell incubation time point. Accordingly, 33 studies fulfilled this criterion, of which 18 used direct contact testing as a method of cell exposure to several materials. From the 33 studies, 10 did not focus on comparing different incubation times.[26,27,32,38,68,78,91–93,119]

A certain heterogeneity was observed in regard to this subject. Some studies showed cell viability recovery over time of exposure for BioRoot™ RCS[69], GuttaFlow® Bioseal and GuttaFlow®2[80], MTA[120], MTA Fillapex® [43] and MetaSEAL™.[65] A recovery of cell viability was also denoted for PMMA after 5 weeks.[65] Other studies showed decreased cell viability over time of exposure for AH Plus™ [31,76,89], AH 26® [89], ProRoot® MTA[35,45], Endocem[35], GuttaFlow® [76], MTA Fillapex® [37], Epiphany® [63,76], Epiphany™ SE[43], RealSeal XT[31] and for other materials, namely IRM® [35,45], ERRM and Cavit™ G.[45]

Key *et al.*[75] showed a recovery of cell viability at 24h for Epiphany® and ThermaSeal®, when compared to 1 hour of exposure time, but a loss of viability for Sealapex™. Jeanneau *et al.*[25] showed an increased proliferation with increasing exposure time only for BioRoot™ RCS, as the inverse relationship was observed for PCS. Bouillaguet *et al.*[79] also showed a higher cytotoxicity for PCS at a second 24h- and 1 week-incubation periods, and also for RoekoSeal and EndoREZ® at 1 week-incubations, with all materials in fresh conditions. Furthermore, some studies showed a maintenance of cytotoxicity over time for PCS[49,56,65], RealSeal™ and EndoREZ®.[56] Mendes *et al.*[83] showed a maintenance of cell viability for PCS and Endofill, which were classified as nontoxic.

Bonson *et al.*[81] suggested that washed materials exhibited a lower cytotoxicity compared to fresh materials, over a 13-day experimental period of observation, in particular for ProRoot® MTA and Geristore®. Other studies that also used “aged” sealers (i.e. sealer specimens immersed in culture media with renewal), also showed a general recovery of cell viability over time for AH Plus™ and Endosequence BC™ after 6 weeks[49], AH Plus™ after 5 weeks[54], AH Plus Jet® after 5 weeks[65] and RealSeal SE™ and MetaSEAL™ over 5 weeks of observation.[56] In fact, these findings appear to be partially confirmed by studies which used different extraction time points.

Studies that performed cumulative extractions (i.e. same culture medium over the entire period of extraction) showed an increase in cytotoxicity over time of extraction for BioRoot™ RCS, MTA Fillapex® and SimpliSeal® [47], ProRoot® MTA and BioAggregate®.[60] Mandal *et al.*[33] showed increasing cell viability over time (72h compared to 24h) for AH Plus™ but decreased for GuttaFlow®2 and MTA in the higher concentration (all materials in set conditions).

On the other hand, studies that performed separate extractions (i.e. culture medium renewed after harvesting the extract from the previous time point) – which simulates periodontal ligament clearance[42] – showed a decrease in cytotoxicity over the time of extraction for several sealers (e.g. GuttaFlow®, AH Plus™).[42,50] Also using similar extraction

methods, Zhou *et al.*[29] showed a recovery of cell viability over time for AH Plus™ but not for MTA Fillapex®, which showed increased toxicity in more concentrated extracts (1:2 and 1:8). Al-Sa'eed *et al.*[66] showed an increased cell viability for 3-day eluates of Retroplast, Geristore® and Ketac™ Fil, as SuperEBA™ remained cytotoxic. Camps *et al.*[82] showed a decrease in cytotoxicity for Sealapex with no difference for AH Plus™ using a root-dipping technique. However, these results were not confirmed by experiments with International Organization for Standardization (ISO) Standards 10993-5 in the same study, as only Cortisomol™ had a decreasing cytotoxicity over time in this technique. Azar *et al.*[88] showed a decrease in cytotoxicity for both AH Plus™ (only toxic in first 4 hours) and AH 26® (toxicity decreased after 1 week), as no decrease was observed for ZOE cement. Other studies did not compare different extraction time points or did not show significant differences.[30,40,51,57,84]

3.2. *In Vivo* Biocompatibility

The general characteristics of the included studies are presented in Table 8. As can be seen, the main reported methods were the subcutaneous tissue response to sealer implants[98–100,102,105,107,108,110–112,115,117] and the periapical tissue response to root canal filling procedure[95–97,101,106,114,116,118]. Specifically, in relation to root filling procedures, these were carried out primarily in premolars (both maxillary and mandibular) and also in maxillary incisors in some studies. One study compared the tissue response by subcutaneous and alveolar socket implantation[113] and showed similar results for both implantation sites. The alveolar socket-implantation method following tooth extraction was also reported by Cintra *et al.*[104] In one study[103], Tanomaru-Filho *et al.* evaluated the periapical tissue response to retrobturation, after the induction of a periapical lesion, by exposure to oral environment for a period of 7 days. Furthermore, Assmann *et al.*[94] and Morinaga *et al.*[109] studied the bone tissue response to intraosseous sealer implants in the femur and the mandible of Wistar rats, respectively.

In regard to setting condition, most studies used the materials in a freshly mixed state, except for Garcia *et al.*[112] and Morinaga *et al.*[109], who only used materials in a set condition after photoactivation or after a period of one day and one night, respectively. Campos-Pinto *et al.*[98] and Ozbas *et al.*[108] used both materials in freshly mixed and in set conditions.

In terms of *in vivo* model, rats of different species or strains were used in 15 studies and dogs in 9. In one study, New Zealand rabbits were used as animal model.

Table 8 General characteristics of included studies in regard to *in vivo* biocompatibility.

Year	Author(s)	Groups (G)	N	Material condition (setting time)	Method	Teeth for root canal filling	Animal model
2019	Santos *et al.*[111]	G1: Empty PE tube (control); G2: GuttaFlow® Bioseal; G3: GuttaFlow®2; G4: AH Plus™	N=16 (4 implants per animal)	Freshly mixed	Subcutaneous tissue response to implant	-	Wistar rat
2015	Assmann *et al.*[94]	G1: MTA Fillapex® G2: AH Plus™ G3: Empty cavity (control)	N=15 (5 per time point)	Freshly mixed	Bone tissue response to implant	-	Wistar rat
2014	Silva *et al.*[95]	G1: Sealapex Xpress™/GP G2: RealSeal XT/Resilon	N=38 canals (SX/GP: 16, RS/R: 22)	Freshly mixed	Periapical tissue response to root canal filling	19 PMs (max. and mand.)	Beagle dog
2011	Suzuki *et al.*[106]	G1: Endométhasone/GP (short of apical foramen); G2: Endométhasone/GP (overfilling)	N=20 canals (10/group)	Freshly mixed	Periapical tissue response to root canal filling	INC (max.) and PMs (max. and mand.)	Mongrel dog (2)
2010	Garcia *et al.*[112]	Epiphany/Resilon (G1: with self-etch primer, G2: without primer); G3: Endofill/GP; G4: Empty dentin tube	N=15 (4 implants per animal)	Set (photoactivated)	Subcutaneous tissue response to implant	-	Rat
	Cintra *et al.*[113]	Empty PE tube (G1: alveolar, G2: subcutaneous); ProRoot MTA (G3: alveolar, G4: subcutaneous	N=40 (10/group)	Freshly mixed	Subcutaneous and alveolar tissue response to implant	-	Wistar rat
	Silva *et al.*[114]	G1: Calen® [a] thickened with ZnO; G2: IRCP paste; G3: ZOE cement; G4: Sterile saline	N=40 canals (8-12/group)	Freshly mixed	Periapical tissue response to root canal filling	PMs (max. and mand.)	Mongrel dog (2)
	Oliveira *et al.*[115]	G1: AH Plus™; G2: AH Plus™ with calcium hydroxide 5% (w/w); G3: Control (n/s)	N=30 (assigned to groups)	Freshly mixed	Subcutaneous tissue response to implant	-	Wistar rat
	Brasil *et al.*[116]	G1: Epiphany®/Resilon system G2: Pulp Canal Sealer/GP	N=30 canals (distributed to 2 groups)	Freshly mixed	Periapical tissue response to root canal filling	PMs (max. and mand.)	Beagle dog (2)

Year	Author(s)	Groups (G)	N	Material condition (setting time)	Method	Teeth for root canal filling	Animal model
	Zmener *et al.*[117]	G1: EndoREZ® + polimerization accelerator; G2: RealSeal™; G3: PCS (positive control); G4: Solid silicone rods (control)	N=8 per group	Freshly mixed	Subcutaneous tissue response to implant	-	Wistar rat
	Suzuki *et al.*[118]	G1: EndoREZ®/GP (short of the apical foramen) G2: EndoREZ®/GP (overfilling)	N=20 canals (10/group)	Freshly mixed	Periapical tissue response to root canal filling	INC (max.) and PMs (max. and mand.)	Mongrel dog (2)
2009	Tanomaru-Filho *et al.*[96]	G1: Intrafill; G2: AH Plus™; G3: RoekoSeal; G4: Epiphany®/Resilon system	N=64 canals (16/group)	Freshly mixed	Periapical tissue response to root canal filling	PMs (max. and mand.)	Mongrel dog (4)
2008	Leonardo *et al.*[97]	G1: RoekoSeal Automix G2: AH Plus™	N=32 canals (16/group)	Freshly mixed	Periapical tissue response to root canal filling	PMs (max. and mand.)	Dog (2)
	Campos-Pinto *et al.*[98]	G1: Epiphany®; G2: Photoactivated Epiphany®; G3: Epiphany® with self-etch primer; G4: Photoactivated Epiphany® with primer; G5: Empty PE tube	N=15 (5 implants per animal)	Freshly mixed & set (photoactivated)	Subcutaneous tissue response to implant	-	Wistar rat
2007	Zafalon *et al.*[99]	G1: Endométhasone; G2: EndoREZ® (Lateral wall outside Teflon tube was the negative control)	N=40 (20/group)	Freshly mixed	Subcutaneous tissue response to implant	-	Rat[b]
	Onay *et al.*[100]	G1: Teflon (negative control); G2: Epiphany®; G3: Gutta-percha; G4: Resilon	N=36 (4 implants per animal)	Freshly mixed	Subcutaneous tissue response to implant	-	Wistar rat
	Holland *et al.*[101]	ProRoot® MTA + Water (G1: cemental canal, G2: overfilling); ProRoot® MTA + Propyleneglycol (G3: cemental canal, G4: overfilling)	N=40 canals (10/group)	Freshly mixed	Periapical tissue response to root canal filling	INC (max.) and PMs (max. and mand.)	Dog (2)
2006	Shahi *et al.*[102]	G1: White MTA (Dentsply); G2: ProRoot® MTA (gray); G3: Sinaalloy; G4: Empty PE tube (control)	N=45 (4 implants per animal)	Freshly mixed	Subcutaneous tissue response to implant	-	Spraghe-Dawley rat

Year	Author(s)	Groups (G)	N	Material condition (setting time)	Method	Teeth for root canal filling	Animal model
	Tanomaru-Filho *et al.*[103]	G1: Sealer 26; G2: Sealapex™ + ZnO; G3: MTA; G4: No retrofilling	N=48 canals (10-14 per group)	Freshly mixed	Periapical tissue response (retrofilling after PA lesion)	PMs (max. and mand.)	Mongrel dog (4)
	Cintra *et al.*[104]	G1: Empty PE tubes (control); G2: ProRoot® MTA; G3: MBPc (new calcium hydroxide-based sealer)	N=48 (equally distributed)	Freshly mixed	Alveolar tissue response to implant	-	Wistar rat
2004	Kim *et al.*[105]	G1: PCS EWT; G2: ARS (type I); G3: ARS (type II); G4: CAPSEAL I; G5: CAPSEAL II; G6: Empty PTFE tube (control)	N=64 (total)	Freshly mixed	Subcutaneous tissue response to implant	-	Spraghe-Dawley rat
	Zmener[107]	G1: EndoREZ® G2: Solid silicone rods	N=24 (5-6 per time period)	Freshly mixed	Subcutaneous tissue response to implant	-	Wistar rat
2003	Ozbas *et al.*[108]	G1: Dyract compomer; G2: F2000 compomer; G3: Valux Plus composite; G4: Oralloy high-copper amalgam; G5: Empty PE tube (control)	N=30 (6 per time period)	Freshly mixed & set (photoactivated)	Subcutaneous tissue response to implant	-	Wistar rat
	Morinaga *et al.*[109]	G1: SuperEBA™ G2: Base liner (cyanoacrylate cement) G3: IRM®	N=60 (distributed)	Set (1d + overnight)	Bone tissue response to implant	-	Wistar rat
2001	Figueiredo *et al.*[110]	G1: *N*-Rickert; G2: AH 26®; G3: Fillcanal; G4: Sealer 26	N=30 (7-8/group)	Freshly mixed	Subcutaneous tissue response to implant	-	NZ rabbit

N represents the number of animals in studies with implantation methods or the number of root canals in studies with root canal filling procedures.
Setting time defined in days (d).
[a] Calen® is a calcium hydroxide + polyethyleneglycol-based paste.
[b] *Calomys callosus* rat.
Abbreviations: ARS, Apatite Root Sealer; GP, Gutta-percha; INC, incisors; IRCP, iodoform, Rifocort and camphorated paramonochlorophenol; max., maxillay; mand., mandibular; n/s, non-specified; NZ, New Zealand; PA, periapical; PE, polyethylene; PMs, premolars, PTFE, polytetrafluoroethylenef RS/R, RealSeal XT/Resilon; SX/GP, Sealapex Xpress/Gutta-percha.

3.2.1. Inflammatory tissue reaction to sealers

All studies showed a generalized inflammatory response to the materials tested, as presented in Table 9. AH Plus™, EndoREZ®, Epiphany® and ProRoot® MTA were the most studied sealers. Relatively to the epoxy resin-based sealer AH Plus™, Oliveira *et al.*[115] reported a nonspecific chronic inflammatory response, which can be reduced with the addition of calcium hydroxide. A slight to moderate inflammatory reaction was reported by other authors.[96] A similar inflammatory infiltrate was shown in comparison with silicone-based sealers RoekoSeal[96,97] and GuttaFlow®2[111], although higher comparing to GuttaFlow® Bioseal within 8 days of exposure.[111] Nevertheless, the same study showed that such difference had disappeared after 30 days. Assmann *et al.*[94] showed a lower neutrophil infiltrate in comparison to MTA Fillapex®, even though both sealers provided the re-establishment of original bone structure.

In regard to the methacrylate resin-based sealer Epiphany®, Garcia *et al.*[112] showed that the addition of its self-etch primer decreases the inflammatory reaction to the Epiphany/Resilon system. Similarly, Campos-Pinto *et al.*[98] showed that photoactivated Epiphany® without primer induced a moderate to severe inflammatory reaction with extensive necrosis, whereas only slight chronic inflammatory reaction was observed in the presence of the primer. Tanomaru-Filho *et al.*[96] showed a slight to moderate inflammatory reaction of Epiphany® comparable to AH Plus™ and RoekoSeal, as Onay *et al.*[100] showed an inflammatory reaction which varied from none to severe at first-week observation to none to slight reaction at eighth-week observation. Comparing the Epiphany/Resilon with a system of PCS/Gutta-percha, Brasil *et al.*[116] showed a similar biocompatibility, as both elicited a mild inflammatory reaction with macrophage and lymphocytes infiltrates.

Concerning EndoREZ®, Suzuki *et al.*[118] showed a mild to severe inflammatory reaction. A severe tissue reaction was also shown by Zmener *et al.*[117] for EndoREZ® combined with an accelerator (ACC, Ultradent Products Inc.), by Zmener[107] at a 10-day observation and by Zafalon *et al.* [99], who showed a high toxicity and late hypersensitive reaction to this sealer.

As to the bioceramic sealer ProRoot® MTA, a mild to moderate inflammatory response was shown by Cintra *et al.*[104,113] In comparison with Sinaalloy, a high-copper amalgam, Shahi *et al.*[102] showed a superior biocompatibility of MTA-based materials in an early phase (i.e. 3 days to 1 week), as no difference was reported for 3 weeks of exposure. Furthermore, Holland *et al.*[101] showed that the biocompatibility of this sealer was not affected by the vehicle, i.e. distilled water or propyleneglycol.

Table 9 Summary of parameters and results collected from included *in vivo* studies.

Year	Author(s)	Groups	Exposure time	Type of analysis	Outcomes	Biocompatibility
2019	Santos *et al.*[111]	G1: Empty PE tube (control); G2: GuttaFlow® Bioseal; G3: GuttaFlow®2; G4: AH Plus™	8d, 30d	Histology (H&E)	Macrophage infiltrate, thickness of fibrous capsule, vascular changes	At 8d, GuttaFlow® Bioseal had lower inflammatory reaction than GuttaFlow®2, AH Plus™. All biocompatible at 30d.
2015	Assmann *et al.*[94]	G1: MTA Fillapex® G2: AH Plus™ G3: Empty cavity (control)	7d, 30d, 90d	Histology (H&E)	Inflammatory infiltrate, fibers and hard tissue barrier formation	Both sealers provided re-establishment of original bone tissue structure. Inflammatory reaction decreased over time.
2014	Silva *et al.*[95]	G1: Sealapex Xpress™/GP G2: RealSeal XT/Resilon	90d	Histology (H&E and IHC for mineralization markers)	Biological apical sealing, inflammatory infiltrate, root and bone resorption	Both sealers allowed biological apical sealing with deposition of mineralized tissue.
2011	Suzuki *et al.*[106]	G1: Endométhasone/GP (short of apical foramen); G2: Endométhasone/GP (overfilling)	90d	Histology (H&E)	Biological apical sealing, root resorption, inflammatory infiltrate, presence of giant foreign-body cells and thickness and organization of PDL	Chronic inflammatory infiltrate in all specimens. Best result obtained with filling short of the apical foramen (vs. overfilling).
2010	Garcia *et al.*[112]	Epiphany/Resilon (G1: with self-etch primer, G2: without primer); G3: Endofill/GP; G4: Empty dentin tube	7d, 21d, 42d	Histology (H&E)	Inflammatory infiltrate, capacity of cellularity and vascularization, macrophagic activity	ER system with primer had lower inflammation, compared to system without primer, but higher compared to Endofill+GP.
	Cintra *et al.*[113]	Empty PE tube (G1: alveolar, G2: subcutaneous); ProRoot MTA (G3: alveolar, G4: subcutaneous	7d, 30d	Histology (H&E)	Inflammatory infiltrate	No differences regarding implantation site. At 30d, a more mature healing and lower inflammatory cell count was seen.
	Silva *et al.*[114]	G1: Calen® [a] thickened with ZnO; G2: IRCP paste; G3: ZOE cement; G4: Sterile saline	30d	Histology (H&E, Mallory Trichrome)	Intensity of inflammatory infiltrate, thickness of PDL, cementum resorption, dentin resorption and bone resorption	Calen®+ZnO > IRCP paste (mild inflammation and bone resorption) > ZOE (altered PA region and PDL, inflammation)

Year	Author(s)	Groups	Exposure time	Type of analysis	Outcomes	Biocompatibility
	Oliveira *et al.*[115]	G1: AH Plus™; G2: AH Plus™ with calcium hydroxide 5% (w/w); G3: Control (n/s)	14d	Histology (H&E, Masson´s Trichrome)	Inflammatory response (lymphocytes, plasmocytes, neutrophils, eosinophils, macrophages, giant foreign-body cells, blood vessels)	All showed nonspecific chronic inflammation. Calcium hydroxide improved biocompatibility of AH Plus™.
	Brasil *et al.*[116]	G1: Epiphany®/Resilon system G2: Pulp Canal Sealer/GP	60d	Radiographic evaluation & Histology (H&E)	Radiographic evaluation (quality of filling, apical limit and extruded material) & Histology (biological apical sealing, PDL thickness, inflammatory reaction, resorption)	Similar biocompatibility between systems: mild inflammatory reaction (macrophages and lymphocytes).
	Zmener *et al.*[117]	G1: EndoREZ® + polimerization accelerator; G2: RealSeal™; G3: PCS (positive control); G4: Solid silicone rods (control)	10d, 30d, 90d	Histology (H&E)	Fibrous capsule formation, inflammatory infiltrate (PMN leukocytes, lymphocytes, plasmocytes, macrophages, giant foreign-body cells), capillaries	EndoREZ® & RealSeal™ had severe inflammation reaction (resolved over time). PCS had severe reaction (over time).
	Suzuki *et al.*[118]	G1: EndoREZ®/GP (short of the apical foramen) G2: EndoREZ®/GP (overfilling)	90d	Histology (H&E, Brown and Brenn staining)	Biological apical sealing, apical cementum resorption, intensity of inflammatory infiltrate, organization and thickness of PDL	Both groups showed inflammation. Best result obtained with filling short of the apical foramen (vs. overfilling).
2009	Tanomaru-Filho *et al.*[96]	G1: Intrafill; G2: AH Plus™; G3: RoekoSeal; G4: Epiphany®/Resilon system	90d	Histology (H&E, Mallory Trichrome)	Intensity of inflammatory infiltrate, PDL thickness, bone and apical cementum resorption, biological apical sealing	AH Plus™, RoekoSeal, Epiphany® (slight to moderate) > Intrafill (severe inflammation and PDL thickening)
2008	Leonardo *et al.*[97]	G1: RoekoSeal Automix G2: AH Plus™	90d	Histology (H&E, Mallory Trichrome, Brown and Brenn staining)	Newly mineralized formed tissue, periapical inflammatory infiltrate, apical PDL thickness, cementum, dentin and bone resorption	For biological apical sealing: RoekoSeal > AH Plus™. Similar infiltrate, PDL thickening and resorption.
	Campos-Pinto *et al.*[98]	G1: Epiphany®; G2: Photoactivated Epiphany®; G3: Epiphany® with self-etch primer; G4: Photoactivated Epiphany® with primer; G5: Empty PE tube	7d, 21d, 42d	Histology (H&E)	Neutrophils, leukocytes, macrophages, lymphocytes, plasmocytes, giant foreign-body cells, dispersed material, necrotic tissue	All groups showed mild inflammation. Group with photoactivation+no primer showed necrosis and more inflammation.

Year	Author(s)	Groups	Exposure time	Type of analysis	Outcomes	Biocompatibility
2007	Zafalon *et al.*[99]	G1: Endométhasone; G2: EndoREZ® (Lateral wall outside Teflon tube was the negative control)	15d, 30d, 60d, 90d	Histology (H&E)	FDI criteria: new bone, neutrophils, macrophages, lymphocytes, plasmocytes, giant foreign-body cells, dispersed material, capsule, necrotic tissue, resorption	Endométhasone (tissue reaction decreased over time) > EndoREZ® (highly toxic and late hypersensitive reaction)
	Onay *et al.*[100]	G1: Teflon (negative control); G2: Epiphany®; G3: Gutta-percha; G4: Resilon	1w, 4w, 8w	Histology (H&E, Masson´s Trichrome)	Stromal inflammatory response, infiltration of mast cells, proliferation of fibroblasts, vascular changes, granulation tissue, giant foreign-body cells	All groups induced inflammation. Tissue reaction decreased over time.
	Holland *et al.*[101]	ProRoot® MTA + Water (G1: cemental canal, G2: overfilling); ProRoot® MTA + Propyleneglycol (G3: cemental canal, G4: overfilling)	90d	Histology (H&E, Brown and Brenn staining)	Thickness and extension of newly formed cementum, biological sealing, resorption, microorganisms, intensity and extension of inflammatory infiltrate, PDL, debris	Vehicle did not influence biocompatibility of MTA. Best result obtained with filling short of the apical foramen (vs. overfilling).
2006	Shahi *et al.*[102]	G1: White MTA (Dentsply); G2: ProRoot® MTA; G3: Sinaalloy; G4: Empty PE tube (control)	3d, 1w, 3w	Histology (H&E)	Inflammatory reaction: accumulation of acute and chronic inflammatory cells, fibrin deposits, tissue edema and vascular congestion	At 3 days and 1 week, MTA-based materials were more biocompatible. At 3 weeks, no difference was observed.
	Tanomaru-Filho *et al.*[103]	G1: Sealer 26; G2: Sealapex™ + ZnO; G3: MTA; G4: No retrofilling	180d	Histology (H&E, Mallory Trichrome)	Periapical inflammatory infiltrate, apical PDL thickness, deposition of cementum on the sectioned apical surface, cementum and bone resorption, apical dentin resorption	Sealer 26, Sealapex™ with ZnO and MTA provided PA repair. Control showed unsatisfactory PA repair.
	Cintra *et al.*[104]	G1: Empty PE tubes (control); G2: ProRoot® MTA; G3: MBPc (new calcium hydroxide-based sealer)	7d, 15d, 30d	Histology (H&E, Brown and Brenn staining)	Extent and intensity inflammatory infiltrate based on cell count and extension beyond implants	All groups showed similar biological response (mild to moderate inflammatory response).
2004	Kim *et al.*[105]	G1: PCS EWT; G2: ARS (type I); G3: ARS (type II); G4: CAPSEAL I; G5: CAPSEAL II; G6: Empty PTFE tube (control)	1w, 2w, 4w, 12w	Histology (H&E)	Thickness of reaction zone, inflammatory infiltrate (macrophages, plasmocytes, lymphocytes, neutrophils	Capseal groups showed lower tissue response than others. In all groups, inflammatory reaction decreased over time.

Year	Author(s)	Groups	Exposure time	Type of analysis	Outcomes	Biocompatibility
	Zmener[107]	G1: EndoREZ® G2: Solid silicone rods	10d, 30d, 90d, 120d	Histology (H&E)	Fibrous capsule formation, inflammatory infiltrate (PMN leukocytes, lymphocytes, plasmocytes, macrophages, giant foreign-body cells), capillaries	Inflammation was observed with EndoREZ® (decreased with time). Control showed mild inflammation only at 10d.
2003	Ozbas *et al.*[108]	G1: Dyract compomer; G2: F2000 compomer; G3: Valux Plus composite; G4: Oralloy high-copper amalgam; G5: Empty PE tube (control)	7d, 15d, 30d, 60d, 90d	Histology (H&E)	Inflammatory infiltrate (lymphocytes, plasmocytes, PMN leukocytes, macrophages, giant foreign-body cells), necrosis, formation of calcifications	All groups showed moderate to severe inflammatory reactions at 7d, which decreased at 60d and 90d.
	Morinaga *et al.*[109]	G1: SuperEBA™ G2: Base liner (cyanoacrylate cement) G3: IRM®	4w, 8w	Histology (H&E)	Presence of connective tissue or bone formation, presence of macrophage infiltration, thickness of fibrous connective tissue	Base liner > SuperEBA™, IRM® (development of fibrous connective tissue and macrophage infiltration).
2001	Figueiredo *et al.*[110]	G1: N-Rickert; G2: AH 26®; G3: Fillcanal; G4: Sealer 26	90d	Histology (H&E)	Histopathologic evaluation (granulation tissue, lymphocytes, PMN neutrophils and eosinophils, plasmocytes, macrophages, giant foreign-body cells)	Sealer 26 (mild irritation) > N-Rickert and AH 26® (moderate) > Fillcanal (severe irritation).

N represents the number of animals in studies with implantation methods or the number of root canals in studies with root canal filling procedures.
Exposure time was defined in days (d) or weeks (w).
Abbreviations: ARS, Apatite Root Sealer; ER, Epiphany/Resilon system; FDI, Féderation Dentaire Internationale; GP, Gutta-percha; H&E, Hematoxylin-eosin; IHC, immunohistochemistry; IRCP: iodoform, Rifocort (5mg prednisolone acetate + 1.5mg Rifamycin SV sodium) and camphorated paramonochlorophenol; n/s, non-specified; PA, periapical; PE, polyethylene; PEG, polyethyleneglycol; PDL, periodontal ligament; PMN, polymorphonuclear; PTFE, polytetrafluoroethylene.

All other materials elicited an inflammatory tissue reaction of variable degree. In a study of retrofilling, Tanomaru-Filho *et al.*[103] showed that Sealer 26, Sealapex™ associated with zinc oxide and MTA provided periapical repair, despite the slight to moderate inflammatory infiltrate.

3.2.2. Time of exposure influence on biocompatibility

In order to understand how the time of exposure influences the biocompatibility of root canal sealers, we focused on studies that reported more than one exposure time points. Based on the results from the included studies, a time-dependency (i.e. resolution of tissue reaction over time) has been shown for the following sealers: AH Plus™ [94,111], Endométhasone[99], GuttaFlow®2[111], GuttaFlow® Bioseal[111], MTA Fillapex® [94] and RealSeal™.[117] The decrease in tissue reaction has also been shown for other materials[105,108], as presented in Table 9.

For Epiphany®, contrary evidence was found as Garcia *et al.*[112] and Onay *et al.*[100] showed a decrease in tissue reaction over time whereas Campos-Pinto *et al.*[98] suggested a resolution of the tissue reaction. Studies on EndoREZ® also showed conflicting results, as the time-dependency has been shown either as isolated sealer [107] or associated with an accelerator[117], whereas Zafalon *et al.*[99] showed evidence of severe inflammatory infiltrate even 90 days after implantation.

Regarding ProRoot® MTA, the maturation of the healing process over time with a decrease in inflammatory infiltrate and improvement of connective tissue organization has been shown.[104,113]

3.2.3. Influence of apical limit of root canal filling on biocompatibility

Three studies aimed to evaluate the influence of the apical limit for root canal filling on biocompatibility to root canal sealers.[101,106,118] The three studies demonstrated a better biocompatibility with root canal filling short of the apical foramen, in comparison with overfilling for all sealers tested, i.e. Endométhasone, EndoREZ® and ProRoot MTA®.

3.3. Risk of bias

The results of the quality assessment of the studies are presented in Appendix I (*in vitro*) and Appendix II (*in vivo*) and are schematically represented in Fig. 3 (*in vitro*) and Fig. 4 (*in vivo*).

Regarding *in vitro* studies, three studies[49,54,65] reported calculation of the sample size. Relatively to the randomization process, only two studies[40,60] reported these items. No studies reported researcher blinding to the procedures. Only few studies reported the estimated size of effect and its precision. All studies reported information relatively to the background and aims, except for one.[27]

Concerning *in vivo* studies, the allocation sequence generation was unclear in several studies. No study reported allocation concealment, random animal housing and caregiver and/or researcher blinding. Only one study reported random outcome assessment. Other sources of risk of bias were found in most of the studies, mainly due to unit of analysis errors (e.g. multiple interventions per animal) and due to addition of animals in replacement of drop-outs from the original sample.

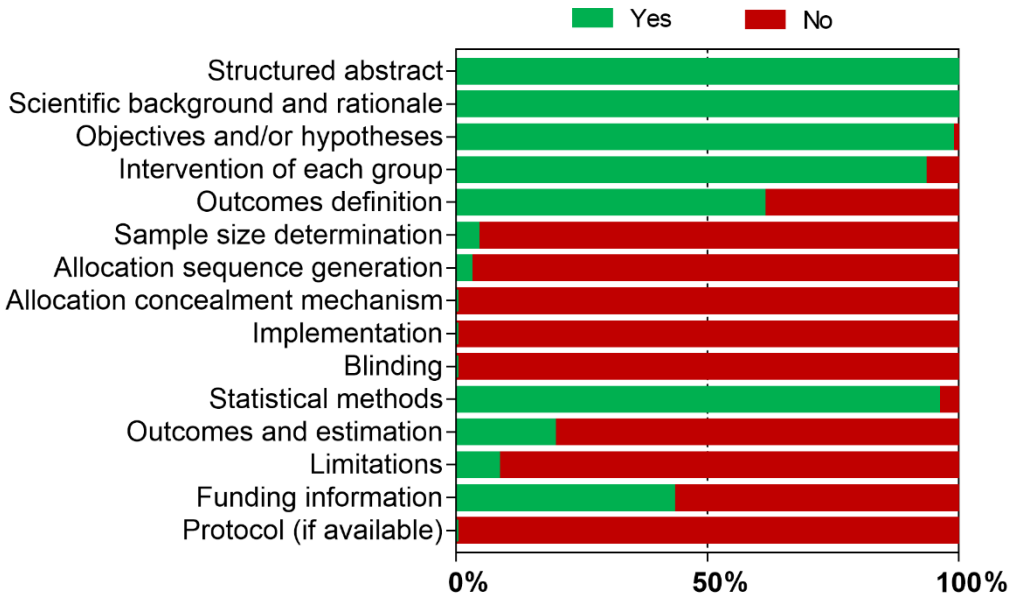

Figure 3 Methodological quality assessment of *in vitro* studies.

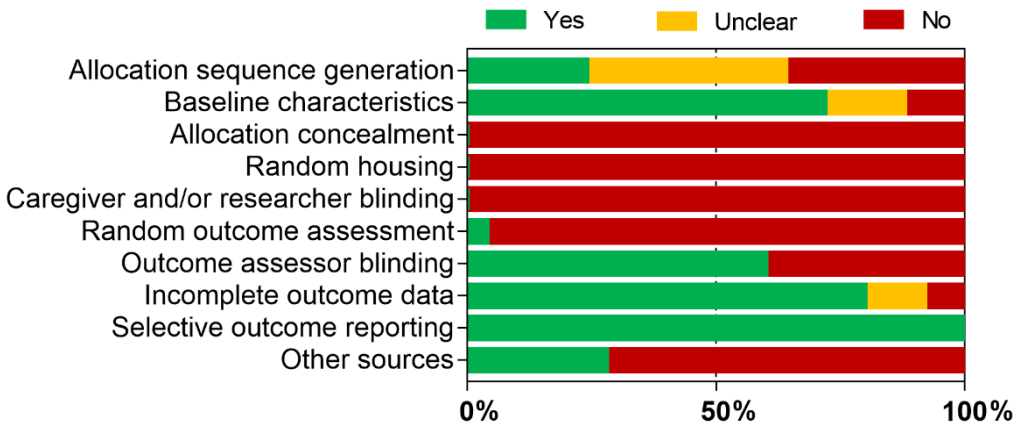

Figure 4 Methodological quality assessment of *in vivo* studies.

4. DISCUSSION

In the context of root canal therapy, materials used for root canal filling may come in contact with the periapical tissue.[4] Ideally, these materials should allow or promote the resolution of periapical inflammatory and/or infectious processes, also preventing further contamination with microorganisms.[4] Among the biological properties desirably shown by sealers (e.g. antimicrobial effect, osteogenic potential), biocompatibility is considered a key property of root canal sealers[4,5,24], thus demonstrating the importance of the study of biocompatibility of different endodontic materials.[33]

For root canal filling, the combination of a sealer with a central core material, such as gutta-percha, has been a standard.[4,7] Several reasons support the widespread use of gutta-percha, namely its plasticity, low toxic potential, ease of manipulation, radiopacity and ease of removal, even though the lack of adhesion to dentin and shrinkage after cooling are known disadvantages of this material.[4] Other core materials and/or obturation systems have also been developed, such as resin-based obturation systems with the high-performance synthetic polyester–based Resilon (e.g. in association with RealSeal™ or Epiphany®) and Activ GP™, which consists of glass ionomer-impregnated gutta-percha cones.[4,7]

Here, we aimed to perform a systematic review of the literature on the cytotoxicity and biocompatibility of root canal sealers, in order to understand how these materials (individually or by type) perform in terms of biocompatibility in experimental cell and animal models. Furthermore, we also aimed to understand how the material setting condition, concentration, time and type of exposure influence the cytotoxicity and biocompatibility of these materials. As a multiplicity of methods and conditions has been reported in previous studies in this area, an overview on this subject could become difficult as well as the interpretation of the results. Therefore, a systematic review of the literature may be a useful tool to integrate such concepts and data.

Over the years, several materials have been developed for root canal filling. According to chemical composition and structure, sealers may be classified into the following types: zinc oxide-eugenol-based, resin-based, glass ionomer-based, silicone-based, calcium hydroxide-based and bioceramic sealers, which includes calcium silicate-based, MTA-based and calcium phosphate-based sealers. In regard to retrograde filling, MTA, amalgam, IRM® and SuperEBA™ are some of the materials that have been used.[3] AH Plus™ has been the most studied sealer over the last two decades, either as a test sealer or as reference

material, thus we applied a date limit to our search in order to retrieve articles since its introduction as a new substitute to AH 26®.[89]

In this systematic review, the set of included studies assessed the cytotoxicity and biocompatibility of multiple sealers of the different types. Among *in vitro* studies, the most studied sealers were the zinc oxide-eugenol-based PCS, the epoxy resin-based AH 26® and AH Plus™, the methacrylate resin-based EndoREZ® and Epiphany®, the calcium hydroxide-based Sealapex™ and the bioceramic sealers Endosequence BC™, MTA Fillapex® and ProRoot® MTA. AH Plus™, EndoREZ®, Epiphany® and ProRoot® MTA were also the most studied *in vivo*.

Concerning *in vitro* cytotoxicity, results suggested a lower cytotoxic potential from bioceramic sealers, even though some conflicting evidence was found, particular in regard to MTA Fillapex®, which may be due to the release of lead in set conditions.[29] This lower cytotoxicity of bioceramic sealers is in accordance with previous systematic reviews on the biological, physiochemical and clinical properties of calcium silicate-based sealers in comparison with conventional materials.[17–19]

A considerable methodological heterogeneity was observed in relation to several parameters, for example material setting condition, setting time and sealer extract dilution. As to setting condition, several studies performed experiments with freshly mixed sealers, others used set materials and others both freshly mixed and set conditions. Moreover, multiple setting times were reported from 1 hour to 1 month. In general, studies that assessed both conditions reported a differential in cytotoxic potential, with freshly mixed materials exhibiting higher cytotoxic potential.

The important role of setting conditions on the biological properties of sealers has been recognized, as differences have been reported between fresh and set sealers[119,120], which may account for some of the heterogeneity in the literature. However, such differences seem to decrease with setting.[4,15] The release of unconverted monomers may play a role in the cytotoxicity of sealers in freshly mixed conditions, whereas, in set condition, a residual toxic effect, amount- and elution kinetics-dependent, may be expected for these compounds.[31] However, the leaching of unconverted or partially converted constituents with potential toxicity may also remain after setting of the material.[58] In fact, the role of setting time has been studied by Camargo *et al.*[34], who suggested further research should be carried out aiming at evaluating this setting time-dependency over longer experimental periods. Arun *et al.*[58] also suggested that long-term clinical studies are important to understand if these materials maintain as cytotoxic over time or loose the initial toxic potential.

From a clinical perspective, the use of freshly mixed sealers is relevant because these materials are applied in an unset condition when introduced in root canals, becoming in contact with the periapical tissues.[26,31]

In studies which tested sealer extracts in multiple concentrations, results suggested a concentration-dependency of the cytotoxicity, i.e. increasing cytotoxic potential with increasing extract concentration, for several sealers.

Furthermore, different contact methods were used, specifically direct contact testing, indirect contact testing with sealer extracts and indirect contact testing with incubation of cells with sealer specimens (without direct contact, using for example inserts). Previous studies have suggested that direct contact exposure may lead to increased toxicity, in comparison with other methods and in spite of the acceptable clinical performance.[56,82] However, as a direct contact between the sealer and the periapical tissue is possible during and after root filling procedures[4,82], such contact method may provide important information on the cytotoxicity of these materials as it simulates the possible extrusion of unset sealer in the periapical tissues.[31,56,65,82] Furthermore, some studies used root models[26,40,60,82,84], which may represent a useful model as it attempts to simulate the reality of endodontic treatments.[26]

Regarding the influence of exposure time on the cytotoxic effects of the materials, studies showed a certain heterogeneity. Interestingly, studies that used washed-out or "aged" sealers reported a general recovery of cell viability over 5-6 weeks of observation.[49,54,56,65] Also, Bonson *et al.*[81] reported a lower cytotoxicity of washed sealers over 13 days of observation.

As mentioned, these findings seem to be supported by studies that tested sealers by extraction methods, with different extraction time points. Methodologically, a difference in studies was observed in this regard, as some studies performed cumulative extractions, i.e. no medium renewal, and others carried out separate extractions, that is with medium renewal. In general, the first method appears to be related to higher cytotoxic effects. In a way, such findings may be related to the time-dependent release of compounds with setting, as previously discussed.

The *in vitro* studies included in this systematic review are indicative of differences between the several root canal sealers. Furthermore, most studies followed the ISO Standards 10993-5:2009, which encompasses direct and indirect contact methods, fresh and set materials and several extract concentrations. However, the concentrations tend to be

higher compared to the clinical context. Therefore, care should be taken when extrapolating these results for clinical practice.

Relatively to the *in vivo* evidence, all studies showed an inflammatory reaction in response to the several sealers, independently of type, that ranged from slight to severe inflammatory reactions. Nevertheless, studies also generally suggested that the tested sealers presented acceptable biocompatibility. The ability to provide the re-establishment of original bone structure was also shown for some sealers, such as AH Plus™ and MTA Fillapex®.[94]

Moreover, different methods were used for the assessment of tissue response to sealers. The ISO Standards 7405 on the biological evaluation of dental materials were followed in most studies. In this context, several studies assessed the tissue response to subcutaneous or intraosseous sealer implantation and others the periapical tissue response to root filling procedure. In one study, Cintra *et al.*[113] compared the subcutaneous implantation and the alveolar socket implantation (i.e. sealer implantation in alveolar socket post-extraction) methods and showed the equivalency between methods. This method was also reported in another study[104], further suggesting it could be an interesting method to study tissue response to dental materials. From the studies that performed root filling procedures, Tanomaru-Filho *et al.*[103] evaluated periapical tissue response to retrofilling, after periapical lesion induction, in order to simulate the clinical conditions of endodontic surgery.

The influence of exposure time on biocompatibility was shown by several studies, which showed that the initial inflammatory reaction tends to subside over time.[94,99,105,108,111,117] In addition to the decrease in inflammatory infiltrate, ProRoot® MTA has been shown to promote the improvement of connective tissue organization over time, thus suggesting the maturation of the healing process.[104,113] However, conflicting results were found for some sealers, specifically Epiphany® and EndoREZ®.

In addition, three *in vivo* studies tested the biocompatibility of root canal fillings by comparison of two apical limits, short of the apical foramen and overfilling.[101,106,118] As expected, a better biocompatibility was shown in fillings short of the apical foramen, in accordance with previous literature.

In regard to the methodologic quality, eligible studies exhibited a considerable risk of bias, with several studies lacking information on randomization processes, blinding and outcome measures, including estimated size of effects and its precision.

5. CONCLUSION

In this study, we carried out a systematic review of the literature on the cytotoxicity and biocompatibility of root canal sealers.

A joint analysis of the included *in vitro* and *in vivo* studies reveals that sealers elicit variable toxic effects at cellular and tissue level. Although a tendency for lower cytotoxicity of bioceramic sealers was noted, such finding was not observed *in vivo*, probably due to the smaller number of studies and sealers and to the variability of conditions tested. Nevertheless, results suggested that several factors may influence the biocompatibility of these materials, particularly setting condition and time and type of exposure, among others.

Further research is warranted to achieve a better understanding of the biological effects of root canal sealers, with precisely designed studies and accurate and complete reporting.

From a clinical perspective, the extrapolation of these results must be taken into caution due to several aspects, namely: biocompatibility was assessed in experimental models; some methods do not correlate directly to the clinical reality of endodontic treatments, e.g. testing only set materials; and other material properties should be taken into account, e.g. antimicrobial and physicochemical properties.

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APPENDIX

Appendix I Results of risk of bias assessment of *in vitro* studies according to the modified CONSORT checklist.[22]

Study	1	2a	2b	3	4	5	6	7	8	9	10	11	12	13	14
Lee *et al.*[24]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	Y	N
Jeanneau *et al.*[25]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	Y	N
Giacomino *et al.*[36]	Y	Y	Y	N	Y	N	N	N	N	N	Y	N	N	N	N
Vouzara *et al.*[47]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	N
Arun *et al.*[58]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	N	N
Collado-González *et al.*[69]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	Y	N
Collado-González *et al.*[80]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	Y	N
Cintra *et al.*[120]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	Y	N
Zhu *et al.*[91]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	Y	N
Cintra *et al.*[119]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	Y	N
Lv *et al.*[92]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	Y	N
Suciu *et al.*[93]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	N	N
Camps *et al.*[26]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	Y	N
Dimitrova-Nakov *et al.*[27]	Y	Y	N	Y	N	N	N	N	N	N	Y	N	N	N	N
Konjhodzic-Prcic *et al.*[28]	Y	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	Y	N
Zhou *et al.*[29]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	Y	Y	N
Jiang *et al.*[30]	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N	Y	N
Cotti *et al.*[31]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	N
Chang *et al.*[32]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	Y	N
Mandal *et al.*[33]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	N
Camargo *et al.*[34]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	Y	N
Choi *et al.*[35]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	N	N
Güven *et al.*[37]	Y	Y	Y	Y	N	N	N	N	N	N	N	N	N	N	N
Willershausen *et al.*[38]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	Y	N
Kim *et al.*[39]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	N	N
De-Deus *et al.*[40]	Y	Y	Y	Y	N	N	Y	N	N	N	Y	N	Y	Y	N
Bin *et al.*[41]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	N
Scelza *et al.*[42]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	Y	N
Salles *et al.*[43]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	N	N
Landuyt *et al.*[44]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	Y	N
Ma *et al.*[45]	Y	Y	Y	N	Y	N	N	N	N	N	Y	N	N	N	N
Mukhtar-Fayyad[46]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	N
Zoufan *et al.*[48]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	N
Loushine *et al.*[49]	Y	Y	Y	Y	Y	Y	N	N	N	N	Y	Y	Y	N	N
Yu *et al.*[50]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	N	N
AlAnezi *et al.*[51]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	N

Zhang *et al.*[52]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	N	N
Huang *et al.*[53]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	Y	N
Bryan *et al.*[54]	Y	Y	Y	Y	Y	Y	N	N	N	N	Y	N	N	N	N
Badr[55]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	N
Ames *et al.*[56]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	Y	N
Donadio *et al.*[57]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	N
Gambarini *et al.*[59]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	N	N
De-Deus *et al.*[60]	Y	Y	Y	Y	N	N	Y	N	N	N	Y	Y	N	Y	N
Camargo *et al.*[61]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	Y	N
Huang *et al.*[62]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	Y	Y	N
Heitman *et al.*[63]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	Y	N	N
Valois *et al.*[64]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	N
Pinna *et al.*[65]	Y	Y	Y	Y	Y	Y	N	N	N	N	Y	Y	N	N	N
Al-Sa'eed *et al.*[66]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	N
Huang *et al.*[67]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	Y	N
Gorduysus *et al.*[68]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	N	N
Lee *et al.*[70]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	Y	N
Lee *et al.*[71]	Y	Y	Y	N	N	N	N	N	N	N	Y	N	N	Y	N
Lee *et al.*[72]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	N	N
Merdad *et al.*[73]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	Y	N
Lodiene *et al.*[74]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	N
Key *et al.*[75]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	N
Bouillaguet *et al.*[76]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	N
Miletic *et al.*[77]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	N
Al-Awadhi *et al.*[78]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	N	N
Bouillaguet *et al.*[79]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	N
Bonson *et al.*[81]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	Y	N
Camps *et al.*[82]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	N	N
Mendes *et al.*[83]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	N	N
Schwarze *et al.*[84]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	N	N
Huang *et al.*[85]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	Y	N
Schwarze *et al.*[86]	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	N	N	N
Keiser *et al.*[87]	Y	Y	Y	N	Y	N	N	N	N	N	Y	N	N	Y	N
Azar *et al.*[88]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	N	N
Huang *et al.*[89]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	Y	N
Schweikl *et al.*[90]	Y	Y	Y	N	Y	N	N	N	N	N	N	Y	N	N	N

Abbreviations: N, No; Y, Yes.

Checklist items:
1 – Structured abstract
2a – Scientific background and rationale; 2b – Objectives and/or hypotheses
3 – Intervention of each group; 4 – Outcomes definition; 5 – Sample size determination; 6 – Allocation sequence generation; 7 – Allocation concealment mechanism; 8 – Implementation; 9 – Blinding; 10 – Statistical methods
11 – Outcomes and estimation
12 – Limitations
13 – Funding information; 14 – Protocol (if available)

Appendix II Results of risk of bias assessment of *in vivo* studies according to the SYRCLE's risk of bias.[23]

Study	1	2	3	4	5	6	7	8	9	10
Santos *et al.*[111]	U	Y	N	N	N	N	Y	Y	Y	N
Assmann *et al.*[94]	Y	Y	N	N	N	Y	Y	Y	Y	Y
Silva *et al.*[95]	N	N	N	N	N	N	Y	Y	Y	N
Suzuki *et al.*[106]	Y	Y	N	N	N	N	Y	Y	Y	N
Garcia *et al.*[112]	U	Y	N	N	N	N	N	Y	Y	N
Cintra *et al.*[113]	N	Y	N	N	N	N	N	Y	Y	Y
Silva *et al.*[114]	N	U	N	N	N	N	Y	Y	Y	N
Oliveira *et al.*[115]	Y	Y	N	N	N	N	Y	Y	Y	Y
Brasil *et al.*[116]	Y	Y	N	N	N	N	Y	N	Y	N
Zmener *et al.*[117]	N	Y	N	N	N	N	Y	U	Y	N[a]
Suzuki *et al.*[118]	Y	Y	N	N	N	N	Y	Y	Y	N
Tanomaru-Filho *et al.*[96]	U	Y	N	N	N	N	Y	Y	Y	N
Leonardo *et al.*[97]	U	Y	N	N	N	N	Y	Y	Y	N
Campos-Pinto *et al.*[98]	U	U	N	N	N	N	N	Y	Y	N
Zafalon *et al.*[99]	N	Y	N	N	N	N	N	Y	Y	N
Onay *et al.*[100]	U	Y	N	N	N	N	N	U	Y	N
Holland *et al.*[101]	U	Y	N	N	N	N	Y	Y	Y	N
Shahi *et al.*[102]	U	Y	N	N	N	N	Y	Y	Y	N
Tanomaru-Filho *et al.*[103]	U	U	N	N	N	N	Y	Y	Y	N
Cintra *et al.*[104]	N	Y	N	N	N	N	Y	Y	Y	Y
Kim *et al.*[105]	N	N	N	N	N	N	N	Y	Y	Y
Zmener[107]	U	U	N	N	N	N	N	Y	Y	Y
Ozbas *et al.*[108]	N	Y	N	N	N	N	N	Y	Y	Y
Morinaga *et al.*[109]	N	N	N	N	N	N	N	N	Y	N
Figueiredo *et al.*[110]	Y	Y	N	N	N	N	N	U	Y	N

[a] The preparation of sealer (EndoREZ with accelerator) was performed with slight modifications of the manufacturer's instructions. Also, one new animal was added to one of the groups (unspecified) to replace a drop-out from the original population (reasons were not specified).

Abbreviations: N, No; U, Unclear; Y, Yes.

Checklist items:
1 – Allocation sequence generation; 2 – Baseline characteristics; 3 – Allocation concealment
4 – Random housing; 5 – Caregiver and/or researcher blinding
6 – Random outcome assessment; 7 – Outcome assessor blinding
8 – Incomplete outcome data
9 – Selective outcome reporting
10 – Other sources of bias