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REVIEW

Genotoxicity of root canal sealers: a literature review

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Abstract

Objectives Root canal sealers are widely used worldwide in endodontics to prevent reinfection and growth of surviving microorganisms. Considering the strong correlation between genetic damage and carcinogenesis, evaluation of genotoxicity induced by endodontic sealers is recommended for elucidating the true health risks to patients and professionals. The purpose of this article was to provide a comprehensive review of studies involving genotoxicity analysis of endodontic sealers and the used methodologies.

Materials and methods A literature search was made in PubMed using the following combination of words "genotoxicity," "mutagenicity," "endodontic sealers," and "root canal sealers." A total of 39 articles with genotoxicity studies were selected for the present study.

Results Sealers have been ranked in decreasing order of their genotoxicity as: ZOE sealers > GIC sealers > S sealers > ER sealers > MR sealers > Novel sealers > CH sealers > CS sealers.

Conclusions All published data showed some evidence of genotoxicity for most of the commercial root canal sealers; however, contradictory results were found, mainly for AH Plus, the most studied sealer.

Clinical relevance The information provided would direct the endodontists to use the less genotoxic materials in endodontic treatment in a way to reduce DNA damage promoting oral healthcare.

Keywords Endodontic sealers · Comet assay · Micronucleus test · Mutagenicity

Abbreviations

BisDMA	Bisphenol-A dimethacrylate
BisEMA	Ethoxylated bisphenol-A dimethacrylate
BisGMA	Bisphenol-A-glycidylmethacrylate
CH	Calcium hydroxide
CS	Calcium silicate
DSB	Double-strand breaks
GIC	Glass ionomer cements
ER	Epoxy resin
hDPF	Human dental pulp fibroblast
hDPSC	human dental pulp stem cells
HEMA	Hydroxyethylene methacrylate
hGF	Human gingival fibroblast
hPB	Human peripheral blood
MR	Methacrylate resin
MN	Micronucleus
MTA	Mineral trioxide aggregate
PBMC	Peripheral blood mononuclear cells
PDL	Periodontal ligament cells
PEGDMA	Polyethylene glycol dimethacrylate
PR	Polyvinyl resin
RCS	Root canal sealer
ROS	Reactive oxygen species
S	Silicone
SHE	Syrian hamster embryo
TEGDMA	Triethyleneglycoldimethacrylate
UDMA	Urethanedimethacrylate
ZOE	Zinc oxide-eugenol

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Introduction

Tooth pulp is protected from injurious elements by enamel, dentin, and periodontium; however, when these barriers are breached, microorganisms and substances may adversely affect the stability, causing inflammation (pulpitis) and even tissue death (pulp necrosis). To overcome this, the recommended procedures are pulpectomy, which involves surgical removal of all the material in the pulp chamber and root canal, or pulpotomy, which refers only to removal of the coronal portion of the pulp [1]. The root canal system is sealed with a filling material to prevent reinfection and growth of surviving microorganisms. The deposition of cementum is considered a desired healing response and a prerequisite for restoring a functional periodontal attachment. The effectiveness of the filling materials is critically dependent on its physical and chemical properties, however being biological safety a prerequisite for its clinical use [2].

Genotoxicity is a critical issue in determining the safety of agents that might contact biological structures and should be considered within a biological risk assessment process [3]. Mutagens or genotoxic substances induce DNA damage directly or indirectly through inactivation of enzymes involved in the maintenance of genome integrity. Mutagen-target interactions may result in different types of DNA damage (DNA adducts, alkali labile sites, strand breaks) that can be pre-mutagenic. Cellular mechanisms to overcome these harmful effects include protective antioxidant activity (mediating elimination/neutralization processes) and the removal of induced lesions by the DNA repair machinery [4]. Nevertheless, insufficient cell-protecting mechanisms and/or high DNA-inflicted damage result in the disruption of the replication and/or transcription processes hindering the cell self-repairing potential leading, ultimately, to cell apoptosis [5].

Several methodologies able of detecting genetic damage and/or mutations have been established and are approved by international regulatory agencies for validation of chemical agents commercially available. The potential health risks are thus elucidated as it has been established that genetic damage is intimately linked to diseases such as cancer [6]. Understanding the impact of exposure scenarios (dose, chronic, acute) on cancer risk is important but remains a scientific challenge. Dental materials persist in the oral cavity for long periods which imply that risk assessment is required to ensure the safety profile of such materials [7].

Due to the current demand of enhanced clinical performance of dental materials, the number of commercial products is continuously increasing. Physical properties, biocompatibility, sealing ability, adhesion, solubility, and antibacterial efficacy results are abundant for root canal sealers, and some reviews on those issues have been written [2, 8–13]. However, genotoxic stress as a reaction to endodontic sealers is also an important parameter to be assessed to validate the safety of biomaterials in clinical practice [14]. So far, limited reviews have reported about the genotoxicity of endodontic sealers [15].

The present review intends to provide detailed information on the genotoxicity of root canal sealers (RCS), displaying the reported results considering the sealer's composition. A comprehensive literature search on "genotoxicity," "mutagenicity," "endodontic sealers," and "root canal sealers" was performed on studies conducted between 1998 and 2020. In brief, a search of PubMed, MEDLINE, Embase, and Google Scholar for a plethora of articles was carried out using the aforementioned keywords. Case reports and articles not written in English were excluded from this review.

Genotoxicity as a biocompatibility requirement

ISO 10993-1 lists two components for biological response evaluation of biomaterials [16]. The first normative component requires a number of aspects, such as physicochemical information, cytotoxicity, sensitization, irritation or intracutaneous reactivity, material-mediated pyrogenicity, toxicity, implantation, hemocompatibility, genotoxicity, carcinogenicity, reproductive/developmental toxicity, and biodegradation. The second one provides suggestions and considerations. These aspects are combined together with the nature of the tissues and contact time to assist in the selection of an appropriate evaluation technique [14]. However, the clinical relevance of these assays is limited because they do not take into account the complexity of a living organism, as well as the clinical presentation of the apical region. Despite that assessment, it is also mandatory to determine the biocompatibility of a material within in vivo setting [16]. Of the above-mentioned aspects, cytotoxicity and genotoxicity are most commonly reported in the literature [10, 11].

Genotoxicity is defined as toxicity that affects DNA structure, i.e., the ability of a substance (genotoxin) to modify the chemical structure of DNA, causing DNA lesions [14]. However, not all genotoxins act directly on the DNA molecule. Some genotoxins interact with DNA repair proteins, increasing mutation rate, or mitotic spindle proteins, leading to chromosomal misaggregation or even with proteins involved in the cell cycle, increasing the proliferation rate [17]. Several assays are addressed to detect DNA damage, i.e., comet assay, sister chromatid exchange, detection of γH2AX or 32P-postlabeling assay, and its transition to mass spectrometry [18]. Once damaged DNA can mispair during replication, an alteration in nucleotide sequence can arise, characterizing the mutation, which can involve a single (point mutation), few base pairs or a whole chromosome. Mutagenicity can be assessed using the Ames test, cytogenetics, or micronucleus

(MN) assay [19]. The aim of genotoxicity assays is the identification of probable mutagens.

Regulatory agencies require testing for biomaterials to be available in the market. However, multiple tests are needed to monitor all potential endpoints related to DNA damage or mutations [14]. Registration, Evaluation, Authorisation, and restriction of Chemicals (REACH) is a European Union (EU) regulation adopted to improve protection of human health and the environment from the risks posed by chemicals. REACH Annexures describe the requirements on genotoxicity, specifying the information that must be submitted for purposes of registration and evaluation [20]. A set of specifications for testing chemicals and also medical devices is established by the Organization for Economic Co-operation and Development (OECD) with appropriate model systems, methodologies, reference standards, and recommendations for statistical analysis [21]. For medical devices, ISO10993-33 provides guidance on tests to evaluate the potential genotoxicity, carcinogenicity, or reproductive toxicity [22]. Table 1 summarizes the methodologies used to assess materials genotoxicity according to EU/OECD/ISO10993-33 guidelines.

Results

Commonly used sealers are based on zinc oxide-eugenol (ZOE sealers), silicone (S sealers), glass ionomer cements (GIC sealers), methacrylate resin (MR sealers), epoxy resin (ER sealers), polyvinyl resin (PR sealers), calcium silicate (CS sealers), calcium hydroxide (CH sealers), and novel sealers (Table 2). Detailed information of the commercial sealers with reported results on genotoxicity is presented in the Supplementary material (Table S1).

The present survey addresses the information on the genotoxicity of available root canal sealers reported from 1998 to the present date. A total of 39 articles were included. Figure 1 shows the publication periodicity grouped over a 4-year time span. On average, 1.9 articles were published per year, with two peak periods (2006 to 2009, 9 articles; 2014 to 2017, 11 articles). The two more recent articles were available in 2018 and 2020. Table 3 presents an overview of the included studies in terms of the experimental protocol (cell line/type, exposure time, concentration range, genotoxicity assay) and the relevant results, considering the sealers' groups. Most of the studies were performed in vitro; the comet assay and the micronucleus test were widely used and broadly applied to assess genotoxic and mutagenic effects, respectively. Relevant results are given below.

Zinc oxide-eugenol-based sealers

Six articles addressed the genotoxicity of eight ZOE sealers (five of them published from 1999 to 2009, and the last one in 2016). One or two studies were performed for each sealer (Table 3). All sealers were considered genotoxic in the tested cell lines through different methods, i.e., comet assay [36, 40], DNA precipitation assay [37], proto-oncogenes expression [38], and micronuclei assay [39]. The Ames test was performed for Tubli-Seal and Endométhasone N, and the results were considered negative [35].

Silicone-based sealers

RoekoSeal demonstrated time-dependent positive genotoxicity results on V79 cells using comet assay [41], and GuttaFlow showed negative results performing chromosomal alteration analysis and comet assay in hPB lymphocytes [40].

Glass-ionomer cement-based sealers

Ketac Endo was not mutagenic on *S. typhimurium* strains [35]. Vitrebond was tested on CHO cells exhibiting a clear genotoxic effect in the *hprt* test and umu chromotest [42]. Additionally, the genotoxic compounds, being hydrophilic, leached out rapidly when in contact with saliva [42], and when tested on V79 cells using the MN test, results were also considered positive [43].

Resin-based sealers

The prototype of R sealers was developed as a bis-phenol resin using methenamine for polymerization; however, the product released formaldehyde during setting [74]. Alternatives were sought and variants include phenol-formaldehyde, resorcin-formaldehyde, and methylmethacrylate, which were strongly antibacterial however presenting shrinkage and discoloration and poor biocompatibility during setting. Genotoxicity results for resin-based sealers were considered for methacrylate resin (MR)-, epoxy resin (ER)-, and polyvinyl resin (PR)-based sealers and are summarized in Table 3.

MR sealers were involved in a variety of studies performed from 2009 to 2018, and a higher number revealed absence of genotoxicity. Several studies that compared single MR sealer (EndoRez) with multi-MR sealer (RealSeal) found no toxic effects on FMM1 cells and transfected COS-7 cells [47] and also hGF [44]; EndoRez caused low double-strand break (DSB) formation in PDL cells while RealSeal did not [46]. EndoRez also showed positive results on V79 cells [41, 45] and on PDL cells [46]. Epiphany, using the comet assay, showed negative effects on hPB lymphocytes [40] and hPB leucocytes [49]; however, results were positive with the micronucleus test on V79 cells [48]. Coming to RealSeal, only one study (out of six) reported dose- and time-dependent DNA damage, namely on BHK-21 cells using the comet assay

Table 1 Methodologies used to assess materials genotoxicity according to EU/OECD/ISO guidelines

Assay	EU guideline	OECD guideline	ISO/TR 10993-33 [22]	Genotoxic endpoints measured	Principle of the test methods
VII. In vitro gene mutation study in bacteria					
Bacterial reverse mutation test—Ames test	B13/B14 [20]	OECD 471 [23]	Chapter 6	Gene mutations	It uses amino-acid-requiring strains of bacteria to detect reverse gene mutations (point mutations and frameshifts).
VIII. In vitro gene mutation study in mammalian cells					
In vitro mammalian cell gene mutation test—*hprt* test	B17 [20]	OECD 476 [24]	N/A	Gene mutations	It identifies substances that induce gene mutations in the *hprt* gene of established cell lines.
In vitro mammalian cell gene mutation tests	B17 [20]	OECD 490 [25]	Chapter 9	Gene mutations and structural chromosome alterations	It identifies substances that induce gene mutations in the *tk* gene of the L5178Y cell line. If colonies are scored using the criteria of normal (large) and slow growth (small) colonies, gross structural chromosome alterations may be measured, since mutant cells that have suffered damage to both the *tk* gene and growth genes situated close to the *tk* gene have prolonged doubling times and are more likely to form small colonies.
Syrian hamster embryo (SHE) transformation assay	B21 [20]	N/A	N/A	Phenotypic alterations	It refers to the induction of phenotypic alterations in cultured cells that are characteristic of tumorigenic cells. Transformed cells with the characteristics of malignant cells have the ability to induce tumors in susceptible animals; this validated the use of phenotypic alterations in vitro as criteria for a carcinogenic potential in vivo.
VIII. In vitro cytogenetics					
In vitro mammalian chromosome alteration test	B10 [20]	OECD 473 [26]	Chapter 7	Structural and numerical chromosome alterations	It identifies substances that induce structural chromosome alterations in cultured mammalian-established cell lines, cell strains, or primary cell cultures. An increase in polyploidy may indicate that a substance has the potential to induce numerical chromosome alterations, but this test is not optimal to measure numerical alterations and is not routinely used for that purpose.
In vitro micronucleus (MN) test	N/A	OECD 487 [27]	Chapter 8	Structural and numerical chromosome alterations	It identifies substances that induce micronuclei in the cytoplasm of interphase cells which may originate from acentric fragments or whole chromosomes, and the test thus has the potential to detect both clastogenic and aneugenic substances.
DNA damage and repair					
In vitro unscheduled DNA synthesis	B18 [20]	OECD 482 [28]	N/A	DNA damage	It identifies substances that induce DNA damage (measured as unscheduled "DNA" synthesis).
In vitro sister chromatid exchange (SCE) assay	B19 [20]	OECD 479 [29]	N/A	Mammalian DNA damage	It detects reciprocal exchanges of DNA between 2 sister chromatids of a duplicating chromosome. SCEs represent the interchange of DNA replication products at apparently homologous loci which presumably involves DNA breakage and reunion.
IX. In vivo cytogenetics					
In vivo mammalian bone marrow chromosome alteration test	B11 [20]	OECD 475 [30]	Chapter 11	Structural and numerical chromosome alterations	It identifies substances that induce structural chromosome alterations in the bone marrow cells of animals, usually rodents. An increase in polyploidy may indicate that a substance has the potential to induce numerical chromosome alterations, but this test is not optimal to measure numerical alterations and is not routinely used for that purpose.
In vivo mammalian erythrocyte micronucleus (MN) test	B12 [20]	OECD 474 [31]	Chapter 10	Structural and numerical chromosome alterations	It identifies substances that cause micronuclei (originated from acentric fragments or whole chromosomes) in erythroblasts sampled from bone marrow and/or peripheral blood cells of animals, usually rodents.
Transgenic rodent (TGR) somatic and germ cell gene mutation assays	B58 [20]	OECD 488 [32]	N/A	Gene mutations and chromosomal rearrangements	Since the transgenes are transmitted by the germ cells, they are present in every cell. Therefore, gene mutations and/or chromosomal rearrangements can be detected in virtually all tissues of an animal, including target tissues and specific site of contact tissues.
In vivo alkaline single cell gel electrophoresis assay—comet assay	N/A	OECD 489 [33]	N/A	DNA strand breaks	It does not only recognize DNA damage that would lead to gene mutations and/or chromosome alterations but will also detect DNA damage that may be effectively repaired or lead to cell death. It can be applied to almost every tissue of an animal from which single cell or nuclei suspensions can be made.

Table 1 (continued)

Assay	EU guideline	OECD guideline	ISO/TR 10993-33 [22]	Genotoxic endpoints measured	Principle of the test methods
In vivo DNA damage and repair					
In vivo unscheduled DNA synthesis (UDS) test	B39 [20]	OECD 486 [34]	N/A	DNA repair	It identifies substances that induce DNA damage followed by DNA repair in liver cells of animals, commonly rats. It is usually based on the incorporation of tritium-labeled thymidine into the DNA by repair synthesis after excision and removal of a stretch of DNA containing a region of damage.

Abbreviations: *EU*, European Union; *ISO/TR*, International Organization for Standardization/Technical Reports; *OECD*, Organization for Economic Co-operation and Development

[50], while others reported negative genotoxicity on several cell types [45, 49]. Hybrid Root Seal, a self-etching hydrophilic material as a result of the inclusion of 4-META, showed significant DSB formation in PDL cells [46].

ER-based sealers have also been extensively tested, with published studies from 1998 to 2020, prevailing the results reporting genotoxicity. Results are available for Topseal, AH26, AH Plus/AH Plus Jet, and Acroseal (Table 3). Most concern AH Plus. AH26 and AH Plus showed positive genotoxicity [36, 37, 51] but negative effects on the Ames test [35, 52–54]. AH Plus was also genotoxic in V79 cells [41, 48, 55], FMM1 cells [56], UO2S cells [38], hDPS cells [57], and, recently, in hGF cells [58]. However, controversial results were found with the same cell lines, i.e., results were negative with V79 cells [45], FMM1 cells [47], hGF cells [44], and PDL cells [46] perhaps due to different methodologies. Other ER sealers have also been investigated. Topseal showed some DNA damage in OC2 cells using the comet assay [36], and Acroseal increased the production of ROS and MN and delayed the cell cycle in G2 phase on V79 cells [48]; however, it did not alter DSB formation on PDL cells [46].

Diaket, a PR sealer, sets by chelation, but contains polyvinyl chloride as polymer and showed positive genotoxicity on hPB lymphocytes [40].

Calcium silicate-based sealers

CS sealers were the most tested, from 2005 to 2020, and only few studies demonstrated genotoxicity in mammalian cells (Table 3). Portland cement (PC) did not cause genotoxic effects in four cell lines, using the comet assay [59–62]. ProRoot MTA was also safe in most studies [43, 63, 65, 66], except for one exhibiting some genotoxicity on L929 cells using comet assay [64], perhaps due to the high concentrations used. Endocem MTA [65] and MTA Plus [50] were not genotoxic using the comet assay on CHO cells and BHK-21 cells, respectively. MTA Fillapex was tested in five cell lines being genotoxic in four of them [46, 50, 55, 57], except with hGF cells [58]. MTA Angelus, the most tested CS sealer, showed absence of genotoxicity, with the comet assay, on L5178Y cells [67], CHO-K1 cells [60, 68], hPB lymphocytes [61, 69], and 3T3-L1 cells [62]. MTA Angelus also showed

Table 2 Endodontic sealers classified by composition

Classes of endodontic sealers	Abbreviation	Examples
Zinc oxide-eugenol	ZOE sealers	Tubli-Seal, N2, Endométhasone N, Canals, Canals-N, IRM, SuperEBA, Hermetic
Silicone	S sealers	RoekoSeal, GuttaFlow
Glass ionomer cements	GIC sealers	Ketac Endo, Vitrebond
Resin derivatives		
Methacrylate resin	MR sealers	EndoRez, Epiphany, RealSeal, Hybrid Root SEAL
Epoxy resin	ER sealers	Topseal, AH26, AH Plus, Acroseal
Polyvinyl resin	PR sealers	Diaket
Calcium derivatives		
Calcium silicate	CS sealers	Portland cement, ProRoot MTA, Endocern MTA, MTA Plus, MTA Fillapex, MTA Angelus, CS cement, TheraCal LC
Calcium hydroxide	CH sealers	Sealapex, Calcibiotic RCS, Apexit, Calcipex II, Vitapex, Calcicur, Hydro C, P.A. CH
Other sealers	Novel sealers	Endosequence BC, iRootSP Injectable, Biodentine, BioRoot RCS, Polifil, Bioseal, CEM, CS+HA

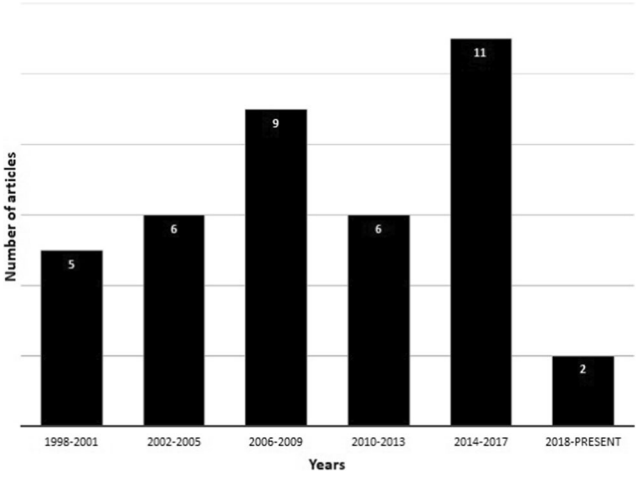

Fig. 1 Number of articles that performed genotoxicity studies with root canal sealers grouped over a 4-year time span, from 1998 to the present

negative results using the MN test with V79 cells [55] and the bone marrow of Swiss mice [39]. Genotoxicity was observed on hDPS cells, evaluated by gene expression analyses [57]. Other CS-based sealers not displaying genotoxicity were a CS cement [63] and TheraCal LC [66], a light-cure single-component material for direct and indirect pulp capping.

Calcium hydroxide-based sealers

CH sealers have a complex and inhomogeneous setting reaction, producing a hard surface with the deeper part retaining a dough-like structure. They perform remarkably well in laboratory leakage tests and also on biological, animal, and human tests [12]. Lack of physical sturdiness, poor clinical and radiographic outcomes when compared with other sealers are causes for concern [75]. CH is also added to other cements such as resins and ZOE sealers [76–79].

As a group, CH sealers appeared safe, as suggested from the studies performed through 1999 to 2018 (Table 3). CRCS [35], Sealapex [36, 38], Hydro C [43], Calcicur [44], P.A. CH [39], and Apexit [47] showed negative results. Positive genotoxicity was only noted for Calcipex II and Vitapex with CHO-K1 cells, observed by a higher incidence of micronuclei and higher tail moment values when compared with MTA [65].

Other novel sealers

Bioceramics exhibit excellent biocompatibility properties due to compositional similarity with biological hydroxyapatite, producing mineral hydroxyapatite with an osteoconductive effect, leading to bone formation at the interface between material and bone. Even though these advantages have contributed to their rapid spread in the dental field, they are not widely used, and products on the market are not yet known or used by many dentists [9]. Other novel sealers include the use of medicinal plants with studies showing the effect of natural products in pulpal and dentin repair with variable effectiveness such as alkaloids, coumarins, saponins, and flavonoids [80].

Genotoxicity results of novel sealers are summarized in Table 3. Studies were reported through 2008 to 2020. EndoSequence BC and iRoot SP are the same product, a hydrophilic sealer where the moisture inside the tubules initiates setting and shows genotoxicity [46], however lower than ZOE with L929 cells [70] and AH Plus with FMM1 cells [56]. Biodentine, a Ca3SiO5-based material indicated as a dentine substitute, was not mutagenic or genotoxic [66, 71]. However, using the MN test, an increase in the frequency of micronuclei was observed [72] which may have occurred because the high concentrated test solution BioRoot RCS demonstrated negligible DSB formation [46]. Two novel natural resin-based sealers have been recently created, namely Polifill [43, 45] (based on a polymer from *R. communis*—castor oil) and Bioseal [45] (based on *Copaifera multijuga* oil-resin—copaiba—with CH and zinc oxide) which tested negative for genotoxicity with V79 cells. Other experimental sealers tested with the comet assay include calcium silicate-hydroxyapatite (CS+HA) which was not genotoxic with hPB lymphocytes [73] and calcium-enriched mixture (CEM) which was genotoxic with L929 cells [64]. Novel sealers with favorable biocompatibility results that require genotoxicity studies include polymer nanocomposite resins [81], PC-based partial stabilized cement with zinc [82], and new resin cement [83].

Discussion

In 1981, Grossman listed requirements for RCS, as they should (1) be tacky when mixed to provide good adhesion to the canal wall when set; (2) make a hermetic seal; (3) be radiopaque to be visualized on radiographs; (4) have fine particles of powder to mix easily with liquid; (5) not shrink upon setting; (6) not discolor tooth structure; (7) be bacteriostatic or at least not encourage bacterial growth; (8) set slowly; (9) be insoluble in tissue fluids; (10) be well tolerated by the periapical tissue; and (11) be soluble in common solvents if it is necessary to remove it [84]. In 2007, de Ingle et al added two more requirements: (1) should not provoke an immune response and (2) should not be mutagenic or carcinogenic [85]. Thus, a RCS should have an acceptable level of biocompatibility and according to regulations, successfully pass a clinical risk assessment before commercialization, fulfilling the technical, biological, handling, and biocompatibility requirements [86].

This review put together the results concerning the studies addressing the genotoxicity of commercially available endodontic sealers and endodontic agents, included in 39 articles published from 1998 to the present data. The results' outcome

Table 3 Overview of genotoxicity/mutagenicity studies addressing endodontic sealers

Sealer	Cell line	Time	Concentration	Genotoxicity assays	Effects	Ref.
Zinc oxide-eugenol-based sealers (ZOE sealers)						
Tubli-Seal	–	24 h	0–0.375 mg/plate	Ames test on *S. typhimurium* strains	Negative (not mutagenic)	[35]
	OC2 cells	24 h	0.1–2.5 mg/mL	Comet assay	Positive (greatest migration length of all ZnOE sealers)	[36]
N2	V79 cells	24 h	1- and 3-day elutes	DNA precipitation assay	Positive (DNA single strand breaks and digestion of genomic DNA)	[37]
	U2OS cells	1, 3, and 6 h	1:2–1:8	Protooncogenes expression analysis	Positive (induced *c-jun* and *c-fos* mRNA expression)	[38]
Endométhasone N	–	24 h	0–0.375 mg/plate	Ames test on *S. typhimurium* strains	Negative (not mutagenic)	[35]
	U2OS cells	1, 3, and 6 h	1:2-1:8	Protooncogenes expression analysis	Positive (induced *c-jun* and *c-fos* mRNA expression)	[38]
Canals	OC2 cells	24 h	0.1–2.5 mg/mL	Comet assay	Positive (induced dose-dependent DNA damage)	[36]
	V79 cells	24 h	1- and 3-day elutes	DNA precipitation assay	Positive (DNA single-strand breaks and digestion of genomic DNA)	[37]
Canals-N	OC2 cells	24 h	0.1–2.5 mg/mL	Comet assay	Positive (genotoxicity at high concentration)	[36]
IRM	Bone marrow of Swiss mice	24 and 48 h	1:10–1:1000	MN test	Positive (induced micronuclei with ZnO sealer)	[39]
	hPB lymphocytes	1 h and 1, 5, and 30 days	0.2–0.8 μg/mL	Comet assay; chromosomal alteration analysis	Negative (slight increase of tail length, at 1 h; no effect, after 1 day)	[40]
SuperEBA	hPB lymphocytes	1 h and 1, 5, and 30 days	0.2–0.8 μg/mL	Comet assay; chromosomal alteration analysis	Positive (significant increase of tail length, after 1 h; no effect, after 1 day)	[40]
Hermetic	hPB lymphocytes	1 h and 1, 5, and 30 days	0.2–0.8 μg/mL	Comet assay; chromosomal alteration analysis	Positive (significant increase of tail length, after 1 h; no effect, after 1 day)	[40]
Silicone-based sealers (S sealers)						
RoekoSeal/RSA	V79 cells	0, 12, and 24 h	1:1–1:32	Comet assay	Positive (time-dependent increased % of tail moment)	[41]
GuttaFlow	hPB lymphocytes	1 h and 1, 5, and 30 days	0.2–0.8 μg/mL	Comet assay; chromosomal alteration analysis	Negative	[40]
Glass ionomer cement-based sealers (GIC sealers)						
Ketac Endo	–	24 h	0–0.375 mg/plate	Ames test on *S. typhimurium* strains	Negative (not mutagenic at all concentrations)	[35]
Vitrebond	CHO-HPRT cells	3 h	2–8%	umu test with *S. typhimurium* TA1535/pSK1002; *hprt* test	Positive (> 3-fold increase in mutation frequency, at 8%, hprt test; genotoxic effects, umu test, induction rate > 1.5, at ≤ 1%)	[42]
	V79 cells	24 h	1:1–1:32	MN test; cell cycle analysis	Positive (increased MN ~ 2.5-fold at 1:32 and 1:16)	[43]
Methacrylate resin-based sealers (MR sealers)						
EndoRez	hGF cells	24 h	1:3–1:300	γ-H2AX assay	Negative (at 1:3, cells with more and larger foci, no statistical significance)	[44]
	V79 cells	0, 12, and 24 h	1:1–1:32	Comet assay		[41]

Table 3 (continued)

Sealer	Cell line	Time	Concentration	Genotoxicity assays	Effects	Ref.
					Positive (time-dependent increased % of tail moment)	
	V79 cells	24 h	1:1–1:8	MN test	Positive (high number of micronuclei)	[45]
	hTERT-expressing PDL cells	6 h	1/3th and 1/10th of EC_{50} value	γ-H2AX assay	Positive (low DSB formation)	[46]
	FMM1 cells	24 h	1:2	MN test (at 1:2)	Negative	[47]
Epiphany	hPB lymphocytes	1 h and 1, 5, and 30 days	0.2–0.8 μg/mL	Comet assay; chromosomal alteration analysis	Negative	[40]
	V79 cells	24 h	1:1–1:32	MN test	Positive (increased number of micronuclei with 1:8 and 1:16)	[48]
	hPB leucocytes	4 and 24 h	0.02 g	Comet assay	Negative (both polymerized and unpolymerized forms)	[49]
RealSeal/RealSeal SE	hPB leucocytes	4 and 24 h	0.02 g	Comet assay	Negative (both polymerized and unpolymerized forms)	[49]
	hGF cells	24 h	1/3th and 1/10th of EC_{50}	γ-H2AX assay	Negative	[44]
	BHK-21 cells (clone CCL-10)	1 and 5 days	50–100%	Comet assay	Positive (dose- and time-dependent tail length and tail intensity)	[50]
	V79 cells	24 h	1:1–1:8	MN test	Negative	[45]
	hTERT-expressing PDL cells	6 h	1:3–1:300	γ-H2AX assay	Negative (induced γ-H2AX foci formation similar to control)	[46]
	FMM1 cells	24 h	1:2–1:16	MN test (at 1:2)	Negative (similar to control)	[47]
MetaSEAL/Hybrid Root SEAL	hTERT-expressing PDL cells	6 h	1/3th and 1/10th of EC_{50}	γ-H2AX assay	Positive (induced γ-H2AX foci formation)	[46]
Epoxy resin-based sealers (ER sealers)						
Topseal	OC2 cells	24 h	0.1–2.5 mg/mL	Comet assay	Positive (high migration values, induced dose-dependent DNA damage)	[36]
AH26	–	24 h	0–0.375 mg/plate	Ames test on *S. typhimurium* strains	Negative (not mutagenic at all concentrations)	[35]
	OC2 cells	24 h	0.1–2.5 mg/mL	Comet assay	Positive (high migration values, induced dose-dependent DNA damage)	[36]
	V79 cells	24 h	1- and 3-day elutes	DNA precipitation assay	Positive (at 3 days, DNA single-strand breaks and digestion of genomic DNA, at 3 days)	[37]
	Rat cerebral astrocytes	24 h	0.01–0.25 mg/mL	Comet assay	Positive (dose-dependent increase in all measures of DNA damage)	[51]
AH Plus/AH Plus Jet	–	12 h	1:1–1:512	Prokaryotic umu test with *S. typhimurium* strain at 4 h; eukaryotic DNA synthesis inhibition test (DIT) with HeLa S3 cells at 24 h; alkaline filter elution (AFE) test	Negative (umu test and DIT; AFE rations < 1.3)	[52]
	–	72 h	0.0–25.0 mg/plate	Ames test on *S. typhimurium* strains	Negative (with physiological saline eluates of the freshly mixed material and of mixed material which was set for 24 h)	[53]
	–	48 h	0.75–3 μL/plate	Ames test on *S. typhimurium* strains	Negative (in some concentrations)	[54]

Table 3 (continued)

Sealer	Cell line	Time	Concentration	Genotoxicity assays	Effects	Ref.
	OC2 cells	24 h	0.1–2.5 mg/mL	Comet assay	Positive (high migration values, induced dose-dependent DNA damage)	[36]
	Rat cerebral astrocytes	24 h	0.01–0.25 mg/mL	Comet assay	Positive	[51]
	V79 cells	24 h	1- and 3-day elutes	DNA precipitation assay	Positive (DNA single-strand breaks and digestion of genomic DNA, at 3 days)	[37]
	U2OS cells	1, 3, and 6 h	1:2–1:4–1:8	Protooncogenes expression analysis	Positive (induced *c-jun* and *c-fos* mRNA expression, within 3 h)	[38]
	V79 cells	24 h	1:1–1:16, 1:32	MN test	Positive (increased number of micronuclei with the extracts 1:16 and 1:8)	[48]
	V79 cells	12, 48, and 72 h	1:1–1:32	MN test	Positive (increased number of micronuclei, at 48 h with the 1:4 extract)	[55]
	hGF cells	24 h	1:3–1:300	γ-H2AX assay	Negative (with 1:10 extract, cells tended to have more and larger foci, 3.0%, without statistical significance)	[44]
	V79 cells	0, 12, and 24 h	1:1–1:32	Comet assay	Positive (time-dependent increased % of tail moment)	[41]
	FMM1 cells	24 h	1:10 of 0.2 g/mL elutes	MN test	Positive	[56]
	V79 cells	24 h	1:1–1:8	MN test	Negative	[45]
	hTERT-expressing PDL cells	6 h	1/3th and 1/10th of EC_{50}	γ-H2AX assay	Negative	[46]
	FMM1 cells	24 h	1:2–1:16	MN test (at 1:2)	Negative	[47]
	hDPS cells	1, 2, 7, and 15 days	50%	Gene expression analysis (qRT-PCR) of DNA damage sensors and DNA damage repair sensors	Positive (increased apoptosis, oxidative stress, and genotoxicity markers)	[57]
	hGF cells	24, 48, and 72 h	Silicone disks	MN test	Positive	[58]
Acroseal	V79 cells	24 h	1:1–1:32	MN test	Positive (increased number of micronuclei, with the lowest concentrations, 1:64 and 1:128)	[48]
	hTERT-expressing PDL cells	6 h	1/3th and 1/10th of EC_{50}	γ-H2AX assay	Negative	[46]
Polyvinyl resin (polyketone)-based sealers						
Diaket	hPB lymphocytes	1 h and 1, 5, and 30 days	0.2–0.8 μg/mL	Comet assay; chromosomal alteration analysis	Positive (increased migration at the highest concentration and slight increase in chromatid breaks and number of aberrant cells, after 1 h; no effect, after 1 day)	[40]
Calcium silicate-based sealers (CS sealers)						
Portland cement (PC); *grey; *white	L5178Y cells	3 h	1–1000 μg/mL	Comet assay	Negative	[59]
	CHO-K1 cells	1 h	1–1000 μg/mL	Comet assay	Negative	[60]
	hPB lymphocytes	1 h	1–1000 μg/mL	Comet assay	Negative	[61]
	3T3-L1 cells	3 h	10–1000 μg/mL	Comet assay	Negative	[62]
	V79 cells	24 h	1:1–1:32	MN test; cell cycle analysis	Negative (all assays)	[43]

Table 3 (continued)

Sealer	Cell line	Time	Concentration	Genotoxicity assays	Effects	Ref.
ProRoot MTA; *grey; *white	MG63 cells	1 and 7 days	1–50 mg/mL	DNA precipitation assay	Negative	[63]
	L929 cells	24 h	0–1000 μg/mL	Comet assay	Positive	[64]
	CHO-K1 cells	24 h	1–100 μg/mL	MN test, comet assay, γ-H2AX assay	Negative (all assays)	[65]
	hDP fibroblasts	24 h	0–1000 μg/mL	Comet assay	Negative	[66]
Endocem MTA	CHO-K1 cells	24 h	1–100 μg/mL	MN test, comet assay, γ-H2AX assay	Negative (all assays)	[65]
MTA Plus; *grey; *white	BHK-21 cells (clone CCL-10)	1 and 5 days	50–100%	Comet assay	Negative	[50]
MTA Fillapex	V79 cells	12, 48, and 72 h	1:1–1:32	MN test	Positive (increased number of micronuclei with 1:4 extracts, at 48 h)	[55]
	BHK-21 cells (clone CCL-10)	1 and 5 days	50–100%	Comet assay	Positive (induced DNA damage, tail length, and tail intensity)	[50]
	hTERT-expressing PDL cells	6 h	1/3th and 1/10th of EC_{50}	γ-H2AX assay	Positive (for 1/3th of EC_{50})	[46]
	hDPS cells	1, 2, 7, and 15 days	50%	Gene expression analysis (qRT-PCR) of DNA damage sensors and DNA damage repair sensors	Positive (all assays)	[57]
	hGF cells	24, 48, and 72 h	Silicone disks	MN test	Negative	[58]
MTA Angelus; *grey; *white	L5178Y cells	3 h	1–1000 μg/mL	Comet assay	Negative	[59]
	L5178Y cells	3 h	1–1000 μg/mL	Comet assay	Negative	[67]
	CHO K-1 cells	3 h	1–1000 μg/mL	Comet assay	Negative	[68]
	CHO K-1 cells	1 h	1–1000 μg/mL	Comet assay	Negative	[60]
	hPB lymphocytes	1 h	1–1000 μg/mL	Comet assay	Negative	[61]
	hPB lymphocytes	1 h	1–1000 μg/mL	Comet assay	Negative	[69]
	3T3-L1 cells		10–1000 μg/mL	Comet assay	Negative (DNA strand breaks)	[62]
	V79 cells	12, 48, and 72 h	1:1–1:32	MN test	Negative	[55]
	Bone marrow of Swiss mice	24 and 48 h	1:10–1:1000	MN test	Negative	[39]
	hDPS cells	1, 2, 7, and 15 days	50%	Gene expression analysis (qRT-PCR) of DNA damage sensors and DNA damage repair sensors	Positive (all assays)	[57]
CS cement	MG63 cells	1 and 7 days	1–50 mg/mL	DNA precipitation assay	Negative	[63]
TheraCal LC	hDP fibroblasts	24 h	0–1000 μg/mL	Comet assay	Negative	[66]
Calcium hydroxide-based sealers (CH sealers)						
Calciobiotic RCS (CRCS)	–	24 h	0–0.375 mg/plate	Ames test on *S. typhimurium* strains	Negative (not mutagenic at all concentration ranges)	[35]
Sealapex	OC2 cells	24 h	0.1–2.5 mg/mL	Comet assay	Negative	[36]
	U2OS cells	1, 3, and 6 h	1:2–1:8	Protooncogenes expression analysis	Negative	[38]
Hydro C	V79 cells	24 h	1:1–1:32	MN test; cell cycle analysis	Negative	[43]
Calcicur	hGF cells	24 h	1:3–1:300	γ-H2AX assay	Negative	[44]

Table 3 (continued)

Sealer	Cell line	Time	Concentration	Genotoxicity assays	Effects	Ref.
P.A. CH	Bone marrow of Swiss mice	24 and 48 h	1:10–1:1000	MN test	Negative	[39]
Calcipex II	CHO-K1 cells	24 h	1–100 μg/mL	MN test, comet assay, γ-H2AX assay	Positive (micronuclei incidence and tail moment)	[65]
Vitapex	CHO-K1 cells	24 h	1–100 μg/mL	MN test, comet assay, γ-H2AX assay	Positive (micronuclei incidence and tail moment)	[65]
Apexit/Apexit Plus	FMM1 cells	24 h	1:2–1:16	MN test (at 1:2)	Negative	[47]
Other/Novel sealers						
Endosequence BC	L929 cells	48 h		Comet assay	Negative	[70]
	FMM1 cells	24 h	1:10 of 0.2 g/mL elutes	MN test	Positive	[56]
iRootSP	hTERT-expressing PDL cells	6 h	1/3th and 1/10th of EC_{50}	γ-H2AX assay	Positive (for the 1/10th of EC_{50})	[46]
	L929 cells	48 h		Comet assay	Negative (compared with ZOE)	[70]
Biodentine	hPB lymphocytes; hDPF cells	–	–	Ames test with *S. typhimurium* strains at 48 h; MN test at 72 h with 1–5% (on hPB lymphocytes); comet assay at 2h (on hDPF)	Negative (all assays)	[71]
	Bone marrow of Wistar rats	–	24-h elute	Comet assay; MN test	Positive (negative on the comet assay; increased micronuclei frequency)	[72]
	hDP fibroblasts	24 h	0–1000 μg/mL	Comet assay	Negative	[66]
BioRoot RCS	hTERT-expressing PDL cells	6 h	1:3–1:300	γ-H2AX assay	Negative	[46]
Polifil	V79 cells	24 h	1:1–1:32	MN test; cell cycle analysis	Negative	[43]
	V79 cells	24 h	1:1–1:8	MN test	Negative	[45]
Bioseal	V79 cells	24 h	1:1–1:8	MN test	Negative	[45]
CS+HA	hPB lymphocytes	1 h	0.01–10 mg/mL	Comet assay	Negative (single cell gel electrophoresis)	[73]
CEM	L929 cells	24 h	0–1000 μg/mL	Comet assay	Positive (single-cell gel electrophoresis)	[64]

Abbreviations: *3T3-L1*, murine fibroblast cells; *BHK-21*, baby hamster kidney fibroblast cells; *CHO-K1*, Chinese hamster ovary cells; *D824*, human dental pulp cells; *FMM1*, immortalized human gingival fibroblast cells; *hDPF*, human dental pulp fibroblast cells; *hDPS*, human dental pulp stem cells; *hGF*, human gingival fibroblast cells; *hPB*, human peripheral blood; *L5178Y*, mouse lymphoma cells; *L929*, mouse fibroblast cells; *MG63*, human osteosarcoma cells; *MN*, micronucleus; *OC2*, oral squamous cell carcinoma cells; *PBMC*, peripheral blood mononuclear cells; *PDL*, periodontal ligament cells; *rPB*, rat peripheral blood; *SHE*, Syrian hamster embryo cells; *tHPFC*, transfected pulp fibroblast cells; *UO2S*, human osteosarcoma cells; *V79*, Chinese hamster lung fibroblast cells

was presented considering the chemical composition of the sealers and are summarized in Table 3. Most studies were performed in vitro, with only three studies being conducted in vivo [39, 72, 87]. A great heterogeneity on the experimental protocols was evident, involving a variety of animal and human cell lines, exposure conditions (sealers' concentration and exposure time) and the genotoxicity assays, hampering the results' analyses and the characterization of genotoxicity patterns. Nevertheless, some trends were clearly noted. As a whole, all sealers' groups presented agents with reported genotoxicity, but differences were observed among the groups, and also within each group. Figure 2 gives a quantitative overview of the reported studies, displaying also the genotoxicity outcome. Resin-based sealers (methacrylate- and epoxy-based) and calcium silicate sealers were addressed in a higher number of studies, followed by the zinc oxide-eugenol sealers and calcium hydroxide sealers.

As displayed in Fig. 2, resin-based sealers were tested in a great number of studies. Detailing the methacrylate-based sealers, EndoRez, Epiphany and RealSeal presented different outcomes depending on the cell line and experimental protocol; however, absence of genotoxic effects was reported in most studies. RealSeal was the most tested sealer of this group, and genotoxicity was rarely observed [50]. Otherwise, MetaSeal was only addressed in one study, being genotoxic [46]. On the epoxy resin-based sealers, AH 26 but, particularly, AH Plus, were thoroughly tested. This sealer yielded different outcomes although prevailing an absence of toxicity [44, 46, 47]. TopSeal and Acroseal were less addressed. The same was verified with Diaket, a polyvinyl resin-based sealer [40]. In the R sealers, the monomers and co-monomers appear potentially associated with genotoxicity, and a relationship between the structural and biological activities has been reported [88]. Resin monomers enhance the formation of ROS affecting the expression and levels of protective antioxidant enzymes as superoxide dismutase, glutathione peroxidase, and catalase [89], and the redox imbalance triggers DNA damage and apoptosis [90]. In methacrylate resin-based sealers, leached compounds as BisGMA, BisDMA, HEMA, PEGDMA, TEGDMA, UDMA, and the photoinitiator camphorquinone [91], possibly due to their solubility [92], alter tightly regulated metabolic pathways, by inducing cellular stress responses, including oxidative DNA damage and DSB [93–95]. In the oral environment, HEMA increases production of ROS and causes oxidative DNA damage through DSB as evidenced by the presence of micronuclei, cell-cycle delay, and apoptosis [96], as well as the transcription of early inflammatory genes [97]. For epoxy resin-based sealers, identified leachable components include bisphenol-A diglycidyl ether and formaldehyde, which are considered carcinogens and probably responsible for cellular toxicity [98] and apoptotic cell death [91].

Calcium silicate-based sealers are mainly composed of mineral trioxide aggregate (MTA) and were described as the most biocompatible material with most predictable outcomes [99], however with some drawbacks [100]. These sealers were tested in a large variety of studies and genotoxicity was observed only occasionally (Fig. 2). The most tested were Portland cement, ProRoot MTA, MTA Fillapex and, particularly, MTA Angelus. Portland cement (and also MTA Plus, Endocem MTA, CS cement) did not reveal genotoxic effects. ProRoot MTA showed toxic effects in only one study, as well

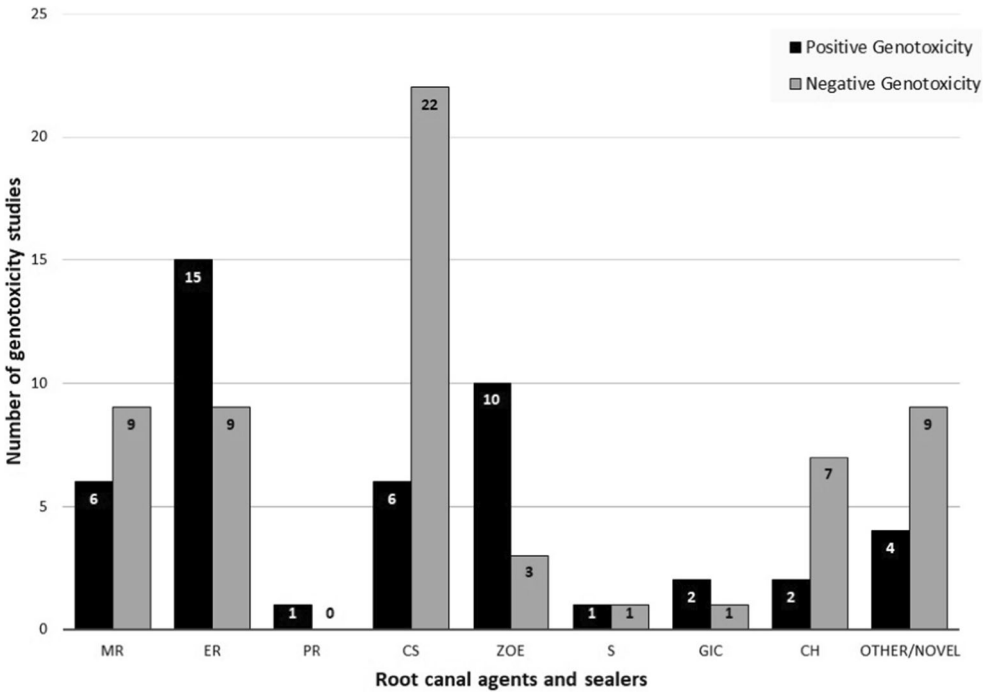

Fig. 2 Root canal sealers' genotoxicity studies presented by composition: methacrylate-based sealers (MR), epoxy resin-based sealers (ER), polyvinyl resin-based sealers (PR), calcium silicate-based sealers (CS), zinc oxide eugenol-based sealers (ZOE), silicone-based sealers (S), glass ionomer cement-based sealers(GIC), calcium hydroxide-based sealers (CH), and other novel sealers

as MTA Angelus. Comparatively, MTA Fillapex displayed higher toxicity. As a whole, MTA-based sealers are associated with low genotoxicity potential [65, 69]. However, calcium-enriched mixtures of MTA were genotoxic [64]. Nevertheless, pure CS-based cements, modified CS-based cements, and three resin-based CS cements showed no genotoxicity in human osteoblast cells [101].

Zinc oxide-eugenol sealers were also involved in a number of studies. Overall, these sealers appeared to be genotoxic. The eight tested sealers presented deleterious effects in several in vitro [51–54] and in vivo [39] assays. In the other way, calcium hydroxide-based sealers showed low genotoxicity [35, 36, 38, 39, 43, 44, 47]. Eight sealers were analyzed, and only Calcipex II and Vitapex were genotoxic [65]. Sealapex did not exhibit genotoxicity [36, 38].

The other sealers' groups were less tested, as shown in Fig. 2. Two silicone-based sealers were tested, i.e., RoekoSeal and Gutta Flow presenting, respectively, positive [41] and negative [40] genotoxicity. For the glass ionomer cement sealers, three genotoxicity studies were reported, namely involving Ketac-Endo [35] and Vitrebond [42, 43]. The presence of the resin component in the sealer composition might play a role in the observed deleterious effect [102, 103]. Miscellaneous commercially available sealers were grouped together, and tested sealers included Endosequence BC [56, 70], iRootSP [56, 70], Biodentine [66, 71, 72], BioRoot RCS, Polifil, CEM, and CS+HA. Although only one or two studies were conducted per sealer, overall, genotoxicity potential was low (Fig. 2).

Considering the three groups of sealers involved in a higher number of studies, calcium silicate-based sealers presented the lower genotoxicity potential, followed by methacrylate resin-based sealers, which appeared slightly lower genotoxic than the epoxy resin-based sealers, whereas zinc oxide based sealers presented the higher genotoxic potential. The other groups of sealers were less addressed. Calcium hydroxide-based sealers appeared to present low genotoxic potential, whereas silicone-based and glass ionomer-based sealers showed variable outcomes. Nevertheless, establishing rankings of genotoxicity is a risky exercise. Major drawbacks are the great heterogeneity of the experimental protocols, namely the use of multiple cell lines, some of them not recommended by OECD guidelines, differences in the exposure conditions and in the performed genotoxicity assays. Also, there is insufficient information to draw firm conclusions concerning the sealers' safety. Gene expression profiles and epigenetic mechanisms for damage response, antioxidant, or DNA repair genes, despite being indirect methods of analysis and not commonly recommended by standard guidelines, could provide relevant information on the involved genotoxicity mechanisms [104].

In a translational view, the reported information on the sealers' genotoxicity must be placed in a proper context. Results were collected mostly from in vitro studies, using static cell culture models, and exposure conditions that are far from those anticipated in a clinical setting. Following the endodontic treatment, the levels of degradable/leachable compounds eventually observed are expectably low due to the very small contact area of the sealer potentially yielding leachable components. Also, due to the dynamic in vivo conditions, namely the continuous extracellular fluid flow, eventual leachates are continuously being cleared decreasing the local levels. The exposure features also deserve some observations. In vivo, cells of the periapical tissues are embedded in a collagenous extracellular matrix organized in a three-dimensional structure, thus with lower susceptibility to deleterious effects from the surrounding environment. Due to these unique physiological features and exposure conditions, the genotoxicity trends observed in vitro might not translated to the periapical environment with, eventually, less noticeable differences and/or outcomes among sealers [105]. Nonetheless, efforts to regulate the in vitro and in vivo genotoxicity assays is critical, in order to standardize protocols allowing representative information and translational usefulness.

Conclusions

An overview of the collected studies suggests that most sealers present some degree of genotoxicity and DNA damage, however with a trend toward low or high genotoxic potential. As the available information is mostly from in vitro studies, and involving a great heterogeneity on the experimental protocols, there is insufficient information to draw firm conclusions concerning the sealers' safety.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent For this type of study, formal consent is not required.

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