The Saudi Dental Journal 36 (2024) 249–257

Contents lists available at ScienceDirect

The Saudi Dental Journal

journal homepage: www.ksu.edu.sa
www.sciencedirect.com

Review Article

Genotoxicity induced by endodontic sealers: A systematic review

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ARTICLE INFO

Keywords:
Genotoxicity
Endodontic sealers
DNA damage

ABSTRACT

Introduction: This systematic review aimed to help further elucidate the following question: are endodontics sealers able to induce DNA damage in vitro or in vivo?
Methods: This study was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement 2020 criteria. A total of 23 studies were carefully selected by the authors.
Results: Regarding the general characteristics, most studies evaluated, on average, 3–5 types of sealers (resin epoxy, salicylate, salicylate + MTA, zinc oxide-eugenol, bioceramic products, calcium hydroxide), performing comparisons between them. Our results demonstrate that endodontic sealers may be a genotoxic agent since most studies demonstrated positive findings, with the resin-based ones being the most potentially genotoxic.
Conclusion: The type of genotoxicity assay, material evaluated, and dilution concentration levels influenced the outcome. This study clarifies whether and to what extent endodontic sealers are capable of inducing DNA injury in oral tissues.

1. Introduction

Nowadays, in endodontic practice, sealers are extensively used in gap filling procedures in which the core material and the root canal walls must be in intimate contact (Kaur et al., 2015). This hermetic contact is achieved by the formation of a homogeneous obturation mass with lack of voids after the elimination of the remaining microorganisms and regularization of canal portions via root canal reshaping (Kaur et al., 2015). Endodontic sealers are categorized by composition based on setting reaction and composition, considering their base components. Although other classification variations containing fillers or ceramic powders may be found in literature (such as MTA), the previously cited matrices continue to be the basis of the compositions (Komabayashi, 2020).

Nevertheless, the attempt to hermetically seal root canals is not always successfully achieved as unintentional (and sometimes inadvertent) sealer biomaterial may extrude during endodontic obturation. In this sense, scientific advances are frequently introducing novel endodontics materials at full speed, raising the question whether such introductions may be too deliberate concerning tissue hazards (Hosseinpour et al., 2022). Considering the non-stop scientific biomaterial evolution, safety must be seriously considered. In this context, biocompatibility is one of the most relevant steps for ensuring safety when endodontic materials are studied and launched since they may have unintentional or inadvertent direct contact with the periapical tissue (Hosseinpour et al., 2022). The underlying reason for biocompatibility studies lies in the fact that these biomaterials are in close contact with several oral tissues rather than the root dentin. This potential contact is likely to induce oxidative stress and to generate genetic damage, endangering long-term use of these products (Eid et al., 2014). In this sense, genotoxicity plays an important role in detecting whether and to what extent endodontic sealers may be able to induce DNA damage (Eid et al., 2014; Pires et al., 2016). To this end, and in line with the objective of identifying genotoxic effects in oral cells and tissues, some assays can be used, such as the micronucleus assay, the comet assay, chromosomal aberration, and sister chromatid exchange tests (Kang et al., 2013).

Concerning the cited tests, it is important to stress that the micronucleus assay, the chromosomal aberration, and the sister chromatid exchange aim to identify chromosome damage, whereas the comet assay is a method that aims to quantify DNA breakage as a result of DNA moving fragments when electrophoresis is performed (Lu et al., 2017).

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https://doi.org/10.1016/j.sdentj.2023.11.019
Received 28 July 2023; Received in revised form 13 November 2023; Accepted 15 November 2023
Available online 17 November 2023

Moreover, regarding the comet assay, the lower molecular weight particles are pulled towards the anionic pole, forming a structure similar to a comet that will be further analyzed considering the tail length and intensity to determine the potential DNA damage (Lu et al., 2017). All things considered, it is coherent to state that all techniques evaluate DNA damage quantitatively and qualitatively by different end-points (Wilson et al., 2007; Moller et al., 2020).

In this context, considering the variety of sealers and their potential genotoxic effects, this systematic review aimed to understand whether endodontics sealers may induce DNA damage in vitro or in vivo.

2. Material and methods

This study was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement 2020 criteria. The PICOS strategy: P (mammalian cells), I (Endodontic sealers), C (Control group), O (Genotoxicity), S (In vitro or in vivo exposure) was used as a guide.

2.1. Inclusion criteria

For the analysis, inclusion criteria were studies that: 1) measured genetic damage in vitro and/or in vivo; 2) were published in English; and 3) provided data that clearly met scientific standards. In accordance with the search strategy, some methods used for measuring genotoxicity were highlighted, being the micronucleus, the comet, the sister chromatic exchange, and the chromosomal aberration assays.

2.2. Exclusion criteria

Exclusion criteria included the following types of studies: 1) conference abstracts, reviews, editorials, and letters; 2) full-text not available in English; 3) studies with unavailable or unextractable data or with combined exposure without control group of endodontic sealer only; 4) multigenerational studies; 5) studies focusing on amelioration of endodontic sealers toxicity; 6) studies that did not measure genotoxicity 7) studies with partial or vague results.

2.3. Data search

In January of 2023, searches were conducted in PubMed, SCOPUS, and Web of Science databases to identify eligible articles, with the following keywords and Boolean operators: (“Sealer” OR “Endodontic sealer”) AND (“Genotoxicity” OR “DNA damage” OR “genetic damage” OR “DNA breakage” OR “genetic injury” OR “DNA injury” “chromosome damage” AND (“comet assay” OR “micronucleus assay” OR “sister chromatid exchange” OR chromosome aberration test”). An additional manual search of references and cited/related articles was performed. Terms were validated by conducting the proper selection of articles, representative of relevant works. Moreover, searches were restricted to the English language and all dates of publication were considered. Abstracts were read and judged independently by two reviewers (TGP and DAR). Boolean operators were used (AND and OR) to combine the descriptors with different combinations, as described elsewhere. First, a manual search by author (TGP) of the reference list of reviews and published articles was conducted; then, texts were selected based on both titles and abstracts. Afterwards, the second stage was conducted, in which two researchers (TGP and DAR) reread the references raised to identify possible lost articles in the very first search. The two aforementioned investigators, in an independent manner, reviewed the full-texts and available studies. Thus, relevant studies and their final evaluation were included for a proper selection of studies related to the research. After that, full-text readings of all selected abstracts were conducted to confirm eligibility. All divergences between the two reviewers (TGP and DAR) were achieved by a consensus after discussion.

2.4. Data extraction and quality assessment

The following data were presented: authors, year and country of study, species, organs or cell types, dose, concentration, exposure time, assay, number of evaluated cells, genotoxicity assay used, blind, statistical analysis, positive and negative control, and main results.

2.5. Risk of bias in individual studies

The score of the individual variables was established to classify each article. For this, the following information from the quality instrument was used: (1) study design, (2) identification and treatment of confounding factors, (3) blind analysis, and (4) data analysis. The considered criteria in the evaluation of the study design were: number of participants per group, statistical analysis, and blind analysis. The considered confounding factors were: cytotoxicity, repetitions number, and positive and negative controls. Moreover, strong, moderate, and weak classifications were used for the articles. Studies that controlled all but one, two, or three or more variables were rated as STRONG, MODERATE, or WEAK, respectively (Malacarne et al., 2022).

3. Results

3.1. Study selection

The data search identified 426 scientific records among which 108 publications were duplicates and, thus, excluded. After evaluating the titles and abstracts, 285 studies did not meet the inclusion criteria due to being literature reviews, case reports, commentaries and editorials, papers written in other languages other than English, or letters to Editor. Full manuscripts from 23 studies were meticulously read by both authors of the present article (Fig. 1).

3.2. General characteristics of the included studies

Table 1 shows the most important characteristics of the evaluated studies. A total of 23 studies were evaluated, with eight studies being conducted in Brazil. Only one study was conducted in Australia, Spain, and Turkey, respectively; two studies were conducted in Germany, Croatia, India, and in the USA, respectively; and four studies were conducted in Taiwan. The year of publication found in articles included in this study ranged from 1999 to 2022.

Regarding the general characteristics, most studies evaluated, on average, three types of sealers (amongst resin epoxy, salicylate, salicylate + MTA, zinc oxide-eugenol, bioceramic products, and calcium hydroxide), performing comparisons between them.

3.3. Variables related to dental sealers and genotoxicity

Table 2 describes the variables related to endodontic sealers and genotoxicity. First, all studies presented control groups for proper comparison. However, some studies presented both positive and negative control, whereas others presented only negative control.

All included studies in this review used a type of test to verify genotoxic outcomes induced by sealers. In total, seven of the evaluated studies performed the micronucleus assay, being these conducted by Erdogan et al. (2021), Silva et al. (2015), Só et al. (2022), Martinho et al. (2018), Candeiro et al. (2016), Bin et al. (2012), and Camargo et al. (2009). The comet assay was performed in ten of these studies, being these conducted by Teixeira et al. (2021), Camargo et al. (2009), Barara et al. (2011), Brzovic et al. (2009), Huang et al., (2001; 2002; 2004), Kim et al. (2022), Dhopavkar et al. (2021), Tai et al. (2002), and Nair et al. (2018). However, some studies used other assays, such as the one conducted by Eldeniz et al. (2016) and Van Landuyt et al. (2012), who used the c-H2AX immunofluorescence assay. Victoria-Escandell et al. (2017), on the other hand, used the flow cytometry for the genotoxicity

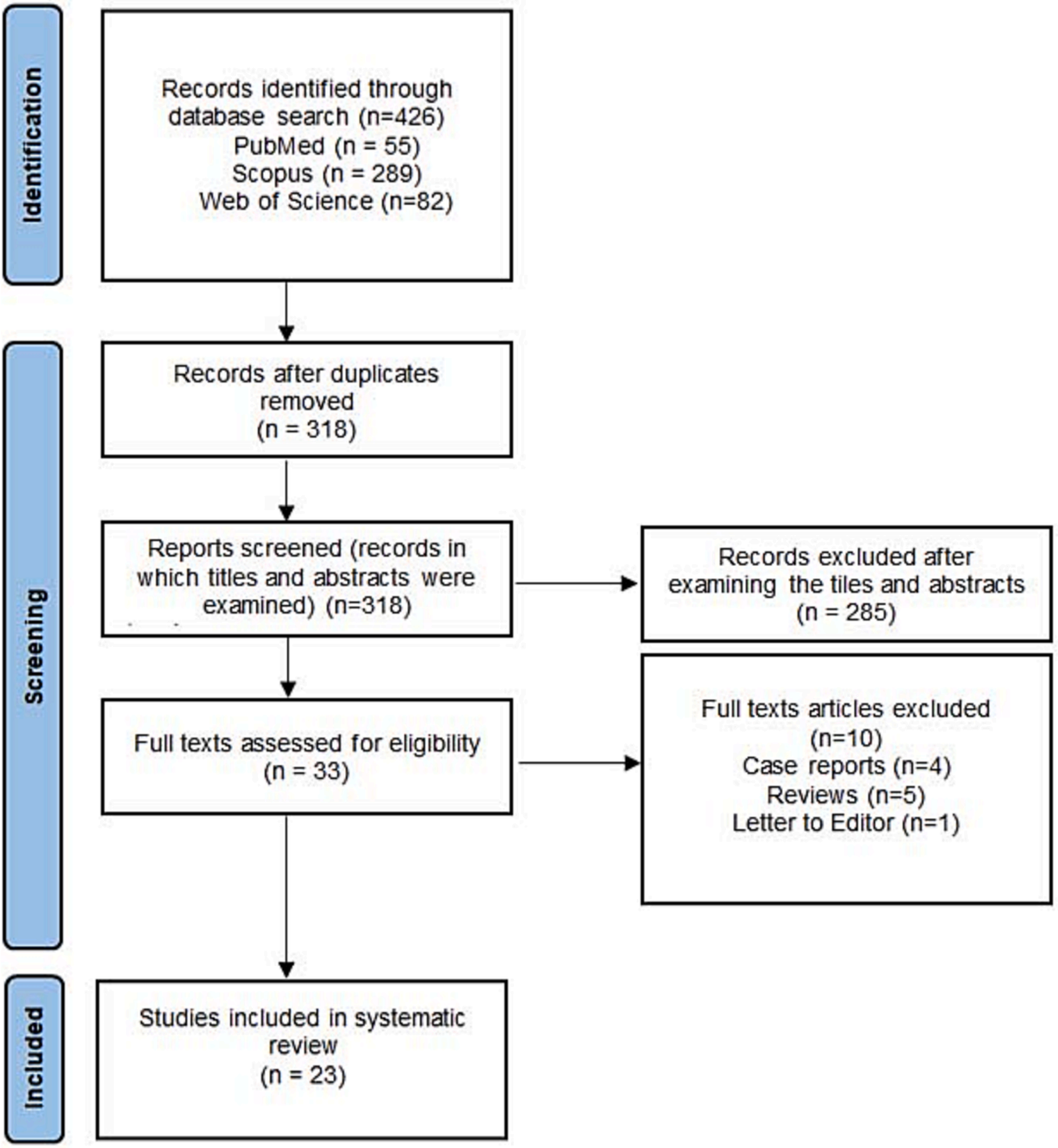

Fig. 1. Flow chart of the study.

analysis, whereas Tai et al. (2002) performed a DNA fragmentation analysis. Contrary to the previously cited authors, to analyze genotoxicity, Leyhaunsen et al. (2022) used the DIT and AFE test for eukaryotic analysis and AMES and UMU tests for procaryotic assessment.

In all in vitro studies, many mammalian cells were exposed to different sealers concentrations. For the in vivo study, clams were exposed to varying concentrations of sealers.

The selected studies presented different exposure times according to the genotoxicity test used. In the micronucleus assay, Erdogan et al. (2021), Silva et al. (2015), Só et al. (2022), Candeiro et al. (2016), Bin et al. (2012), and Camargo et al. (2014) adopted a 24-h period, whereas only the study by Martinho et al. (2018) adopted 55 weeks. In the same sense, comet assays also presented different exposure times: Teixeira et al. (2021) adopted 1, 7, and 30 days; Camargo et al. (2014), Baraba et al. (2011), and Huang et al., (2002; 2004) adopted 24 h; Dhopavkar et al. (2021) adopted 24 h and 48 h; and Nair et al. (2018) adopted 48 h.

Some studies were conducted in healthy human periodontal fibroblasts, from which three had third molars as specific sources and five had either other human teeth as sources or did not have a specific third molar source description. Moreover, only one study specifically informed the

Table 1
Most important characteristics of the included studies regarding genotoxicity induced by sealers in chronological order.

Authors	Year	Country	Compound tested (commercial name)	Seal base
Só et al. (2022)	2022	Brazil	Sealer Plus BC AH Plus MTA-Fillapex	Bioceramic Epoxy resin Salicylate resin + MTA
Leme and Salvadori (2022)	2022	Brazil	MTA-Fillapex	Salicylate resin + MTA
Kim at al. (2022)	2022	Brazil	Adseal AH Plus Dia-Proseal	Epoxy resin Epoxy resin Epoxy resin
Erdogan et al. (2021)	2021	Australia	AH Plus MTA-Fillapex IRootSP	Epoxy resin Salicylate resin + MTA Calcium hydroxide
Dhopavkar et al. (2021)	2021	India	AH Plus MTA-Fillapex GuttaFlow 2 Sealer r	Epoxy resin Salicylate resin + MTA Bioceramic
Teixeira et al. (2021)	2020	Brazil	AH Plus Sealer 26 Endomethasone N	Epoxy resin Calcium hydroxide Zinc oxide eugenol
Martinho et al. (2018)	2018	Brazil	AH Plus EndoREZ Apexit Plus RealSeal SE	Epoxy resin Methacrylate resin Calcium hydroxide Methacrylate resin
Nair et al. (2018)	2018	India	Endosequence BC Sealer Tubli-seal IRootSP	Calcium hydroxide Zinc oxide Calcium hydroxide
Victoria-Escandell et al. (2017)	2017	Spain	AH Plus MTA-Fillapex MTA Angelus White	Epoxy resin Salicylate resin + MTA Salicylate resin + MTA
Eldeniz et al. (2016)	2016	Turkey	AH Plus Jet Acroseal Acroseal EndoREZ RealSeal RealSeal SE Hybrid Root SEAL BioRoot RCS IRootSP MTA-Fillapex	Epoxy resin Calcium hydroxide Zinc oxide Methacrylate resin Methacrylate resin Methacrylate resin Bioceramic Bioceramic Salicylate resin + MTA
Candeiro et al. (2016)	2016	Brazil	Endosequence BC Sealer AH Plus	Calcium hydroxide Epoxy resin
Camargo et al. (2014)	2014	Brazil	AH Plus EndoREZ RoekoSeal	Epoxy resin Methacrylate resin Silicone
Silva et al. (2013)	2013	Germany	AH Plus EndoREZ RealSeal SE Copaifera Polifill	Epoxy resin Methacrylate resin Methacrylate resin Zinc oxide Zinc oxide
Van Landuyt et al. (2012)	2012	USA	AH Plus Jet EndoREZ RealSeal SE	Epoxy resin Zinc oxide Methacrylate resin
Barara et al. (2011)	2011	Croatia	EpiphanyRealSeal SE (Sybron Endo, USA)	Methacrylate resin Methacrylate resin
Bin et al. (2011)	2011	Brazil	MTA-Fillapex AH Plus White MTA	Salicylate resin + MTA Epoxy resin Calcium hydroxide
Brzovic et al. (2009)	2009	Croatia	Guttaflow Epiphany Diaket IRM SuperEBA Hermetic	Zinc oxide Methacrylate resin Zinc oxide Zinc oxide eugenol Zinc oxide eugenol Zinc oxide eugenol
Camargo et al. (2009)	2009	Germany	AH Plus Epiphany Acroseal	Epoxy resin Methacrylate resin Calcium hydroxide
Huang et al. (2004)	2004	Taiwan	Sealapex Canals Canals-N Tubilseal TopsealAH26 (Silver free) AH Plus	Calcium hydroxide Zinc oxide eugenol Zinc oxide Zinc oxide eugenol Epoxy resin Epoxy resin
Huang et al. (2002)	2002	Taiwan	Sealapex AH Plus Canals	Calcium hydroxide Epoxy resin Zinc oxide eugenol
Huang et al. (2001)	2001	Taiwan	AH 26 AH Plus	Epoxy resin Epoxy resin

(continued on next page)

Table 1 (*continued*)

Authors	Year	Country	Compound tested (commercial name)	Seal base
Tai et al. (2001)	2001	Taiwan	AH Plus AH 26 N2 Canals	Epoxy resin Epoxy resin Zinc oxide eugenol Zinc oxide eugenol
Leyhaunsen et al. (1999)	1999	USA	AH Plus	Epoxy resin

volunteer's age.

Regarding the number of cells evaluated in the micronucleus assay, a total of five studies evaluated 1,000 cells per slide. For the alkaline comet assay, a total of three studies evaluated 100 randomly selected comets per slide, totaling 300 comets (Teixeira et al., 2021; Baraba et al., 2011; Dhopavkar et al., 2021), whereas 50 comets per treatment were evaluated in four studies (Leme et al., 2022; Huang et al., 2002; 2004; Dhopavkar et al., 2021). The study conducted by Nair et al. (2018) evaluated from 50 to 100 cells per sample, whereas the study by Camargo et al. (2014) evaluated only 25 cells and three studies failed to inform the amount of analyzed cells. For the c-H2AX immunofluorescence assay, Eldeniz et al. (2016) evaluated 100 cells per slide, whereas Van Landuyt et al. (2012) evaluated at least 200 cells per group. Regarding the study by Leyhaunsen et al. (1999), four clams were analyzed per assay in the AFE test.

The tests were performed in duplicate in two studies, in triplicate in 11 studies, in quadruplicate in five studies, and in quintuplicate in one study. The other four studies failed to inform the number of replicates.

Concerning data analysis, all micronucleus assay studies used cell count in the measurement of genotoxicity. Regarding the comet assay, the use of software programs was the basis of some parameters analysis. While Teixeira et al. (2021) and Leme and Salvadori (2022) evaluated tail intensity and Nair et al. (2018) only evaluated tail moment, all the other comet assay studies evaluated tail intensity and tail moment. As for the c-H2AX immunofluorescence assay studies, cell count and standardized foci quantification were considered. The assay that performed flow cytometry used cell count as a quantitative biological parameter. At last, the study conducted by Leyhaunsen et al. (1999) used different parameters, depending on the evaluated test (DIT, AFE: AFE, UMU, and AMES).

The adoption of blind analysis was observed in the methodology of five studies, whereas 18 of them did not provide such information. Lastly, all the included studies properly described the applied statistical test concerning the data analysis.

3.4. Main results

Regarding cytotoxicity, except for the study conducted by Huang et al. (2000;2002;2004), all selected studies evaluated cell death parameters, such as XTT, MTT, MTS, SRB, Trypan Blue assays and other cell viability tests. Considering chromosome damage, four studies showed that AH Plus was able to induce chromosome damage as analyzed by micronucleus assay (Erdogan et al., 2021; Candeiro et al., 2016; Bin et al., 2012; Victoria-Escandell et al., 2017). Furthermore, chromosome damage induction was also showed in one study for MTA-Fillapex and one for Acroseal and Epiphany, also analyzed by micronucleus assay (Bin et al., 2012; Hubbe et al., 2016). Additionally, sealers genotoxicity (AH Plus, Endorez, RoekoSeal, AH 26, N2, Canals, MTA-Fillapex, and GutaFlow 2) were measured in three studies (Brzovic et al., 2009; Dhopavkar et al., 2021; Van Landuyt et al., 2012). Moreover, at high concentrations, BioRoot RC and RealSeal SE presented genotoxicity in vitro (Huang et al., 2001; Dhopavkar et al., 2021. Table 3 summarizes these findings.

3.5. Quality assessment

Regarding the quality assessment, ten, seven, and six studies were classified as Strong, Moderate, and Weak, respectively, as shown in Table 4.

4. Discussion

Endodontic sealers are widely used worldwide in the attempt to combat and prevent canal reinfection or growth of the remaining surviving microorganisms by residual bacteria entombment and nutrients leakage prevention (Camargo et al., 2014 and Munitić et al., 2019). Nonetheless, it is not rare to observe extrusion by apical constriction and by lateral and secondary canals with consequent contact between sealers and periradicular tissues, posing potential risks concerning genotoxicity in human cells (Dos Santos Costa et al., 2020).

In accordance with the potential risks, different tests can be used to evaluate genotoxicity, each with their own advantages. Nonetheless, currently, the most used ones worldwide converge in some aspects, such as simplicity, robustness and time- and cost-effectiveness in targeting toxicity. Nevertheless, the aforementioned assays require very specific parameters to achieve proper evaluations with reliable results. In this study, micronucleus assay, comet assay, and other tests (c-H2AX, UMU, and AMES) were considered in the review as tests capable of detecting genotoxicity induced by endodontic sealers.

While micronucleus assay can be considered a widely used sensitive method capable of detecting both chromosome or fragments in the cytoplasm of eukaryotic cell, comet assay is also comprehensively used, especially in vivo, as it is considered versatile concerning the evaluation of genotoxicity in different organs and tissues (Hubbe et al., 2016). Our results indicated that, from the 23 included studies, seven studies conducted the micronucleus assay, with positive genotoxicity being encountered in four. Moreover, 12 studies used the comet assay, with systematic results indicating positive genotoxicity in seven.

Additionally, we highlight that, to properly perform the comet assay without compromising the found results, the minimum of 50 cells must be evaluated and the parameter must be tail intensity (Cordelli et al., 2021). While only tail moment and tail intensity evaluations were conducted by one and two studies, respectively, all the others included both analyses. In this sense, it is coherent to state that studies that used scores or any other unmentioned evaluation parameters may compromise the results (Cordelli et al., 2021). Moreover, four studies performed other tests, such as c-H2AX immunofluorescence assay, flow cytometry, DIT test, AFE test, and AMES besides UMU tests. By using these assays, the results also showed that positive genotoxicity was detected in half of them.

Additionally, more than 50 % of the analyzed studies (13 out of 23) suggested genotoxicity increase in at least one of the evaluated sealers. More specifically, among the different evaluated categories according to the sealer base, the resin-based group (AH Plus) was the most genotoxic and cytotoxic across studies. We also highlight that, in the analyzed studies, different parameters were considered to determine the dose of endodontic sealers and most of the studies presented higher cytotoxicity in higher dilution concentrations.

Regarding the final ratings given by the authors of the present systematic review, ten, seven, and six studies were classified as Strong, Moderate, and Weak, respectively (in accordance with the previously described methodology). Overall, we assumed a good quality for most analyzed studies when evaluating genotoxicity, confirming, therefore, that the found results are reliable.

Furthermore, we highlight that an important parameter to be considered in genotoxicity studies is the presence of cytotoxicity, as genotoxicity tests should not be performed under conditions in which cell death is present. Moreover, it is known that cytotoxicity can induce fragmentation of the genetic material by caspases, which could lead to false-positive results (Tice et al., 2000). In this sense, it is reasonable to

Table 2
Variables analyzed in the studies regarding genotoxicity induced by sealers in chronological order.

Author	Concentration	Exposure time	Cell line/ species	Study design	Genotoxicity assay	Number of cells	Cytotoxicity assay	Reproduction number	Evaluated parameters	Blind analysis	Proper statistics description	Positive control	Negative control
Só et al. (2022)	1:10	24 h		In vitro	Micronucleus assay	100 cells/ slide	MTT assay	Triplicate	CellCount	–	Yes	No	Yes
Leme and Salvadori (2022)	5 %, 10 %, 20 % and 40 %	24 h		In vitro	Comet assay	50 cells/ slide	MTS assay	Triplicate	Tail intensity	–	Yes	Yes	Yes
Kim at al. (2022)	100 %, 50 %, 25 %, 12.5 %, 6.25 %, 3.13 %	50 min (Adseal); 8 h (AH Plus); 7.5 h (Dia-Proseal)		In vitro	Comet assay	–	MTT assay	Quadruplicate	Tail moment and tail intensity	–	Yes	No	Yes
Erdogan et al. (2021)	1:1, 1:2, 1:4, 1:8, 1:16, 1:32	24 h		In vitro	Micronucleus assay	100 cells/ slide	XTT	Triplicate	CellCount	–	Yes	No	Yes
Dhopavkar et al. (2021)	1.25 cm2/ml	24 h; 48 h		In vitro	Comet assay	50 cells/ sample	MTT assay	Triplicate	Tail moment and tail intensity	–	Yes	Yes	Yes
Teixeira et al. (2021)	2,5%; 5 %; 10 %	1 day; 7 days; 30 days		In vitro	Comet assay	100 cells/ slide	XTT	Triplicate	Tail intensity	–	Yes	Yes	Yes
Martinho et al. (2018)	1:2	24 h		In vitro	Micronucleus assay	100 cells/ slide	MTT assay	–	CellCount	–	Yes	Yes	Yes
Nair et al. (2018)	4 × 103 cells per mL	48 h		In vitro	Comet assay	50 to 100 cells/ sample	MTT assay	Triplicate	Tail moment	Yes	Yes	Yes	Yes
Victoria-Escandell et al. (2017)	1:2	24 h		In vitro	Flow cytometry	4000 cells/ sample	SRB assay	Triplicate	CellCount	–	Yes	No	Yes
Eldeniz et al. (2016)	1/3 and 1/10 (both EC50)	24 h		In vitro	c-H2AX immunofluorescence assay	100 cells/ slide	XTT	Triplicate	CellCount	–	Yes	Yes	Yes
Candeiro et al. (2016)	1:10	24 h		In vitro	Micronucleus assay	100 cells/ slide	MTT assay	Triplicate	CellCount	–	Yes	No	Yes
Camargo et al. (2014)	1:2, 1:4, 1:8, 1:16, 1:32	24 h		In vitro	Comet assay	25 cells/ slide	MTT assay	Quadruplicate	Tail moment and tail intensity	–	Yes	Yes	Yes
Silva et al. (2013)	1:1, 1:2, 1:4, 1:8	24 h		In vitro	Micronucleus assay	1000 cells/ slide	MTT assay	Quadruplicate	CellCount	–	Yes	Yes	Yes
Van Landuyt et al. (2012)	1/3 and 1/10 (both EC50)	24 h		In vitro	c-H2AX immunofluorescence assay	At least 200/ group	XTT	Quadruplicate	Standardized foci quantification	–	Yes	Yes	Yes
Barara et al. (2011)	0,02 g/4,5 ml	24 h		In vitro	Comet assay	100 cells/ slide	count of viable, apoptotic and necrotic cells	Duplicate	Tail moment and tail intensity	Yes	Yes	Yes	Yes
Bin et al. (2011)	1:1, 1:2, 1:4, 1:8; 1:16; 1:32	24 h		In vitro	Micronucleus assay	1000 cells/ slide	MTT assay	Quadruplicate	CellCount	–	Yes	Yes	Yes

(*continued on next page*)

Table 2 (*continued*)

Author	Concentration	Exposure time	Cell line/ species	Study design	Genotoxicity assay	Number of cells	Cytotoxicity assay	Reproduction number	Evaluated parameters	Blind analysis	Proper statistics description	Positive control	Negative control
Brzovic et al. (2009)	1:4, 1:8, 1:16	1 h, 1 day, 5 days, 30 days		In vitro	Comet assay	100 cells/ slide	Trypan Blue ex lusion test	–	Tail moment and tail intensity	Yes	Yes	Yes	Yes
Camargo et al. (2009)	Acroseal (1:64 and 1:128), AH Plus (1:8 and 1:16), andEpiphany (1:8 and 1:16)	24 h		In vitro	Micronucleus assay	1000 cells/ slide	MTT assay	Quadruplicate	CellCount	–	Yes	Yes	Yes
Huang et al. (2004)	0.02, 0.1, 0.5, 2.5, 12.5 mg/100uL	12 h and 24 h		In vitro	Comet assay	–	MTT assay	–	Survival rate	–	Yes	Yes	No
Huang et al. (2002)	0.01, 0.05, and 0.25 mg/ ml	24 h		In vitro	Comet assay	50 cells/ slide	MTT assay	–	Tail moment and tail intensity	Yes	Yes	Yes	Yes
Huang et al. (2001)	0.1, 0.5, and 2.5 mg/ml	24 h		In vitro	Comet assay	50 cells/ slide	–	–	Tail moment and tail intensity	Yes	Yes	Yes	Yes
Tai et al. (2001)	2,5 ug/ul and 5ug/Ul	24 h		In vitro	Comet assay	–	MTT assay	Triplicate	Count of H activity	–	Yes	No	Yes
Leyhaunsen et al. (1999)	EUCARYOTIC DIT 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128 AFE 1:40, 1:80, 1:100 and 1:200 PROCARYOTIC AMES 1:5, 1:10, 1:20, 1:40 and1:80 UMU 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:245 and 1:512	DIT: 1,5h UMU test: 2 h and 4 h; AMES test: 48 h; AFE test: 2 h		In vitro/ In vivo	all in vivo EUCARYOTIC: DIT test and AFE test PROCARYOTIC: AMES and UMU tests	AFE: 4 clams/ assay	Growth inhibition test	AFE test: triplicate UMU test: Triplicate AMES test: Duplicate	DIT: cellcount AFE: AFE factor (single DNA breaks: treated/control) UMU: induction rate and growth factor AMES: revertants counts	–	Yes	Yes	Yes

Table 3
Main genotoxicity findings of studies in chronological order.

Authors	Genotoxicity findings
Só et al. (2022)	No significant differences
Leme and Salvadori (2022)	↑ CA: MTA-Fillapex
Kim at al. (2022)	↑ MN: AdSeal, AH Plus and Dia-Proseal
Erdogan et al. (2021)	↑ MN: AH Plus
Dhopavkar et al. (2021)	↑ MTA-Fillapex
Teixeira et al. (2021)	No significant differences
Martinho et al. (2018)	No significant differences
Nair et al. (2018)	No significant differences
Victoria-Escandell et al. (2017)	↑ MN: AH Plus and MTA-Fillapex
Eldeniz et al. (2016)	↑ c-H2AX: BioRoot RC and RealSeal SE
Candeiro et al. (2016)	↑ MN: AH Plus
Camargo et al. (2014)	↑ CA: AH Plus, Endorez and RoekoSeal
Silva et al. (2013)	No significant differences
Van Landuyt et al. (2012)	No significant differences
Barara et al. (2011)	No significant differences
Bin et al. (2011)	↑ MN: AH Plus and MTA-Fillapex
Brzovic et al. (2009)	No significant differences
Camargo et al. (2009)	↑ MN: AH Plus, Acroseal and Epiphany
Huang et al. (2004)	No significant differences
Huang et al. (2002)	↑CA: AH Plus and AH 26
Huang et al. (2001)	↑ CA: AH Plus, AH 26 and TopSeal
Tai et al. (2001)	↑ CA: AH Plus, AH 26, N2 and Canals
Leyhaunsen et al. (1999)	No significant differences

↑: increase; CA: comet assay; MN: micronucleus assay.

Table 4
Quality assessment and final rating of the studies in chronological order.

Author	N° of non controlled confounders	Final rating
Só et al. (2022)	3	Weak
Leme and Salvadori (2022)	2	Moderate
Kim at al. (2022)	2	Moderate
Erdogan et al. (2021)	2	Moderate
Dhopavkar et al. (2021)	2	Moderate
Teixeira et al. (2021)	2	Moderate
Martinho et al. (2018)	3	Weak
Nair et al. (2018)	1	Strong
Victoria-Escandell et al. (2017)	3	Weak
Eldeniz et al. (2016)	1	Strong
Candeiro et al. (2016)	2	Weak
Camargo et al. (2014)	1	Strong
Silva et al. (2013)	1	Strong
Van Landuyt et al. (2012)	1	Strong
Barara et al. (2011)	1	Strong
Bin et al. (2011)	1	Strong
Brzovic et al. (2009)	1	Strong
Camargo et al. (2009)	2	Moderate
Huang et al. (2004)	5	Weak
Huang et al. (2002)	1	Strong
Huang et al. (2001)	2	Moderate
Tai et al. (2001)	3	Weak
Leyhaunsen et al. (1999)	1	Strong

say that disregarding some data about cytotoxicity may lead to interpretation bias and that the approach for cytotoxicity is crucial for genotoxicity evaluation (Tice et al., 2000). In this study, most authors evaluated cytotoxicity to ensure the quality of the results regarding genotoxicity of sealers. We also highlight that only the smallest portion of the studies clearly mentioned the use of blind analysis methodology (five out of 23 studies), what interfered in the final rating of most articles.

To summarize, our results demonstrate that endodontic sealers may be considered genotoxic since most studies indicated positive findings and 17 showed a Moderate or Strong final rating. The resin-based sealers were found to be the most potentially genotoxic. The type of genotoxicity assay, material evaluated and dilution concentration levels influenced the outcome. Considering that some studies show that the contact extruded sealers did not impair the repair of endodontic lesions (Li et al., 2022; Shashirekha et al., 2018), further studies (mainly in vivo) should be conducted, especially in humans, elucidating the role of genotoxicity induced by endodontic sealers.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Data sharing are not available to this article.

Funding

The authors acknowledge research grants received from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant Number #001) for productivity fellowship.

Author contributions

Study design: TGP and DAR. Data search: TGP and DAR. Data analysis: TGP, ACMR, JNS, PRC and and DAR. Writing the paper: all authors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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