REVIEW ARTICLE

Evaluation of Cytotoxicity of Calcium Silicate-based Mineral Trioxide Aggregate Sealers: A Systematic Review of *In Vitro* Studies

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Abstract

Aim: This review aimed to evaluate the *in vitro* studies done with regard to the cytotoxicity associated with mineral trioxide aggregate (MTA)-based root canal sealers.

Background: Root canal sealers are used during endodontic treatment as fillers to seal the gaps between the canal gutta-percha cone and canal walls. It is necessary to understand the cytotoxicity of these materials on human-derived cells as these materials interact with human cells periapically.

Review results: Six *in vitro* studies were chosen for review. In these selected studies, along with MTA-based root canal sealers, other sealers were tested for cytotoxicity on human periodontal ligament (PDL) stem cells, human PDL fibroblasts, and human osteoblast cells. Regarding cytotoxicity, the studies were diverse, and most were based on 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide) (MTT) assay. In general, the studies suggested that root canal sealers cause mild to severe cytotoxic effects and that several factors influence this effect, such as material setting time, concentration, and duration of exposure.

Conclusion: All studies in the review indicated that MTA. Fillapex must be used cautiously as it exhibited the highest cytotoxic effect compared to other MTA-based and non-MTA-based sealers.

Clinical significance: Endodontic sealers do serve the purpose of bridging the gaps between the gutta-percha cone and the canal wall but knowing its biocompatibility becomes important as the material is extruded beyond the apical foramen where it comes in contact with the surrounding tissues. The effect of sealers on the surrounding tissues affects the healing and prognosis of the treatment.

Keywords: Cytotoxicity, Human periodontal stem cells, Mineral trioxide aggregate, MTT assay, Root canal sealers, XTT assay.

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Introduction

Endodontic therapy aims to sterilize the root canals and reduce pain and infection. The long-term objective for good clinical success is to seal and plug root canal systems to stop future microbial contamination. Endodontic materials with physical and chemical characteristics that are antimicrobial, hermetic, robust, and particularly biocompatible are used to achieve this goal.[1]

During biomechanical preparation, the root canals are cleaned and simultaneously shaped to receive a standardized cone-shaped restorative material that would fit the canal, such as silver cones or gutta-percha. However, root canal anatomical irregularities such as accessory canals, voids, isthmus, and foramen prevent gutta-percha from completely sealing the canals.[2] This makes it necessary for a flowable sealing agent to bond with the canal walls and the restorative cone while adding anatomical irregularities to the restorative material. This flowable agent is called an "endodontic sealer."[3]

Many endodontic sealers are available, but their use depends on multiple factors, such as the case to be treated, the cost of the sealer, etc.[4] Table 1 provides details of all the commercially available endodontic sealers.

All endodontic sealers have advantages and disadvantages, but none meet all the criteria for an ideal sealer. Biocompatibility is an essential property of sealers because when a sealer comes in contact with periapical tissues, it can have undesired outcomes like lower healing effects, delayed sealer resorption in the periapical region, nerve injuries, and so on.[5] Among all the sealers, mineral trioxide aggregate (MTA) sealers have been of profound interest to researchers.

Bioceramic sealers are classified into the following two types: Calcium silicate-based and calcium phosphate-based root canal sealers. The calcium silicate sealers are additionally classified into MTA-based and non-MTA-based sealers.[6] The MTA-based sealers are ideal bioceramic materials that offer biocompatibility and biological activity in endodontics.[7,8] In the past two decades, it has emerged as one of the endodontic materials that have been studied most.[9,10] It was after first development at Loma Linda University (the 1990s) and acceptance by the US Food and Drug Administration (1993) as a root-end filling cement that it became commercially useful as ProRoot MTA™ which is a calcium

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Table 1: Commercially available endodontic sealers

S. No.	*Name of the sealer*	*Name of manufacturer*	*Class*	*Composition*
1	AH26	DeTrey/Dentsply, Konstanz, Germany	Epoxy resin-based sealer	Bisphenol-A-diglycidylether, bizmut oxide, titanium oksit methenamine, silver
2	AH Plus	DeTrey/Dentsply, Konstanz, Germany	Epoxy resin-based sealer	Epoxy resins, calcium tungstate, oxide, silicone oil, iron oxide zirconium
3	AH Plus Jet	Dentsply Sirona, Germany	Epoxy resin-based sealer	Bisphenol A epoxy resin, zirconium oxide, bisphenol F epoxy resin, calcium tungstate, iron oxide, silica
4	Diaket	ESPE, Seefeld, Germany	Polyketone-based sealer	Isobutyl ether, zincoxide, bismuth phosphate, vinyl chloride, triethanolamine propionylacetophenone, copolymers of acetate, vinyl isobutyl ether
5	DiaRootBioaggregate	DiaDent Group International, Burnaby, Canada	Calcium silicate-based MTA sealer	Amorphous silicon oxide, dicalcium silicate, calcium phosphate monobasic, tantalum pentoxide, tricalcium silicate, amorphous silicon oxide
6	EndoRez	Ultradent, South Jordan, Utah USA	Resin-based sealer	Barium sulfate, zinc oxide, urethane dimethacrylate resin, pigment, resin
7	EndoSequence BC	Brassler, USA	Tricalcium Silicate based MTA sealer	Calcium hydroxide, calcium phosphate, calcium silicate, filler, thickening agents, zirconium oxide
8	Epiphany	Pentron, LLC, Wallingford, Connecticut, USA	Resin-based sealer	Barium sulfate, bismuth, bisphenol-A-glycidyl dimethacrylate, calcium oxide, polyethylene glycol dimethacrylate, ethoxylated bisphenol-A dimethacylate, dimethacrylate, silica, pigment, urethane
9	EndoSeal	EndoSeal, Maruchi, Seoul, Korea	Pozzolan cement-based sealer	Aluminum oxide, iron oxide, sodium oxide, calcium oxide, potassium oxide, magnesium oxide, titanium dioxide, zirconium oxide, silicone dioxide
10	Endo-CPM	Egeo, Buenos Aires, Argentina	Calcium silicate-based sealer	Addition of barium sulfate and calcium chloride to the original composition of MTA
11	FibreFill	Pentron, LLC, Wallingford, CT, USA	Resin-based sealer	Benzoyl peroxide, calcium hydroxide, HDDMA, PEGDMA, Proprietary Carboxylic Acid Functional Resins, Silanereated bariumborosilicate glasses, UDMA, barium sulfate, silica, pigment, UDMA
12	GuttaFlow	Roeko, Langenau, Germany	Silicone-based sealer	Gutta-percha, polydimethylsiloxane, zirconium oxide, silicone oil
13	GuttaFlow 2	Coltene/Whale dent, USA	Silicone-based sealer	Microsilver, zirconium oxide, gutta-percha, polymethylvinylsiloxane, polymethylhydrogensiloxane
14	iRoot SP	Innovative BioCeramix, Inc., Vancouver, Canada	Calcium silicate-based sealer	Calcium hydroxide, calcium silicate, calcium phosphate, niobium oxide, zirconium oxide

(Contd...)

Table 1: *(Contd...)*

S. No.	*Name of the sealer*	*Name of manufacturer*	*Class*	*Composition*
15	Kerr Pulp Canal Sealer	Kerr Sybron, Romulus, Michigan, USA	Zinc oxide, eugenol	Canada balsam, resin thymol iodide, zinc oxide, silver, eugenol, Canada balsam
16	MTA Fillapex	Angelus, Londrina, Paraná, Brazil	Resin-based sealer	Bismuth trioxide, diluting resin, natural resin, salicylate resin, nanoparticulated silica, pigments, MTA
17	MTA Plus	Avalon Biomed	Calcium silicate – MTA based sealer	A recent hydraulic calcium silicate cement mixed with a polymer gel and proposed as an endodontic sealer
18	NeoMTAPlus	Avalon Biomed	Tricalcium silicate-based sealer	Powder and a water-based gel
19	Ortho MTA	*BioMTA*, Seoul, Korea	Calcium silicate – based MTA sealer	Tricalcium aluminate, free calcium oxide, powder (tricalcium silicate, dicalcium silicate, tetra calcium aluminoferrite, bismuth oxide) and liquid (deionized water)
20	ProRoot Endo Sealer	DENTSPLY Tulsa Dental Specialties	Calcium silicate-based sealer	A bit of tricalcium aluminate liquid (viscous aqueous solution of a water-soluble polymer), calcium sulfate, dicalcium silicate, tricalcium silicate, bismuth oxide
21	RoekoSeal	Roeko, Langenau, Germany	Silicon-based sealer	Hexachloride platinum acid, Polydimethylsiloxane, silicone oil, paraffin, zirconium dioxide

HDDMA, hexanediol dimethacrylate; PEGDMA, polyethylene dimethacrylate

silicate-based root canal sealer either in white or grey color.[11] The MTA sealers' composition varies from one manufacturer to another, such as Fillapex manufactured by Angelus, Londrina, Paraná, Brazil contains resins, such as salicylate resin, diluting, and natural resin, bismuth oxide, nanoparticles silica, MTA, pigments, and Endoseal MTA™ manufactured by Maruchi, Wonju, Korea contains calcium silicate, calcium aluminoferrite, calcium aluminate, Calcium sulcate, radio pacifier, and a thickening agent (Table 1).

Bioactivity, biocompatibility, solubility in water, radiopacity, sealing ability, and low solubility are among MTAs most desired characteristics. The two most crucial qualities of MTA essential in dentistry are the ability to seal root canal spaces and biocompatibility. High biocompatibility reassures optimal healing responses, and optimal sealing provides a good prognosis.[12,13] The significant disadvantages of MTA are discoloration of teeth from releasing ferrous ions, extended setting time, improper handling time, etc. Furthermore, MTA's physical, chemical, and hydration properties may be negatively impacted by any pH variation brought on by inflammatory changes in periradicular lesions.[14] Several case reports and case series have reported that unintentional extrusion of MTA-based sealers didn't cause any untoward effects or affect the prognosis of the tooth, yet extrusion of sealers is not recommended.[15] While few studies have reported cytotoxicity associated with MTA-based root canal sealers.[16,17] In the present review, *in vitro* studies that tested the cytotoxicity of MTA were selected for the review as they help to study the individual components of the sealers.

Methods

This systematic review was done according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[18] (Flowchart 1). Since the systematic review was non-clinical, the population, intervention, comparison, and outcome (PICO) research question was selected from the PICO framework[19] (Table 2). It was framed as follows: Are MTA-based root canal sealers cytotoxic to human cells compared to non-MTA based sealers?

A thorough literature search was done from January 2018 to January 2023. This study span was considered because very few reviews were conducted on the studies published during this period.

The following keywords and Boolean operatives were applied in the search: Root canal sealers, MTA, Cytotoxicity, *in vitro* studies, cytotoxicity assays, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) (MTT) assay.

The literature search was conducted in January 2023 utilizing the databases such as PubMed, Scopus, and Google India (http://www.google.co.in).

All citations were combined and uploaded to EndNote v.X9.3.3 (Clarivate Analytics, Pennsylvania, USA) to reject duplicate articles. Two reviewers separated all the headings and abstracts of the studies selected for full-text review. Two independent reviewers (NB and MAQ) autonomously assessed the full text of the articles chosen from the bibliography, which differed from the inclusion criteria.

Flowchart 1: PRISMA flowchart

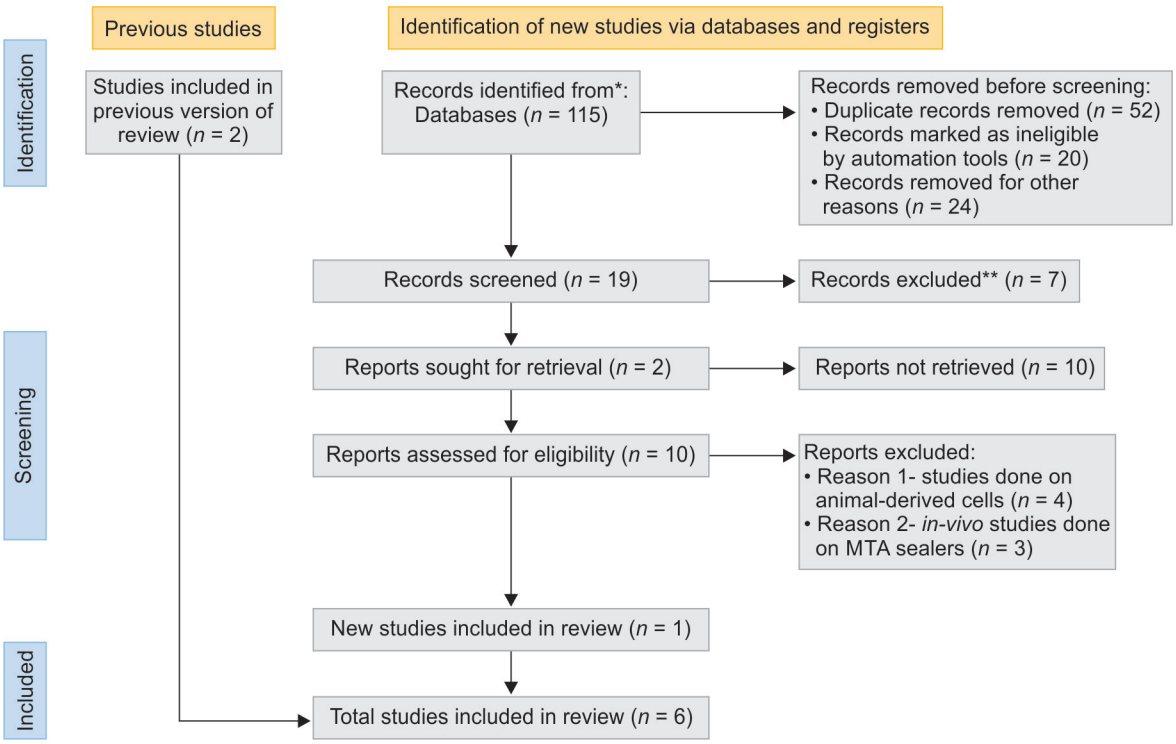

Table 2: Population, intervention, comparison and outcome (PICO) strategy for valuation of scientific literature

Parameters	*Assessment tool*
Population	Human cell models assessed with sealers or sealer extracts
Intervention	Nil
Comparison	Other root canal sealers or unexposed control group
Outcome	Cytotoxicity was measured by cell viability or proliferation count

Additional records were obtained by searching through the bibliography of the selected studies. The Medical Subject Headings (MeSH) and keywords were formed during the search. The focus of the inquiry involved *in vitro* experimental studies (evidence levels IIb). The level of evidence of selected studies was classified according to the guidelines of the Oxford Centre for Evidence-Based Medicine (http://www.cebm.net/levels_of_ evidence. asp).[20]

In vitro studies, retrospective and prospective studies with placebo or blank control, and parallel group designs were combined in the review with the following inclusion and exclusion criteria:

Inclusion Criteria

- Population: The *in vitro* studies on human cell models assessed with sealers or sealer extracts were included.
- Interventions: The *in vitro* studies that tested the cytotoxicity of MTA-based root canal sealers or compared the cytotoxicity of different root canal sealers including MTA were considered for review.
- Control group
  - Non-MTA-based root canal sealers.
  - Blank (no treatment).
  - Negative (such as placebo treatment).
- Outcomes: Cytotoxicity was measured by cell viability or proliferation count.

Exclusion Criteria

- Studies with descriptive analysis and no quantitative outcome.
- Unrelated studies.
- Studies that were published in languages other than English.
- *In vitro* studies performed on cells other than human cell models.
- Systematic reviews, *in vivo* studies, and randomized controlled trials.
- Unpublished studies.
- Studies with improper statistical analysis.

Risk of Bias

Questionnaires were used to assess the quality of each study considered for this review. The questions looked at the accuracy and validity of the outcomes and results and whether a focused research topic had been addressed in each study. The questionnaire was based on the guidelines for reporting pre-clinical *in vitro* studies on dental materials[21] (Table 3).

Data Extraction

Two authors (LMMF and SOZ) extricated data from the selected studies in terms of publication year, author's name, study design, cell models used for cytotoxicity examination, the types of root canal sealers assessed, the experimental groups in the *in vitro* study, the sample size of all groups, cytotoxicity assays used, and outcomes.

Meta-analysis could not be accomplished in the present review due to the assortment in the selected studies, based on the assay used, cells used for testing cytotoxicity, etc.

Table 3: Quality assessment of the selected studies

S. No.	*Domain*	*Items*	*Pritesh Jagtap et al.*[22]	*Susanne Jung et al.*[23]	*Ju Kyung Lee et al.*[24]	*Alexis Gaudin et al.*[25]	*Hilal Erdogan et al.*[26]	*Bruna Barcelos Só et al.*[27]
1	Abstract	Item 1. Structured summary of trial design, methods, results, and conclusions	Yes	No	No	Yes	No	Yes
2	Introduction	Item 2a. Scientific background and explanation of rationale	Yes	Yes	Yes	Yes	Yes	Yes
		Item 2b. Specific objectives and/or hypotheses	Yes	Yes	Yes	Yes	Yes	Yes
	Methods	Item 3. The intervention for each group, including how and when it was administered, with sufficient detail to enable replication	Yes	Yes	Yes	Yes	Yes	Yes
		Item 4. Completely defined, pre-specified primary and secondary measures of outcome, including how and when they were assessed	Yes	Yes	Yes	Yes	Yes	Yes
		Item 5. How sample size was determined	Yes	Yes	Yes	Yes	Yes	Yes
		Item 6. Method used to generate the random allocation sequence	No	No	No	No	No	No
		Item 7. Mechanism used to implement the random allocation sequence	No	No	No	No	No	No
		Item 8. Who generated the random allocation sequence, who enrolled teeth, and who assigned teeth to intervention	No	No	No	No	No	No
		Item 9. If done, who was blinded after assignment to intervention and how	No	No	No	No	No	No
		Item 10. Statistical methods used to compare groups for primary and secondary outcomes	Yes	Yes	Yes	Yes	Yes	Yes
3	Results	Item 11. For each primary and secondary outcome, results for each group, and the estimated size of the effect and its precision	Yes	Yes	Yes	Yes	Yes	Yes
4	Discussion	Item 12. Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	Yes	Yes	Yes	Yes	Yes	Yes
5	Other information	Item 13. Sources of funding and other support	Yes	Yes	Yes	Yes	Yes	Yes
		Item 14. Where the full trial protocol can be accessed, if available	Yes	Yes	Yes	Yes	Yes	Yes

Table 4: Assessment of selected *in vitro* studies

S. No.	*Study*	*Type of assay*	*Types of human cells*	*Materials evaluated*	*Sealer–cell contact type*	*Extraction time (hour)*	*Extract concentration*	*Cell exposure time*	*Time dependent cytotoxicity potential*	*Conclusion*
1	Pritesh Jagtap et al.[22]	MTT assay and trypan blue exclusion method	The PDL fibroblast cells	1. The MTA-based sealer (MTA Fillapex) 2. Calcium hydroxide-based sealer (Apexit Plus) 3. Resin-based sealer (AH Plus) 4. ZnO eugenol-based sealer (Tubli Seal)	Indirect contact	24	10 mL	1 hour; 7 and 14 days	MTA Fillapex was found to be most toxic. The order of cytotoxicity at the end of second week was observed as MTA Fillapex > Tubli Seal > Apexit Plus > AH Plus	MTA Fillapex > Tubli Seal > Apexit Plus > AH Plus
2	Susanne Jung et al.[23]	MTT assay, and living/dead-staining, cytotoxicity by LDH-assay, and changes by Richardson-staining	Human osteoblasts cells	1. Resin-based sealer (AH Plus) 2. Pulp Canal sealer (Kerr) 3. The MTA-based sealer (MTA Fillapex) 4. Silicate-based sealers (BioRoot RCS)	Indirect contact	24	1:1, 1:2, and 1:10	24 hours; 7, 14, and 21days	The different sealers exhibited different levels of cytotoxicity Cytotoxicity decreases over time	Pulp Canal Sealer and MTA Fillapex or freshly mixed AH Plus to osteoblasts should be avoided
3	Ju Kyung Lee et al.[24]	MTT assay, Scanning electron microscopy, Flow cytometry assay, Real Time qPCR, Alkaline phosphate staining	hPDLSCs	1. Calcium silicate-based sealers (EndoSeal MTA) 2. Nanoceramic Sealer 3. Wellroot ST 4. Two epoxy resin-based sealers (AH-Plus, AD Seal)	Indirect contact	24	1:4	24, 48, and 72 hours	There is no significant cytotoxicity at 48 hours but at 72 hours Wellroot ST shown highest cytotoxicity	EndoSeal MTA, Nanoceramic Sealer, Wellroot ST appear to be more biocompatible and less cytotoxic than epoxy resin-based sealers
4	Alexis Gaudin et al.[25]	MTT assay	Human periodontal ligament stem cells	1. Silicate based sealers (BioRoot RCS) 2. ProRoot ES (Dentsply Sirona) 3. The MTA-based sealer (MTA Fillapex)	Indirect contact	24	1:1, 1:2, 1:4, and 1:8	48 hours	Sealers had no cytotoxic effect at 24 hours at high concentration	BioRoot RCS, ProRoot ES, MTA Fillapex, and AH Plus had no toxic effects on cell viability
5	Hilal Erdogan et al.[26]	XTT cytotoxicity and micronucleus (MN) genotoxicity test	Human periodontal ligament fibroblast cell.	1. Epoxy-resin based sealer (AH Plus) 2. Salicylate-based sealer (MTA-Fillapex) 3. Calcium silicate-based sealers (iRootSP)	Indirect contact	24	1:2, 1:4,1:8, 1:16, and 1:32	6, 12, 24, 48, and 72 hours	Sealers shown highest cytotoxicity at 0 hour whereas significantly less at 6 hours and 12 hours at high conc.	Genotoxicity potential of AH Plus is high and iRootSP has no effect
6	Bruna Barcelos Só et al. (2022)[27]	MTT-based assay	Human periodontal ligament dental stem cells	1. Sealer Plus BC (SBC) 2. Resin-based sealer (AH Plus) 3. The MTA-based sealer (MTA Fillapex)	Indirect contact	24	1:10	24, 46, and 72 hours	BCS showed highest absorbance in all periods followed by MTT, AH showed lowest absorbance.	Plus BC presented the lowest cytotoxicity & all the sealers are equally low genotoxic

LDH, lactate dehydrogenase; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide); XTT, methoxynitrosulfophenyl-tetrazolium carboxanilide

Results

In the present review, 115 articles on the cytotoxicity of MTA were found in databases. After filtering the articles for duplicates and eligibility, 19 articles were assessed. Studies that were done on animal-derived cells and *in vivo* studies, systematic reviews, randomized controlled trials (RCTs) were excluded and only *in vitro* studies on Human derived cells were selected for the review. Thus, in the present systematic review, six studies were included[22–28] (Table 4). It was observed that in these studies, along with MTA-based root canal sealers, calcium hydroxide-based sealers, resin-based sealers, zinc oxide eugenol-based sealers, silicate-based sealers, calcium silicate-based sealers, nanoceramic sealers, epoxy resin-based sealers, and other sealers were tested for cytotoxicity. In these studies, human periodontal ligament (PDL) stem cells, human PDL fibroblast cells and Human osteoblasts cells were used to assess these sealers' cytotoxicity. During the study, the cells were in indirect contact with the sealers. The sealer extracts were diluted at a concentration of 1:1 to 1:32, depending on the studies. The cytotoxicity tests used in the studies were MTT assay, cytotoxicity by lactate dehydrogenase (LDH-assay), scanning electron microscopy, flow cytometry assay, real-time qPCR, alkaline phosphate staining, and methoxynitrosulfophenyl-tetrazolium carboxanilide (XTT) cytotoxicity, and micronucleus (MN) genotoxicity test.

Risk of Bias

In the present review, it was observed that of the six studies, three had structured abstracts, and the remaining had unstructured abstracts. Random allocation sequencing and blinding were not done for any of the studies. Majority of the studies in the present review lacked information on randomization procedures, blinding, and outcome measurements, which highlights the necessity for well-designed and well-reported preclinical and clinical studies. The eligible studies also showed a high risk of bias which was the major drawback of the review along with methodological heterogeneity. A high degree of variability was also seen in the cell model employed to measure cytotoxicity, which ranged from osteoblasts or fibroblasts to stem cells.

In the present review, one study had high risk of bias while three studies had medium risk of bias, and two studies had low risk of bias.

Meta-analysis could not be accomplished in the present review due to the assortment in the selected studies, based on the assay used, cells used for testing cytotoxicity, etc.

Discussion

The aim of using an endodontic sealer is to accomplish a three-dimensional obturation of the root canals.[29] Gutta-percha is traditionally used to obturate root canals, while the root canal sealers act as fillers between the root canal walls and gutta-percha. Many root canal sealers have been developed and marketed under various brand names (Table 1). Most of these root canal sealers demonstrate insufficient biological activity and have been cytotoxic when cultured immediately after being mixed.[30] Delayed wound healing and cellular degeneration have been reported when these sealers come in contact with periapical tissues.[31] Furthermore, fluid or blood contamination at the apical third of the canals makes setting of hydrophobic sealers difficult.[32] Thus, there was a necessity for a sealer that could set in a humid environment and overcome the drawbacks of the existing root canal sealers.

Mineral trioxide aggregate is one such material that can overcome the drawbacks of other sealers.[33] When MTA is applied as a root canal sealant and compressed on the dentin in the presence of phosphate, a dentin–MTA interface layer is formed. This layer, when examined, has a composition similar to hydroxyapatite. With this interstitial layer, marginal adaption is better than average. The MTA particles penetrate the dentinal tubules and impede them. This wards off harboring of microorganisms in the tubules.[33] Moisture is necessary for the setting reaction of MTA to occur and for the bioactivity process to be induced, which results in the formation of apatite precipitates.[34] Additionally, the hydration of MTA results in the formation of a tacky calcium silicate hydrate (CSH) gel which binds to the gutta-percha cone. Initially, the CSH is porous, solidifying to form a strong network in 4–6 hours. The complete setting of CSH occurs for several days. Thus, the long final setting time of MTA may prove to be advantageous for enhancing the sealing ability of MTA.[35]

Various types of MTA-based root canal sealers are ProRoot Endo Sealer (Dentsply Tulsa Dental Specialties, Dentsply/Maillefer, Ballaigues, Switzerland), which contains major components such as tricalcium silicate and dicalcium silicate, with addition of calcium sulfate as setting retardant, bismuth oxide as radio pacifier and a minor amount of tricalcium aluminate.[36] It is known for biocompatibility and for having a relatable sealing ability to epoxy resin-based sealers superior to zinc oxide eugenol-based root canal sealers when evaluated using a fluid filtration system.[37] Also, MTA Fillapex®, Angelus Soluções Odontológicas, Londrina, Paraná, Brazil is another type of sealer made up of Salicylate resin, natural resin, bismuth, and silica. It is the first paste-based, MTA-based salicylate resin root canal sealer that may be used with any obturation technique. It has outstanding handling characteristics, delivers quickly and wasteless, and has a quick setting time.[38] The CPM Sealer (EGEO SRL, MTM Argentina S.A., Buenos Aires, Argentina) has a composition of tricalcium silicate, tricalcium oxide, tricalcium aluminate, and other oxides. It is known for its biocompatibility, antimicrobial activity, and less setting time.[39] Furthermore, MTAS Experimental Sealer was developed at Endo Araraquara Dental School UNESP, University of Estadual Paulista, Sao Paulo, Brazil. Approximately, 80% white Portland cement, zirconium oxide, which acts as a radioopacifying agent, calcium chloride as an additive, and resinous vehicle are its components. It has got a good capacity for the release of hydroxyl ions.[40] Moreover, MTA Obtura is a sealer developed by adding liquid resin as a curing initiator to replace saline. The development of MTA Obtura aimed to create an endodontic sealer compounding the biological and sealing features of MTA. This sealer has a good sealing ability.[39] In the present review, it was observed that five studies of the six studies that were selected assessed MTA Fillapex, while one study assessed EndoSeal MTA.[22–28]

Before using any endodontic sealers clinically, it is necessary to test the material for genotoxicity, cytotoxicity, and biocompatibility, as these sealers can potentially interact with periapical tissues extruded due to condensation forces or overfilling the canals. The anatomy of the root canal also contributes to the extrusion of the sealer in the periapical space.[41] To evaluate any biological and cytotoxic concerns associated with products, such as root canal sealants, *in vitro* assays are regarded as screening tests. The advantage of *in vitro* trials is that they are cost-effective, rapid assessment can be done, and predictivity of the material over a duration of time can be done.[42] Thus, the present review selected

all the *in vitro* studies on root canal sealers done on human cells published between 2018 and 2022.

Cytotoxic sealers cause the deactivation of enzymes such as zinc-dependent enzymes and alkaline phosphatase that are essential for bone formation, bone remodeling, wound healing, and collagen and gelatine degradation.[43] Four methods test the cytotoxicity of endodontic sealers:

1. MTT assay: This assay is also called Mosman's tetrazolium toxicity assay. It is a colorimetric assay that checks the mitochondrial activity of the cells to know their viability. It is created on the conversion of MTT into formazan crystals by living cells.
2. Zygomorphy test: This test assesses the matrix metalloproteinases (MMPs) activity in cells based on which cytotoxicity is decided.
3. Cytotoxicity assay: This test assesses the toxicity of the sealers.
4. The XTT assay is a ready-to-use assay for the biological evaluation and screening of chemical, pharmaceutical, cosmetic, and medical device substances. It contains a tetrazolium-based compound sensitive to redox reactions and alive respiring cells. It is considered an early, straightforward, quick, and sensitive assay.

In the present review, five studies employed the MTT assay, while one study by Hilal Erdogan et al.[27] used the XTT assay. The difference between MTT and XTT assay is that in the MTT assay, cells must be lysed to dissolve formazan salt before measuring the absorbance. In contrast, XTT assay allows kinetic monitoring of the sample at different periods, and lysing of cells is not needed.[44]

Studies on human-derived cells were only considered in this review due to the biochemical differences between human and animal-derived cells. When a root canal sealer extrudes from the canals, it comes in contact with PDL fibroblasts and other cells like osteoblasts and mesenchymal cells. When in communication, the sealers cause inflammation and necrosis of these cells, which could lead to delayed periapical healing.[45] Among the studies considered for this review, Susanne Jung et al. conducted a cytotoxicity test on human osteoblast cells.[23] Pritesh Jagtap et al.[22] and Hilal Erdogan et al.[27] conducted cytotoxicity tests on human PDL fibroblast cells, while Ju Kyung Lee et al.,[24] Alexis Gaudin et al. and Bruna Barcelos Só et al.[28] conducted cytotoxicity tests on human periodontal ligament stem cells (hPDLSCs).

In the present review, MTA Fillapex was the most cytotoxic compared to other MTA-based sealers. In the study conducted by Pritesh Jagtap et al.,[22] when human PDL fibroblast cells were bathed with freshly derived elute of the sealers within the first hour, MTA Fillapex displayed potent cytotoxicity, and during the first and second weeks, this toxicity did not lessen.[24] The results of this study were consistent with those of an earlier investigation done by TH Huang et al. that found that MTA Fillapex significantly reduced cell viability utilizing various techniques.[46] In a recent report demonstrating the long-lasting cytotoxic effects of sealers, similar outcomes were observed regarding MTA Fillapex cytotoxicity assessed using the MTT assay.[47]

Conferring to a study by Jung et al.,[23] the solubility of sealers before and after setting greatly affected the cytotoxicity of the sealers. It was reported that sealers such as AH Plus were insoluble after setting the reaction. This explains the cause of the difference in cytotoxicity of AH Plus before and after the setting response.[48] Similarly, the solubility of MTA Fillapex increases after setting, which means that the cytotoxicity of MTA. Fillapex also increases, which is in contrast to AH Plus. This observation concluded that freshly mixed AH Plus and MTA Fillapex must be used cautiously, and contact of these sealers with cells like osteoblasts must be avoided.[23] In another study conducted by Ju Kyung Lee et al., epoxy resin-based sealers (AH Plus and AD Seal) and calcium silicate-based sealers (Endoseal MTA, Wellroot ST, and nanoceramic sealer) were tested for toxicity. It was observed that toxicity was highest when the sealers were mixed.[24] This initial toxicity of AH Plus can be explained by the fact that the sealer's amine or epoxy resin components are released into the adjacent tissues, while the toxicity of calcium -silicate resins is due to the high pH.[49,50] The rise in pH is due to the formation of calcium hydroxide when these resins dissolve in the tissue fluid. Although the high pH of root canal sealers may harm cell viability, they may also have certain biological benefits. These sealers' high alkalinity could alter the environment to encourage the growth of hard tissues and interfere with osteoclastic action, which promotes tissue healing.[50] Endoseal MTA and other calcium silicate-based sealers were reported to be more biocompatible than epoxy resins.[22] Comparable outcomes were testified by Diomede et al.[51]

According to Gaudin et al., MTA Fillapex enhanced the proinflammatory mediators' productions among all the calcium silicate-based sealers. These mediators trigger an innate immune response that adversely affects local wound healing.[25] This was proven by another study by Liu J et al.[52] MTA Fillapex is a disalicylate-resin-based root canal sealer containing 13% MTA-like material. It is not a calcium silicate-based sealant or a "bioceramic" sealant. It has the physicochemical properties of resin-based sealers. Still, the biological properties of MTA.[53] The cytotoxic effect of MTA Fillapex can be attributed to its resinous components such as salicylate resins and silica. It is reported that salicylate resins that prolong the working time and setting time of MTA and render it better flowability also increase the cytotoxicity of MTA[54–56] According to Hilal Erdogan et al.,[27] MTA Fillapex displays time-dependent toxicity. It must be used with utmost care as it can cause an increase in micronuclei formation in cells that come in contact with MTA Fillapex.[27] Bruna Barcelos Só et al., in their study, mention that although MTA is present in MTA Fillapex, the salicylate resin component induces cell apoptosis by precipitating in the cell cytoplasm leading to fragmentation of DNA. However, in this study, the cytotoxicity of MTA Fillapex was comparatively lower than AH Plus.[28] This statement is in strong disagreement with other studies.[57–59] This disagreement can possibly be explained by the composition of the sealers. Thus, it can be mentioned that great caution needs to be exercised when using MTA Fillapex.

The present review is considerably significant since no recent reviews have analyzed the cytotoxicity and bioactivity of MTA-based root canal sealers. Furthermore, in the present study, a more laborious and objective defined procedure for the data collection, extraction, and compilation was followed, which helped disparagingly inspect the study methodologies and eliminate the ambiguous study designs and unclear protocols.

The limitation of the present review was that meta-analysis could not be accomplished due to the assortment in the selected studies, based on the assay used, cells used for testing cytotoxicity, etc. Since meta-analysis was not carried out, an overview of the study's findings could not be given. The present review found very few studies in which MTA was tested as a root canal sealer. Most of the studies used MTA sealer for comparison. Thus, the available literature evidence was insufficient to conduct a more

reliable review and reach a consensus. However, the present review confirms that caution must be exercised when using resin sealers, such as MTA Fillapex.

Conclusion

When a sealer is extruded into the periapical space beyond the apical foramen, cytotoxicity of endodontic sealers can lead to delayed healing of periapical lesions in a tooth that has undergone root canal therapy. Therefore, it is advisable to keep the obturant and sealer within the premises of the apical foramen and use a sealer that has the least proven cytotoxicity and is suitable for the selected case. The available literature suggests that MTA Fillapex has mild to moderate cytotoxicity and must be used cautiously. However, there is a need for more human cell-based studies to help arrive at a general agreement regarding the existing root-filling materials.

Clinical Significance

It is of utmost importance for the dentist to understand the properties of materials that are frequently used in dentistry. Endodontic sealers do serve the purpose of bridging the gaps between the gutta-percha cone and the canal wall but knowing its biocompatibility becomes important as the material is extruded beyond the apical foramen where it comes in contact with the surrounding tissues. The effect of sealers on the surrounding tissues affects the healing and prognosis of the treatment.

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