Review Article

Evaluation of genotoxicity induced by endodontic materials: A systematic review

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Abstract

The ability of a substance to cause harm to the DNA molecule is known as genotoxicity. Because there is significant evidence of link between genetic damage and carcinogenesis, determining the real health hazards to patients and dental workers requires a thorough examination of genotoxicity caused by various dental materials. The aim of this paper is to provide a thorough overview of the genotoxicity induced by various endodontic materials. A systematic electronic search and screening of reference list were undertaken until November 2019. A meticulous search confined to English language articles was carried out in PubMed, Web of Science, and Scopus databases from their inception. The electronic search method retrieved 338 papers after the removal of duplicates, only 25 papers met the inclusion requirements following a thorough review of the findings. All evidence of genotoxicity has been found in the literature notably in relation to bleaching agents, restorative materials, and resin-based sealers. Such data will definitely be included to that already established for regulatory purposes as a secure way to enhance oral healthcare and avoid oral carcinogenesis. Reported information of genotoxicity induced by various endodontic materials is crucial for validating their safety in clinical practice as well as for identifying potential gaps in knowledge that must be studied further in future studies.

Keywords: Biocompatibility, cytotoxicity, dentistry, endodontic materials, genotoxicity

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Submission: 30-07-21 **Revision:** 15-09-21 **Acceptance:** 15-09-21 **Web Publication:** 20-04-22

INTRODUCTION

Over the past decade, toxicological research has changed dramatically, with an increased emphasis on chronic toxicity, carcinogenicity, teratogenicity, and mutagenicity. Various materials used in dentistry and medicine have to undergo biocompatibility testing in order to be approved for being used on human subjects. The constituents of dental material play a crucial role in determining its interaction with surrounding cells as well as the microenvironment.[1] Although multiple studies have shown that certain endodontic materials are genotoxic, such findings should be taken with prudence. While these studies had essentially comparable reported outcomes, the review of current published data indicated that there are insufficient solid scientific guidelines to justify a single test that may offer the most beneficial conclusion for genotoxicity evaluation.[2] In endodontic literature, the toxic effects of root canal irrigant, sealers, and medicines have always been a source of debate. With a few exceptions, these

Access this article online	
Quick Response Code:	Website: www.saudiendodj.com
	DOI: 10.4103/sej.sej_163_21

How to cite this article: Tamanna S, Iftekhar H, Fatima A, Rehman A. Evaluation of genotoxicity induced by endodontic materials: A systematic review. Saudi Endod J 2022;12:158-67.

materials were implanted into the subcutaneous tissues in several studies (animal model),[3] *in vitro* genotoxicity tests on mammalian cell cultures, and conjunctival inflammatory tests[4] These techniques are great for assessing the toxicity of several materials, although, extrapolating the results of these tests to the clinical scenario might be deceptive.[5]

Many dental materials have the potential to come into prolonged contact with oral tissues.[6] DNA damage and the potential for genotoxic consequences such as cancer may be influenced by both material characteristics and the length of time spent in contact with it. Toxic substances like endodontic sealers seep into the periapical tissues through apical foramen and elicit a local immune reaction culminating in a complicated inflammatory response involving a wide range of cytokines.[7] Even if no extrusion occurs, these materials can allow soluble components to be released, which can be harmful to adjacent cells and disrupt local metabolism.[8] Cytotoxicity of dental materials is a proven fact in the literature, however, experimental data support the genotoxic events by the formation of reactive oxygen species and DNA damage and repair along with gene expressions for DNA damage. Previous studies suggested that various dental materials (such as sealers and denture base materials) increase intracellular reactive oxygen species,[9] which is a potential genotoxic agent linked to a variety of degenerative diseases including cancer and lead to oxidative damage of human RNA or DNA. As a result, biochemical tests evaluating the impact of dental materials on DNA are important for reducing possible hazards to both patients and doctors. Studies done previously had suggested that intracanal medicament concentration being used currently had detrimental effect on survival of human stem cells of apical papilla and pulp stem cell.[9] Cell survival along with elimination of microorganism is a crucial factor for success of regenerative endodontic procedure as well for the healing of any periapical infection. Many studies have been conducted regarding the cytotoxicity of these medicaments, but there is lack of evidences regarding genotoxicity of these medicaments in endodontic literature.[10]

A multitude of novel materials have been introduced into the realm of endodontics in recent decades. Because of the present requirement for clinical performance in the oral cavity, several of them have been enhanced. It is crucial to note that almost all of these materials can dwell in the mouth for months or even years.[11] Furthermore, dental clinicians work with these materials on a daily basis in their practice. In this approach, a risk evaluation of such materials' genotoxicity and mutagenicity is critical for guaranteeing the safety of persons who are exposed to them on a regular basis. This implies that all materials must be examined for genotoxicity, as genetic harm has been related to chronic degenerative illnesses such as cancer.[12] Genotoxic damage does not always result in cell death or any other immediately visible consequence. Damage to the cell genome, on the other hand, may severely reduce the tissue's ability to self-repair or, in the long run, result in the formation of neoplasia or other chromosomal abnormalities.[13]

The purpose of this systematic review is to describe and evaluate genotoxicity induced by various endodontic compounds. Such information is crucial for validating their safety in clinical practice, as well as for identifying potential gaps in knowledge that must be studied further in future studies. The purpose of this systematic review was to describe and evaluate genotoxicity induced by various endodontic compounds.

MATERIALS AND METHODS

Search strategy and outcome measures

Literature search strategy was performed, and the protocol of the systematic review was prepared using the established preferred reporting items for systematic reviews and meta-analyses (PRISMA) checklist. A systematic electronic search and screening of reference list were undertaken until November 2019. A meticulous search confined to English language articles was carried out in PubMed, Web of Science, and Scopus databases from their inception. The terms employed in the electronic research were "genotoxicity," "intracanal medicament," "cytotoxicity," and "Endodontic sealer."

Inclusion criteria

The inclusion criteria were framed as follows: studies published in the English language, studies evaluating genotoxicity, materials used in dentistry, studies containing two or more genotoxicity assay, studies performed on mammalian cells or animal models. The publication year was not restricted in any way.

Exclusion criteria

Exclusion criteria were framed as, studies performed on nonmammalian cells, studies using single genotoxicity assay, and materials used other than in dentistry.

The major question under consideration was organized based on the material, the exposure of interest, and the format of the outcome. The following questions were asked to answer in order to lead the systematic review: (i.e., why genotoxicity is important? What are the methods employed for genotoxicity testing till date? What are the Endodontic

materials tested for genotoxicity till date?). Because most of the research is *in vitro* or *ex vivo*, a secondary goal was to check into other results reported to determine the validity of these genotoxicity tests in human participants.

Evaluation of the selected studies

Two investigators assessed the titles and abstracts of published research, and if the titles and abstracts were not precise, the whole paper was examined for data correctness. Following the first screening of the title and abstract to assess their eligibility against the inclusion criteria, the full-text examination of the specific articles was conducted. Two reviewers were in-charge of data extraction.

Author(s), year of publication, journal, materials tested, assay employed, cells to be used for study, dose of medicaments protocol, periapical influence of medicaments, time frame for medicaments to be in contact with tissues, and other result findings were accumulated and documented on a data collection sheet for each article. The authors of the papers included in the review were approached for clarification and/or to provide supplemental information if necessary.

Data synthesis

Overall, if the research provided relevant data and the outcome criteria were equivalent, the genotoxicity test(s) performed, and results obtained were computed for the various outcome measures. In the lack of raw data and in the presence of quantitative data and/or a variety of outcome definitions, the study results were presented as narrative and compiled according to the various outcome measures.

RESULTS

The electronic search method retrieved 338 papers after duplicates were removed. Only 25 of the 338 papers met the inclusion requirements following a thorough review of the findings. The relevant papers and review articles were also screened to calculate the exact data, and the final number of studies was selected after applying and framing PRISMA checklist. The data were framed in the form of PRISMA flowchart [Figure 1]. All the included studies reference lists were searched for citations.

The studies that were included was four *in vitro* (cell culture)[14-17] four *in vivo* (one bacterial cell, two lower animal cells, and one animal model,[13,18-20] and seventeen *ex vivo* (one artificial culture, four animal cell culture, and twelve human cell line) [3,12,16,21-34] [Figure 2].

Analysis of outcome measures

What is genotoxicity and why is genotoxicity important?

Genotoxicity is described as a devastating effect on a cell; s genetic material such as DNA or RNA that compromises its integrity. It can be assessed as mutations, changes in chromosomal shape or number, DNA damage, repair, or a combination of these factors. In general, genotoxicity refers to the existence of a DNA-reactive constituent that has the potential to cause mutagenicity and carcinogenicity.[35] A mutagen (genotoxin) is a physical or chemical substance that alters an organism's genetic material, often DNA, increasing the incidence of mutations over the natural background level.[31] Mutagens are likely to be carcinogens since numerous mutations can lead to cancer.[36] The human genome is thought to be constantly destroyed by many chemical agents. Biological units in eukaryotic cells, however, are extremely specialized for neutralizing genotoxic shocks by encouraging DNA repair. The integrity of the human genome depends on a xenobiotic metabolizing system and DNA repair machinery. However, if any genetic damage is not properly repaired, mutagenicity might result in a permanent lesion in the genetic machinery following cell replication. As a result, genotoxicity testing is critical for determining the dangers posed by hazardous compounds to human genetic material and preventing problems. The bacterial reverse gene mutation assay (Salmonella reversion assay or Ames test), the chromatid sister exchange, the mouse lymphoma gene mutation assay, the micronucleus test, the chromosomal aberration test, and the comet assay are all used to evaluate genotoxicity.[37]

Materials tested till date

Over the last few decades, advancement in genotoxicity testing of endodontic materials has been minimal, and few

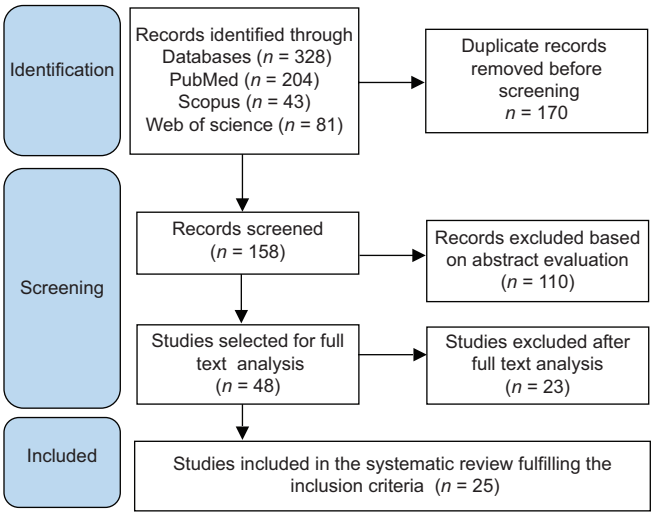

Figure 1: Flowchart for article selection according to preferred reporting item for systematic review and meta-analysis guidelines

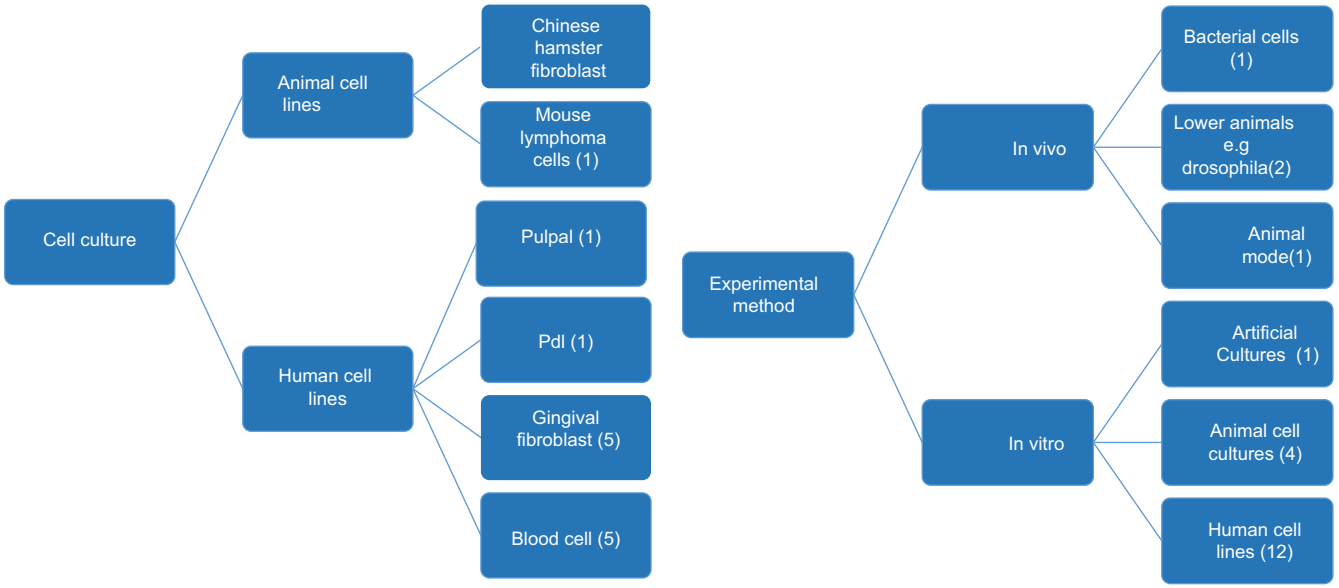

Figure 2: All included experimental and cell culture studies for the procedure

research in the field of Endodontics have not taken full advantage of the various testing systems that have been developed in the general field to probe different facets of genotoxic events. Until 2010, only a few articles have been published regarding genotoxicity.[38] Since 2010 onward, a tremendous research has been carried out upon various dental materials showing a growing concern regarding the biocompatibility and awareness in the field of dental research, but still endodontic materials were minimally being investigated.

In endodontic literature, the irrigant's toxicity, endodontic sealers, and resin materials have been a source of debate. The investigations were based on implantation into animal subcutaneous tissues, *in vitro* genotoxicity testing, and conjunctival inflammatory tests, with a few outliers. These techniques are great for assessing the toxicity of two or more agents, although extrapolating the results of these tests to the clinical scenario might be deceptive. Of all the endodontic materials tested for genotoxicity, mostly were endodontic sealers such as resin-based sealers. As root canal sealers, most commonly have a chance of coming in contact with periapical tissues due to extrusion beyond root canal confines.[39] Even if these materials are not extruded, eluents from them may come into direct contact with periradicular tissues for long duration, causing irritation and delaying wound healing. Apart from sealers, various medicaments, pulp capping materials, calcium silicate cements, bleaching agents, Glass ionomer cements, mineral trioxide aggregate (MTA), and composite resin have also been tested for genotoxicity.[16,40,41] The idea behind the test is to know about the biocompatibility (in terms of genetic damage) of these materials which are placed in contact with pulp/periapical tissues for a comparatively long period of time and may elicit DNA changes if genotoxic to the human cells. In the long run, genotoxic damage may reduce tissue's ability to self-repair or lead to the formation of neoplasia. Since the last few decades, many tests regarding cytotoxicity of these medicaments are conducted, but for genotoxicity, there is a lack of evidence in the literature.

Tests used for genotoxicity testing

Endodontic materials' biocompatibility is determined by a number of factors including genotoxicity, mutagenicity, carcinogenicity, histocompatibility, and immunological effects. DNA precipitation tests are simple and quantitative indicators of DNA damage that may be used to identify viable DNA lesions in eukaryotic cells. The simplicity, speed, and precision of this approach are its benefits. An experimental setup like this might be utilized to assess the genotoxicity of a range of dental materials as a preliminary assay. Various studies published have used different methods for genotoxicity testing. However, the main aim of all the studies was to detect any DNA changes caused by these materials. Most commonly used test was comet assay for testing the genotoxicity as it gives a more reliable results and is being widely used in the field of toxicology testing. Many National toxicological programs are using comet assay as a parameter for toxicity testing. Apart from comet assay, micronuclei formation (MN) and MTT reduction (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, gamma-H2AX assay, somatic mutation and recombinant test (SMART), and gene enzyme assay have been used to assess the genotoxic potential of various dental materials.[42] Micronuclei are strongly indicative of

chromosomal aberrations. The MN formation assay is a valid test for testing carcinogens that cause genetic harm and is the Organization for Economic Co-operation and Development recommendation for chemical testing. The erratic (third) nucleus that forms during the anaphase of mitosis or meiosis is known as the micronucleus. However, the production of micronuclei is not the sole way that a chemical exposure may cause genotoxicity.

At the single-cell level, the comet test assesses DNA damage and DNA repair capability. The numerous mutagenicity and genotoxicity assays examine several types of possible DNA damage that might occur as a result of a chemical or radioactive substance. As a result, multiple tests need to be performed to determine whether DNA damage has occurred and, if so, which particular damages were caused by a chemical or radioactive agent. gamma-H2AX assay, which detects the phosphorylated gamma-H2AX histone,[43] appears rapidly after exposure of cell cultures to ionizing radiation; half-maximal amounts are reached by 1 min and maximal amounts by 10 min. At the maximum, approximately 1% of the H2AX becomes γ-phosphorylated per gray of ionizing radiation, a finding that indicates that 35 DNA double-stranded breaks.[44] This test employ phospho-histone gamma-H2AX specific antibody binding, which may be identified by immunofluorescence, to distinguish DNA double-strand breaks. The detection of DNA double-strand breaks can be made more accurate and sensitive by quantifying the foci.[45]

In Drosophila melanogaster SMART was utilized to assess genotoxicity as homologous mitotic recombination, point, and chromosomal mutation. SMART detects the loss of heterozygocity of marker genes expressed phenotypically on the fly's wings. This fruit fly shares a lot of genetic similarities with mammals, making it a good model organism for genotoxic research.[19]

DNA damage sensors ATM and RAD53 genes and DNA damage repair sensors such as RAD51 and PARP-1 were also measured. Geno modifier capacity assay (GEMO) is a novel test that seeks to determine the genotoxic potential of specific chemicals or extracts. A highly specialized double-stranded DNA dye and pure calf thymus DNA are used in this ultrasensitive and fast method. Pico Green dye is a fluorescent reagent for counting the number of double-stranded DNA molecules in a solution.[46]

Hence, based on the existing evidence, the comet assay and the micronucleus test are commonly used assays for detecting genotoxic effects. However, few studies on immunological elements are carried out such as the study of inflammatory mediators like interleukin (IL)-1,-8,-12, and tumor necrosis factor, which mediate periapical inflammation, bone resorption, and bone repair inhibition.[47] The immunological aspect must also be included as a part of genotoxicity testing.

Cell culture used for genotoxic testing

According to the guidelines of the International Organization of Standardization, *in vitro* genotoxicity investigations should be designed as a series of at least three tests, with at least two of the experiments utilizing mammalian cells as a target.[25](https://www.iso.org/obp/ui#iso: Std: Iso:10993:-1:Ed-5:V2:En).[48] Most of the studies have used mammalian cell culture including human and animal cell lines.[24] Human cell lines being used are pulp cells, periodontal cells, gingival fibroblasts, and blood cells. Various animal models have been tested such as hamster fibroblasts, Wistar rat, etc., Apart from these animals and human model, various nonmammalian cells such as salmonella and lower animal model such as drosophila melanogaster have been used for *in vitro* studies. Ames test is being used for mutagenicity testing for various drugs in medical science; it can be used in mutagenicity testing in the field of dentistry. Following decades of research and testing, it has been determined that the substances to which people are exposed, which are obviously positive in bacterial tests, should be considered possible human health risks. This supports the validity of bacterial tests for genotoxicity. Many of the National and International toxicological programs are using lower animals such as Drosophila for the genotoxicity testing of various drugs. Hence, in coming future, they can also validate genotoxic tests, if being conducted on nonmammalian models. Although the findings of these tests cannot be directly extended to clinical settings in humans, they are clinically beneficial since they aid in a more accurate assessment of the potential health risk associated with endodontic materials. *In vivo* testing is crucial for evaluating a product's genotoxic and mutagenic potential since it can disclose effects that are not visible in *in vitro* studies. *In vivo* tests can be used to analyze a product's metabolic and pharmacodynamic effects, as well as the impact of the gut microbiota on the drug. In studies that look for even a slight effect on DNA level, primary cultures of isolated diploid cells, such as human leucocytes, are preferred. Normal diploid cells have mitotic rates and mitochondrial activity that are comparable to those observed *in vivo* but differ from altered or tumour cells,[21] as a result, their reaction and sensitivity to xenogens will be more akin to that of cells *in situ*.

Concentration of the agents being tested

Most of the studies evaluated the genotoxic tests at the concentration being used clinically on the cell culture (usually monolayer cells).[49] The dilutions of the tested materials were determined based on the findings of a preliminary research, and only the most cytotoxic concentrations were tested. However, studies show that when a chemical agent is injected into animal subcutaneous tissue or comes into contact with a monolayer of cells, it can cause significant toxicity, although its clinical toxicity within the confines of the root canal system and periapical tissue may be well within acceptable parameters.[31,50] Similarly in case of animal models (lower animals), concentration is important for their survival as well, that's why in many of these studies' lower concentrations have been used compared to clinical use. Apart from concentration of these materials, time period of contact is of paramount importance. Usually, at lower contact time, cytotoxic effects are evident as compared to a longer exposure. Genotoxicity testing at various time intervals allows for the assessment of materials' early and late harmful effects, as well as cell recovery. Apart from the end product, each component of the material and their permutual combinations were evaluated to determine the level of toxicity of each component individually or in combination.

Result obtained from various studies

The result concluded from various tests done are variable in context with materials and the test employed. Since each study utilized a different test for genotoxicity testing, the result came out to be variable. However, to a large extent, studies were carried out on the endodontic sealers, which most commonly comes in contact with periapical tissues. The result varied with the type of sealer, and for resin-based sealer, it came out positive on micronuclei formation test, but comet assay did not reveal any genotoxicity. The combination and separate components also showed different reactions. The tested materials' primers and unpolymerized sealants significantly increased the capacity of DNA to migrate. All combined treatments resulted in a significant increase in tail length and tail intensity. Both comet assay values increased significantly when primers and thinning resins were tested alone and in combination, demonstrating the potential for toxicity even after curing,[51] demonstrated that the concentration of Eugenol released after 24 h of desorption from ZnOE based sealers was $(0.46 \times 10^{-5}$ mol L$)^{-1}$. And this concentration of Zn^{+2} and Eugenol was able to cause cyto/genotoxic effects. Imazato[52] found that 400-50 μg mL^{-1} of HEMA, 100-10 μg mL^{-1} of TEGDMA were present in restorative materials eluates. In osteoblast-like cells, the researchers observed that the monomer quantities they examined had detrimental effects. TEGDMA was shown to be cytotoxic at a concentration of 500 g mL^{-1} in this study.[52]

Another study comparing MTA-based sealer with resin sealer concluded that there is increase in micronuclei formation in resin sealers treated groups.[53] The most genotoxic products were 1:4 dilutions of AH Plus (resin sealer) and MTA Fillapex (resin sealer), producing micronuclei 8 times higher than the untreated control and similar to the positive control group. This depicts the material's concentration and dilution effects. The release of resinous chemicals found in the cement composition as salicylate may be responsible for the genotoxic effects found in this investigation. This component may have induced apoptosis in human fibrosarcoma cells and caused cell genetic material fragmentation, resulting in its precipitation in the cytoplasm.[53] When compared to zinc oxide-based sealers, another study revealed that resin-based sealers, such as AH26 and AH Plus were cytotoxic as well as genotoxic. Genotoxicity was evaluated by both breaking the DNA chain and digestion of the genomic DNA. The release of formaldehyde from AH26 sealer may be linked to the genotoxic potential of a resin-based sealer.[54] One study determining the mutagenicity of resin sealers by using gamma-H2AX focus assay concluded that endodontic sealants do not cause DNA double-strand breaks.[13] Another research showed that Diaket, Hermetic, IRM, and Super EBA had a low genotoxic effect on peripheral blood cells *ex vivo*, indicating that there was no genotoxicity.[55] Because the impact lasted <5 days after polymerization and at the highest dose tested (0.8 g/mL), it should not pose a substantial harm to the human DNA.

Another study evaluated the genotoxic and cytotoxic capabilities of three commercially available GICs in the Chinese hamster ovary using the single-cell gel (comet) assay and the Trypan blue exclusion test. The research investigated the genotoxicity of GIC powder and liquid components and discovered that some of the components were both genotoxic and cytotoxic.[16] Iodoform pastes did not trigger DNA damage in human peripheral cells *in vitro*, according to a recent study by Pires *et al*.[56] Zinc oxide Eugenol-based sealers (Canals, Canals-N, and Tubilseal) were shown to exhibit minimal genotoxicity in mammalian cells.[57] Similarly, when human peripheral blood cells were subjected to MTA and Portland cements and genotoxicity testing was performed using the comet assay,[32] none of them were seemed to be genotoxic.[58] Calcium-enriched mixtures, on the other hand, were genotoxic at concentrations of 15.6 and 250 g/ml, which was lower than MTA.[25]

Extrapolation of results on human

The observed effect may not be directly inferred to the effects of endodontic material *in situ*, because unreacted primers and thinning resins do not stay in close contact with living tissue for extended period, and the potential of cells to reduce toxic agent damage *in situ* and *ex vivo* varies markedly. When a genotoxic agent comes in contact with lymphovascular system, the body's defense mechanism tries to eliminate the toxic agent, and the blood also dilutes the concentration being encountered. Hence the concentration of product to be genotoxic is also different in comparison with various *in vitro* studies. Therefore, *in vivo* studies and clinical trials needs to be conducted to validate these tests.

DISCUSSION

Components of composites and other dental materials is suspected of having deleterious repercussions since they may be released into the saliva during implantation or after polymerization and diffuse into the tooth pulp, gingiva, mucosa, and salivary glands. Residual monomers from polymerized dental resin materials may cause cytotoxicity in pulp cells through the production of reactive oxygen species, which might exert genotoxic effects. Dentin bonding agents" genotoxic effects on human cells are poorly defined and disputed.[59] Methacrylates including TEGDMA, UDMA, Bis-GMA, and HEMA caused substantial DNA migration at high concentrations in human salivary glands and lymphocytes. However, a study comparing the genotoxicity of two dental bonding agents Adper Single Bond Plus (Bis-GMA, HEMA, and UDMA) and PrimeandBond 2.1 (UDMA and HEMA) by Drosophila wing spot test suggested Adper Single Bond Plus increased homologous recombination (HR) in the Drosophila wing spot test, whereas PrimeandBond 2.1 produced recombinogenic and, to a minimal extent, mutational events.[20] These findings imply that the dental adhesives tested cause initial DNA damage, which is thereafter processed to a large degree by recombinational DNA repair mechanisms. Previously, several research groups used SMART to show that the molecular foundation for UDMA's genotoxicity is linked to HR and gene/chromosomal mutation. Regardless of the fact that SMART is a very accurate instrument for detecting genotoxic chemicals, there were no positive findings for Bis-GMA or HEMA, implying that neither compound can induce DNA damage.

Using both chromosomal aberration analysis and comet assay found that Epiphany, a resin-based sealer which contains UDMA, PEGDMA, EBPADMA, and Bis-GMA, as well as GuttaFlow, have no genotoxic potential, whereas zinc oxide–Eugenol-based sealers, such as Hermetic and SuperEBA, have slight genotoxic activity on peripheral blood lymphocytes *ex vivo*.[55] Calcium silicate-based sealers (bioceramicbased sealers) showed promising results as root canal sealers, as their cytotoxicity decreased over time.[60]

According to the alkaline comet test, all calcium hydroxide pastes were capable of causing DNA damage in peripheral blood mononuclear cells. Because these pastes produced breakage in dsDNA, the GEMO assay indicated that they were genotoxic. GEMO assay is a low-cost, quick test that might be used in conjunction with a standard genotoxic test like the alkaline comet assay.[56]

When analyzing the cytotoxicity of various root canal irrigants 5.25% of sodium hypochlorite, 2% of chlorhexidine gluconate and mixture of a tetracycline isomer, an acid and a detergent (MTAD) by checking for hemolysis of human red blood corpuscles, sodium hypochlorite was found to be the most cytotoxic solution followed by MTAD and chlorhexidine.[61,62]

Laurent *et al*.[63] investigated Ca3SiO5's a posterior restorative material based on Portland cement, for its genotoxicity and cytotoxicity, as well as its impact on particular functions in target cells. The death rate of cells exposed to this cement was comparable to that of cells exposed to other biocompatible materials, as MTA and less as compared to Dycal. Similarly, the Ames test, which employed lymphocytes and fibroblasts from human dental pulp, found no evidence of mutagenicity in this new material.

Using the single-cell gel (comet) assay and the Trypan blue exclusion test in the Chinese hamster ovary, one research looked at the genotoxic and cytotoxic potential of different commercially available GICs. The study looked at the genotoxicity of the powder and liquid components of GICs, and found that some of them were both genotoxic and cytotoxic.[16,64]

Several authors have investigated the mutagenicity and genotoxicity of MTA, a cement having a composition comparable to Ca3SiO5. MTA undergoes a hydration process after manipulation, resulting in the production of calcium hydroxide and subsequent dissociation of Ca^{+2} ions and hydroxyl, which causes a rise in pH and increased calcium content in the medium.[14,65] The micronucleus test and the comet assay, however, revealed that MTA had no mutagenic or genotoxic effects.[66] Because of its high pH (12.5) and strong biocompatibility in cell cultures,

the MTA exhibits antibacterial capabilities in an aqueous environment. As a result, it has been utilized in apical surgery as a root-end filling material, as a sealing material, and for pulp capping and pulpotomy.[20,67] When compared to calcium hydroxide, MTA produces a hard-tissue barrier at the pulp exposure site but with a mild inflammatory response.[7] MTA and calcium hydroxide formulations have both shown to be effective in clinical trials. In numerous cell lines and test methods, MTA is commonly referred to be a nontoxic or low-cytotoxic root canal cement.[15,17,68]

Collado-González *et al*.[69] investigated the cytotoxicity and bioactivity of several pulpotomy materials: Biodentine, MTA (Angelus, Londrina, PR, Brazil), Theracal LC, and IRM in contact with human stem cells from deciduous teeth using MTT assay and found Biodentine exhibited better cytocompatibility and bioactivity than MTA Angelus, Theracal LC, and IRM.

While no morphologic tests were performed on Chinese hamster fibroblast (V79) cells, Bin *et al*.[3] assessed the cytotoxicity and genotoxicity of MTA canal sealer (Fillapex) compared with white MTA cement and AH Plus and revealed that the cell viability remained above 50% in white MTA group for all dilutions. AH Plus induced an intermediate cytotoxicity in a dilution-dependent manner, followed by Fillapex MTA. Other investigations have found that MTA and Portland cement (White and Gray) did not cause genetic damage in human lymphocytes at any of the doses tested.[15] It might be because the chemical compositions of both Portland cements and MTA are identical,[17] with the exception of the white cement's reduced iron concentration, which gives it its color.

Chloroform is another endodontic irrigant that has been classified as hazardous by the National Cancer Institute (National Cancer Institute. Carcinogenesis bioassay of chloroform)[70] Using mutant S. Typhimurium and *Escherichia coli* strains, Araki *et al*.[71] revealed that chloroform's mutagenesis impact was dose-dependent. Chutich *et al*.[72] found that the quantity of chloroform leaking into the periapical tissues was negligible and that the patients were not at danger. Both authors came to the conclusion that the amount of irrigant used was may be more significant than its mutagenic potential. Patil *et al*.[18] conducted a study on mutagenic potential of precipitate formed by chlorhexidine and NaOCl concluding that precipitates produced by different doses of NaOCl with 0.2% CHX induced no substantial mutagenic changes in the DNA of S. Typhimurium strains TA100 and TA98. The use of lower amounts of NaOCl and CHX was one of their study's drawbacks. The S. Typhimurium strains could not survive at the higher doses employed in their pilot research. Clinical investigations have indicated, however, that chemomechanical debridement with as low as 0.12% CHX and 2.5 percent NaOCl is effective.[73]

This systematic review puts together the studies addressing the genotoxicity of almost all commercially available commonly used endodontic materials. However, most studies of the studies were performed *in vitro*, with only a few studies being conducted *in vivo*. In order to standardize the protocols allowing representative information for clinical usefulness, more *in vivo* studies should be done.

CONCLUSION

Based on the above study, it can be concluded that, at lower concerntration, dentin bonding agents exert no genotoxic effects. Resin-based sealer and calcium silicate-based sealers (bioceramic-based sealers) showed promising results as their cytotoxicity decreased over time. Both MTA and biodentin have no mutagenic or genotoxic effects. More studies using multiple end points and focusing on the function of oxidative stress and DNA repair mechanisms using *in vivo* test systems are required to explain the human health risks posed by these dental materials. As a consequence, additional study is needed in this domain to ensure the safety of both professionals and patients in an attempt to improve oral health as these materials remain in the mouth for long durations.

Financial support and sponsorship
Nil

Conflicts of interest
There are no conflicts of interest.

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