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Cytotoxicity and Genotoxicity of Commonly Utilized Endodontic Sealers: A Review

Habib Fazlullah[1*], Salamzai Ali Mohammad[2]

[1]*Department of clinic, Stomatology Faculty, Rokhan Institute of Higher Education, Jalalabad City, Afghanistan.*

[2]*Department of clinic, Stomatology Faculty, Rokhan Institute of Higher Education Jalalabad City, Afghanistan.*

***Corresponding Author:** Habib Fazlullah, Department of Clinic, Stomatology Faculty, Rokhan Institute of Higher Education, Jalalabad City, Afghanistan.

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Abstract

Radicular pulp cavity filling materials (root canal sealer) are a crucial component after cleaning and shaping for an effective obturation procedure. The germs that are still present in the auxiliary canals after cleaning and shaping may also be killed by radicular pulp cavity filling materials. The most frequently used sealers in endodontic procedures are ZOE (zinc oxide-eugenol) bases, calcium hydroxide bases, and resin bases. Endodontic sealants may come into touch with the root apex before setting or may be extruded into the periapical space through the apical foramen and auxiliary (lateral and accessory) canals, both of which can have adverse biological effects. They may irritate, inflame, or impede wound healing. Which depend on the cytotoxic and genotoxic qualities of the sealer. A substance's capacity to damage or kill cells is referred to as cytotoxicity (programmed cell death or reproductive cell death), and its capacity to harm a cell's DNA molecule is referred to as genotoxicity (toxic effects on DNA structure or DNA damage). Evaluation of cytotoxicity and genotoxicity caused by root canal sealer is required to clarify the genuine health hazards to patients and professionals because there is strong evidence linking genetic damage and carcinogenesis. The utilized endodontic materials are likely to cause some degree of cytotoxicity and genotoxicity. This present paper's goal is to study the cytotoxicity, genotoxicity, and characteristics of various (zinc oxide eugenol, calcium hydroxide, and resin bases) endodontic sealers.

Keywords: *root canal sealer, obturation material, Cytotoxicity, Genotoxicity, formaldehyde.*

Introduction

Enamel, dentin, and periodontium shield the tooth pulp from harmful chemicals; nevertheless, if these barriers are broken, bacteria and substances may negatively influence its stability, resulting in irritation (pulpitis) and even tissue loss (pulp necrosis), which need pulpectomy (removing all the debris from the coronal and radicular pulp cavities) as a remedy for this (Miguel et al, 2020). A filling material is used to close the root canal system, preventing reinfection and the growth of any remaining bacteria (Gatewood, 2007). A necessary condition for reestablishing a functioning periodontal attachment is the deposition of cementum, which is seen as a beneficial therapeutic (healing) response (Kangarlou et al, 2016). The physical and chemical qualities of the filler materials are crucial for effectiveness, but biological safety is a need for clinical use (Kaur et al, 2015).

Obturation in endodontics primarily aims to completely obliterate the root canal system and create a fluid-tight closure at the apical foramen, which creates a biological environment for periapical tissue recovery (Tyagi et al, 2013). The most common technique for filling root canals involves using a root canal sealer to cement a semisolid, solid, or stiff core material into the canal (Camps et al, 2004). Endodontic sealing done improperly might prolong inflammation and infection, among other negative outcomes (De-Deus et al, 2022). There are currently several different filler materials available for canal obturation (Zhou et al, 2013). GP (gutta percha) points are by far the most widely used of all the root canal filler materials, and authors have demonstrated that teeth with gutta percha points and sealer exhibit less leakage after obturation (Garg et al, 2014). In the treatment process, some root canal sealants may need to come into contact with the root apex, or they may mistakenly enter the radicular apex and arise into interaction with the surrounding tissues for a few minutes to hours before strengthening, which has negative biological effects on interacted tissues such as irritation, inflammation, and impede wound healing (Giacomino et al, 2019).

The ISO (International Organization for Standardization) in 1942 states that when biocompatible materials are in contact with the biological environment, they do not have any negative or undesirable side effects (Jafari et al, 2017).The optimum radicular pulp cavity filling material is antibacterial and biocompatible, allowing for substantially longer tooth retention and inducing biological cell responses that aid in regeneration (Komabayashi et al, 2020). For the purpose of selecting an endodontic sealer for clinical use, it is crucial to determine if the pulp is alive or necrotic (Porter et al, 2010). Under the asepsis technique, in cases of symptomatic irreversible pulpitis, the healing effects of sealers are not required because the healing (therapeutic) benefits of sealers are beneficial only in cases of necrotic pulp, particularly in cases with significant periapical radiolucency (Kim et al, 2022).

Understanding their biological effects is necessary because cytotoxic materials can destroy the periradicular structures, and genotoxic substances could lead to DNA changes (Al-Hiyasat et al, 2010).This review analyzes the composition, characteristics, cytotoxicity, and genotoxicity of frequently used endodontic (based on zinc oxide eugenol, calcium hydroxide, and epoxy resin) sealers.

Materials and methods

For the current review, the following key terms were used to conduct an electronic literature search in PubMed, Google Scholar, and ResearchGate: "genotoxicity of endodontic materials," "cytotoxicity of endodontic sealers," and "endodontic sealers (calcium hydroxide, resin, zinc oxide eugenol).

Zinc oxide-Eugenol-Based Sealers:

Since the 1890s, ZOE cement has been broadly used in dental medicine for its exceptional cavity sealing ability, is bactericidal, and has a sedative effect on sensitive teeth (He et al, 2010). Because of their slow setting, low price, antibacterial qualities, and simplicity of use, ZOE sealers continue to be widely used, and over numerous decades, different ZOE sealers have been introduced (Javidi et al, 2014). ZOE sealers include eugenol liquid, an essential oil made from cloves, and zinc oxide powder (Estrela et al, 2014). When it is considered that ZOE has an inhibiting effect on resin bonding, ZONE (zinc oxide non-eugenol) cement has been created and is employed in certain situations (Kwon et al, 2013).

Calcium hydroxide-Based Sealers:

The use of calcium hydroxide as a sealer is hypothesized to encourage a sterile biological closure of the apical region, improving the seal and the effectiveness of the therapy (Garg et al, 2014). Because of its effective healing properties, which may be connected to ion diffusion, Sealapex (a calcium hydroxide-based sealer) is employed in therapeutic procedures to promote healing by raising PH through the diffusion of ions from the sealer (Habib & salamzai, 2023). The bacterial lipopolysaccharide degradation, the promotion of hard tissue development, and the regulation of inflammatory root resorption are all made possible by the high pH (Filho et al, 2011). A sealant with a foundation of calcium hydroxide, zinc oxide, sulfonamide, and zinc stearate, Sealapex (Kerr) also has a catalyst made of barium sulfate, titanium dioxide, and resin (Dummer, 2011). While calcium hydroxide-based sealers have high biological characteristics, they also have certain drawbacks, like weak cohesive strength, increased solubility, and minimal leakage (Desai et al, 2009). Because water is absorbed during the hardening process, Sealapex experiences volumetric expansion, and as a result, its sealing ability may be impacted (Tanmi et al, 2021).

RESIN-BASED SEALERS

Resin-based sealers are divided into two types: epoxy resin-based and methacrylate resin-based (Subbiya et al, 2021).

EPOXY RESIN-BASED SEALERS:

Schroeder introduced epoxy resin-based sealers to endodontics in 1957 (Lee et al, 2017). The advantageous traits include their ability to adhere to tooth structure, their extended working period, ease of mixing, and their positive physical and biological characteristics (Lim et al, 2020). Three different kinds of epoxy resin-based sealants exist: Diaket, AH26, and AH PLUS (Ioannidis et al, 2013).

Diaket

Diaket is a polyketone (polymeric resin) and was considered by Scheufele in 1952 (Economides et al, 2004). To produce a cemented chelate, diaket (a liquid and powder system) is used with GP (gutta percha) points (Rathi et al, 2020). Powder composed of bismuth phosphate and zinc oxide, while the liquid is composed of propionylacetophenone (copolymers of vinyl chloride, vinyl isobutyleether, vinyl acetate, triethanolamine, caproic acid, dichlorophen, and B-diketone) (Ioannidis et al, 2013).

AH 26

When AH 26 (powder and liquid mixed) is created, formaldehyde is released, which has an antimicrobial effect (Miletić et al, 2003). Hexamethylenetetramine, titanium oxide, bismuth oxide, and silver powder are all present in powder form, and the liquid is composed of bisphenol-diglycidyl ether. It flows better, settles over a 24- to 36-hour period, and has a film thickness of 39µm (Adanir et al, 2006).

AH PLUS

AH Plus is a two-component (A&B) paste sealant that has the features of a quick setting time and is simple to prepare, facilitating tight root canal adaptation and avoiding decreasing after setting (Resende et al, 2009). AH Plus Paste A is composed of epoxy resins, calcium tungstate, zirconium oxide, silica, and iron oxide pigments, while Paste B of AH Plus is composed of aminoadamantane, dibenzyldiamine, calcium tungstate, zirconium oxide, silica, and silicone oil (Nisha Garg & Amit Garg, 2019).

METHACRYLATE RESIN-BASED SEALERS

Methacrylate resin-based sealants are divided into four groups based on their generational history (1st, 2nd, 3rd, and 4th generations) (Subbiya et al, 2021). Hydron (1st generation) biocompatible substance was introduced in 1960 as a HEMA (hydroxyl ethyl methacrylate) polymer, which is a hydrophobic, air-injected, rapid-setting root canal filler substance that serves as a root canal sealer without the need for a core (Bhor et al, 2023). Endorez (2nd generation)is a hydrophilic, non-etching, double-cure opaque sealer that is composed of zinc oxide, barium sulfate, and urethane dimethacrylate resin (Zmener et al, 2008). When Endorez is utilized in the wet condition of the root canal system, this sealer's hydrodynamic character can be particularly successful at infiltrating the tubules of dentin and creating lengthy resin tags (Sousa et al, 2006). Epiphany (3rd generation) is a hydrophilic, dual-curing resin sealant whose monobloc construction and ability to adhere to dentinal walls give it outstanding sealing ability (Bhor et al, 2023). Metaseal (4th generation) is a self-adhesive, dual-cure, insoluble, radiopaque sealer that comes in powder and liquid form and can be utilized with either Resilon or regular gutta percha (Lawson et al, 2008).

Definition of Cytotoxicity

Cytotoxicity is the capacity to harm living organisms, including the hazardous property that a chemical exhibits toward the cells of the body. It can have a variety of effects on a cell, which can result in diverse cell fates (Freshney, 2005). The most frequent of these outcomes are various forms of cell death, such as apoptosis (cell death that is genetically predetermined) and necrosis (celik, 2018). Examining a substance's capacity to kill cells is the focus of cytotoxicity tests, which involve the biological assessment of tissue cells in vitro for the purpose of detecting the effects of the cell to ascertain whether a specific material (drug or chemical) can kill a cell either directly or via the leakage of a number of poisonous compounds (Fellows & Donovan, 2007).

Cytotoxicity of Root Canal Sealers

Numerous unique root canal sealers have recently been made available under a variety of brand names and are believed to have varying amounts of cytotoxicity on structures, which may result in slow wound healing, inflammation, and bone resorption (Camps & About, 2003). Sealants should ideally have biocompatibility as well as tolerable biological and physicochemical qualities and must undergo a biocompatibility assessment before being used on patients (Mondal et al, 2023).

Cytotoxicity of Zinc Oxide Eugenol

A lengthy history of successful use of ZOE sealers has been established (Ranade & Kamra, 2003). Despite their benefits, ZOE cements displayed variable levels of cytotoxicity and were shown to be moderately to highly harmful in implantation tests (Huang et al, 2004). In a study after two years, moderate inflammation was found in the apical and periapical tissues of baboons (Angelo et al, 2017). ZOE sealers have been demonstrated to be a major fibroblast cytotoxic and irritant, as well as to trigger a complement-mediated immunological response (Komabayashi et al, 2020). Eugenol had a more cytotoxic effect on the human periodontal ligament when implanted subcutaneously (Ho et al, 2006). According to researchers, when ZOE cement came into contact with the periapical tissues, it was extremely irritating (inflammation), allergic, and caused necrosis of the bone and cementum (Ghosh et al, 2005). The causes of the cytotoxicity and inflammation are eugenol and zinc ions from both ZOE and ZONE cements (Kwon et al, 2013), (Ranade & Kamra, 2003).

Additionally, it was discovered that eugenol has the power to stop melanoma cells from proliferating (Amandeep et al, 2015). A 40% reduction in tumor size and a significant delay in tumor growth were the outcomes of such an effect (Bohrer et al, 2018).

Cytotoxicity of Calcium hydroxide

Calcium hydroxide sealants have osteogenic and cementogenic potential as well as antibacterial activity and are believed to satisfy the majority of endodontic treatment requirements (Huang et al, 2004). In another study, it was explained that calcium hydroxide-based root canal sealant caused mild to moderate tissue irritation (Tanmi et al, 2021). Sealapex is a calcium hydroxide-based sealer that is predominantly composed of polymethylene methyl salicylate resin and isobutyl salicylate (Loushine et al, 2011). Numerous studies have demonstrated that Sealapex is highly cytotoxic, which is mainly because of salicylate, but calcium hydroxide may show cytotoxicity itself as a result of its high pH (Desai & Chandler, 2009). In a different animal study, the calcium hydroxide sealer (Sealapex) caused more severe inflammation on the third day of evaluation, which didn't go away until the fourteenth day (Hee et al, 2006). Since solubility is necessary for the release of calcium hydroxide and ongoing action, and it revealed cytotoxic effects, it is incompatible with the goal of a perfect sealer and contradicts the previously held belief that calcium hydroxide-based sealants are biological sealers (Eldeniz et al, 2007).

Cytotoxicity of Epoxy resin-based sealers

Endodontic practice uses epoxy resin-based sealers because of their beneficial traits, including adherence to tooth structure, extended operational period, simplicity in mixing, and effective sealing ability (Huang et al, 2004). Due to the discharge of extractable monomers and/or other mineral and organic substances that can deteriorate the tissue beneath the radicular sealer, epoxy resin-based radicular cavity filling materials may have negative local and/or general effects on root-surrounded tissues and tooth-supporting bone (Garg et al, 2014).The emission of formaldehyde by resin-based sealers may be connected to their cytotoxicity (Subbiya et al, 2021). This sealer was hazardous to root-surrounded (periradicular) structures and was not advised because formaldehyde produces coagulative cell death and persistent formaldehyde prevents local healing of injured areas (Komabayashi et al, 2020).

Additionally, bisphenol diglycidyl ether was found to be a possibly cytotoxic component of resin-based products (Kim et al, 2022). To examine toxicity, a variety of test techniques are needed because cytotoxicity results do not necessarily coincide with genotoxicity results (Loushine et al, 2011). In comparison to AH 26, there has recently been the introduction of AH Plus, which is a new epoxy resin sealer that claims to be more biocompatible, less mutagenic, less cytotoxic, and does not emit formaldehyde (Gebel, 2004). The unset sealer's negative effects can be reduced thanks to its quick working time (Lodienė et al, 2007). When oral mucosa comes into contact with unset pastes with resin-based sealers, acute inflammation, such as irritation and discomfort, in the involved area may develop (Pinna et al, 2008). Local and systemic allergies have also been documented in some instances (Öztan et al, 2003). The generation of formaldehyde during the setting reaction was thought to explain the initial significant cytotoxicity (Özdemir & Kopac, 2022). In comparison to epoxy resin-based (AH26), Epiphany has better biocompatibility and is less irritating, while hydron causes persistent periapical irritation when it crosses the apical foramen or is overfilled (Cintra et al, 2017). Metaseal has been shown to be non-mutagenic and harmless (Ashraf et al, 2018).

Definition of Genotoxicity

Genotoxicity is a destructive process in which the genetic material of cells loses its integrity through chemical or radiation exposure (Turkez et al, 2017). Genotoxic substances are those that interact with DNA with a high affinity, making them possibly mutagenic or carcinogenic (Miguel et al, 2020).

Genotoxicity of Root Canal Sealers

Since it is considered that the placed sealant material comes into contact with the periradicular tissue, it is crucial to understand the genotoxic effects of the utilized material because it has been closely linked to illnesses like tumors (cancer) (Miguel et al, 2020). It is advised to do some in vitro experiments to determine the genotoxicity of such items (Mohammadi et al, 2015).

Genotoxicity of Zinc Oxide Eugenol

Zinc oxide eugenol has been shown to produce moderate to severe toxic effects in the V79 cell line, and researchers have also shown that these properties are dose-associated, which suggests that eugenol has genotoxic properties (De et al, 2013). Various phenolic chemicals, including eugenol, isoeugenol, and safrole, have been tested for their ability to cause genotoxicity in Drosophila melanogaster using the wing spot test, and the outcomes clearly showed that isoeugenol was not genotoxic at the same concentrations (NEJAD et al, 2017).

According to the data, eugenol has the potential to be mutagenic in male mice and can cause mutations, especially in the anaphase of polychromatic erythrocytes (Hee et al, 2006). Eugenol may have dose-related antigenotoxic effects (Ulanowska & Olas, 2021). Eugenol was effective in preventing deoxyribonucleic acid (DNA) loss as a chemopreventive agent by inhibiting dimethylbenz (a) anthracene (V Nair et al, 2018).

Genotoxicity of calcium hydroxide-based sealers

The investigations conducted revealed that calcium hydroxide-based sealers appeared safe as a whole (Miguel et al, 2020). No genotoxic effects were found with the calcium hydroxide-based sealer (Sealapex) (Hee et al, 2006).

Genotoxicity of Epoxy resin-based sealers

According to studies, sealers that contain three substances such as formaldehyde, paraformaldehyde, and bisphenol A diglycidyl are genotoxic (Miletić et al, 2003). Even though the amount of formaldehyde produced from various epoxy-based sealers is less than that of paraformaldehyde, it nevertheless reaches its maximum rate after 48 hours (Ündeğer et al, 2011). In vitro, under strictly regulated circumstances, it was discovered that there is no mutagenicity of AH26 and AH Plus sealers on human lymphocytes (ØRSTAVIK, 2006). The systemic distribution of formaldehyde from the root canal system following pulpotomy treatments has also been seen in VIVO (Tai et al, 2002). Unset epoxy resin-based sealers generally have a low level of biocompatibility and reveal higher DNA damage (genotoxic) because of the remaining monomer and formaldehyde (Jukić et al, 2003). However, epoxy sealants like AH 26 have been seen to produce formaldehyde even two days after mixing and produce ambiguous genotoxic results, and no genotoxic activity was detected.(Loushine et al, 2011). Since the application of epoxy resin-based sealers, the high levels of inflammation in the periapical and subcutaneous tissues have been revealed (Mohammadi et al, 2015).

The newly substituted (AH plus) material is produced to prevent the release of formaldehyde, which generates a less severe inflammatory reaction in the periapical region than AH 26 (Costa et al, 2020).

Conclusion

Evaluation of cytotoxicity and genotoxicity caused by root canal sealers is required to clarify the genuine health hazards to patients and professionals. Before using dental materials, researchers and doctors should take into account the probability of the various types of toxicity they may cause.

Endodontic sealers are likely to cause some degree of cytotoxicity (cell death) and genotoxicity (toxic effects on DNA structure or DNA damage).

Zinc oxide eugenol-based cements show dose-dependently irritative, allergic, cytotoxic, and genotoxic effects on periradicular tissues.

Sealapex (calcium hydroxide-based sealers) is a regenerative material that does not have genotoxic characteristics but has cytotoxic characteristics, which are not only due to salicylate but can also be due to its high PH.

Due to formaldehyde, epoxy-based sealants (AH26) are mutagenic and genotoxic, while AH PLUS shows no evidence of mutagenicity or genotoxicity because it does not emit formaldehyde.

Unset epoxy sealers (AH26) are more genotoxic in comparison to mixed ones, so when this sealant cement comes into contact, oral mucosal discomfort happens. However, quick working times can reduce the negative effects of unset sealers.

Because these materials stay in the mouth for extended periods of time, additional investigation is needed in this field to assure the security of both professionals and patients while working to enhance oral health.

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