Review

Characterization of the Cytotoxicity of Materials in Dental Medicine

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Abstract

The increasing use of synthetic and metallic materials in modern dentistry has raised growing concerns about their potential biological impact on oral and systemic health. Many of these materials can release toxic components into the oral environment due to incomplete polymerization, mechanical wear, or enzymatic degradation. Resin monomers such as TEGDMA, BisGMA, and EGDMA have shown dose-dependent cytotoxic effects, often through mechanisms involving oxidative stress and apoptosis. Similarly, metals such as nickel, mercury, cobalt, and components of titanium and zirconium implants can provoke allergic or systemic reactions and lead to cellular damage. Furthermore, impression materials and substances like eugenol also demonstrate cytotoxic effects. Cytotoxicity can vary based on the dosage, duration of exposure, and combination of materials used. This review is based on a systematic analysis of peer-reviewed literature from major biomedical databases, focusing on studies published between 2000 and 2024 that examined the cytotoxicity of dental materials in vitro and in vivo. It emphasizes the importance of standardized testing (ISO 10993, ISO 14971, ISO 7405) to ensure material safety and guide clinical decision-making in dentistry.

Keywords: Cytotoxicity; Dental materials; Resin monomers; Oxidative stress; Metal alloys; Biocompatibility

1. Introduction

Biocompatibility of dental materials is essential for ensuring clinical safety and long-term success in dental treatments. To be considered clinically safe, dental materials must exhibit high biocompatibility, which includes the absence of cytotoxic, pro-oxidative, inflammatory, and mutagenic effects, as well as the ability to support normal tissue repair without interfering with physiological processes. Cytotoxicity is defined as a specific aspect of toxicity that refers to a substances ability to damage or kill cells, often measured in vitro (Shahi et al., 2019). The aim of this review is to characterize the cytotoxicity of commonly used dental materials, such as resin composites, metal alloys, impression materials, cements, and others. Understanding the cytotoxic potential of these materials is essential for optimizing clinical choices and ensuring patient safety.

The first national system for reporting adverse effects of dental materials was established in Norway in 1993. Between 1993 and 1999, a total of 899 reports were received, of which 253 were referred for clinical evaluation. According to the study, amalgam was responsible for the highest number of adverse reactions, followed by metals and resin-based materials (Lygre et al., 2003).

2. Design of the Review

A systematic literature search was conducted using the PubMed and Web of Science databases, focusing predominantly on articles published between 2000 and 2024. These databases were selected due to their comprehensive indexing of peer-reviewed biomedical literature and extensive coverage of dental, toxicological, and clinical research. The search strategy included combinations of the following keywords: cytotoxicity, dental material, biocompatibility, resin monomers, metal alloys, impression materials, toxicity, in vitro, and in vivo. Inclusion criteria were: (1) clinically documented case reports describing adverse biological reactions during or after the use of dental materials, (2) studies that assessed the cytotoxic effects of dental materials, (3) both in vitro and in vivo studies involving human or animal models, and (4) peer-reviewed articles published in English.

3. Types of dental materials and associated cytotoxic risks

Resin-based dental materials, such as composite materials, bonding agents, resin-based cements, acrylic resins, sealants, are not inert in the oral environment and can release various components, initially due to incomplete polymerization and later as a result of mechanical wear and enzymatic degradation (Emmler et al., 2008; Wiertelak-Makala et al., 2024). These materials remain in prolonged and close contact with the oral tissues in multiple ways. This includes direct contact with dentin, enamel, or adjacent gingiva, as well as indirect contact with the dental pulp via dentin tubules or with soft tissues through saliva (Wiertelak-Makala et al., 2024). This prolonged exposure increases the likelihood of adverse biological responses. Based on a national survey of adverse reactions to dental materials conducted in the USA, resins were identified as the leading cause of adverse reactions among dental technicians (Scott et al., 2004). Some studies have shown that the most common resin monomers, EGDMA (ethylene glycol dimethacrylate), BisGMA (bisphenol A-glycidyl methacrylate), TEGDMA (triethylene glycol dimethacrylate) cause dose-dependent cytotoxicity. Among these, TEGDMA is considered the most cytotoxic, primarily due to its ability to induce oxidative stress, deplete intracellular glutathione (GSH), impair mitochondrial function, and activate apoptotic signaling pathways, such as caspase activation and MAPK cascade (Schweikl et al., 2005; Eckhardt et al.; 2009; Stanislawski et al., 2003). Considering that BisGMA is a derivative of bisphenola-A, which is known to be associated with developmental issues of the genital organs, immune system dysfunction, thyroid disturbances, and neurological development problems in children, its cytotoxicity is understandable (Jung et al., 2023). Moreover, several studies have demonstrated that these resins can interfere with hormonal signalling. It has also been demonstrated that resins can interact with hormone receptors and negatively affect human health by disrupting the normal endocrine function (McLachlan,1993). Therefore, adverse reactions of dental materials can be classified as either local or systemic. Local adverse reactions are assessed from two distinct aspects: mucosal toxicity and pulpal toxicity. Resin-based dental materials can cause numerous adverse effects on the oral mucosa, including epithelial proliferation, asthma, oral lichenoid reaction, pain, burning sensations, epithelial desquamation, contact allergies, etc.

(Marquardt et al., 2009; Syed et al., 2015). In more severe cases, systemic symptoms may also develop. There have also been reports of generalized neuropathy after 14 years of exposure to methacrylates (Sadoh et al., 1999). Possible effects of monomers on cells include apoptosis, loss of cell viability, oxidative stress, glutathione depletion, and reduced mitochondrial activity. It has been established that the cytotoxicity of a dental material can be influenced by several factors, including the material's dosage, duration of exposure, and the combination of materials used during treatment. For example, an in vitro study demonstrated that combining Durafill (microfilled composite resin) with Dycal (calcium hydroxide-based dental material) did not significantly alter cytotoxicity. In fact, the rate of cell death decreased to approximately 15% when Flow Line (flowable composite) was combined with Dycal. However, the combination of MTA (mineral trioxide aggregate) with Durafill increased the cytotoxicity to approximately 85% (Agnes et al., 2017). Special Clinic of Children's Dentistry in Sweden reported an isolated case in which adverse effects such as asthma and urticaria occurred following fissure sealing (Hallström, 1993)

Alloys are frequently used in dentistry due to their strength and durability. Among all metals, nickel is the most common sensitizer, known to cause immediate allergic reactions (Syed et al., 2015). The Co-Cr (cobalt-chromium) alloy also contains metals such as manganese, molybdenum, and nickel, which have been shown to exert cytotoxic effects on human growth factors and osteoblasts, primarily through increased production of reactive oxygen species (ROS) (Shahi et al., 2019). The first reported case of a metal allergy in dentistry was associated with amalgam fillings in the oral cavity. The toxicity of mercury was the main reason for the eventual replacement of amalgam. Allergic reactions may present as urticaria, rhinorrhea, and swelling, as well as erythematous, erosive, and atrophic changes or ulcerative lesions. In severe cases can lead to life-threatening conditions such as laryngeal edema, anaphylaxis, and cardiac arrhythmias (Karabucak et al.,2007; Ramnarayan et al., 2014). Mercury exposure has also been linked to oral lichenoid lesions and burning mouth syndrome (Syed et al.. 2015). In addition, mercury can enter the bloodstream after being absorbed from saliva and can cause toxicity in various organs. It is well known for its neurotoxic and nephrotoxic effects. It induces different signaling pathways leading to cell death, DNA damage and liver dysfunction (Shahi et al.,2019). After the placement of titanium dental implants, orthodontic appliances, or endoprostheses, 56 patients developed severe health problems, including muscle and joint pain (Müller et al., 2006). Titanium and zirconium implants can form an oxide layer upon exposure to oral fluids. The accumulation of titanium and zirconium ions in tissue, particularly in lymph nodes and lung tissue may occur as a result of corrosion, degradation of the oxide layer, and the subsequent release of ions into the oral cavity. The accumulation of titanium particles within lysosomes and macrophages has been reported to trigger hypersensitivity reactions (Chaturvedi et al., 2013; Mitchell et al., 1990).

Due to their small size and chemical properties, silver nanoparticles (AgNPs), which are gaining momentum in dentistry because of their antimicrobial and anti-inflammatory characteristics, have the potential to cross the blood-brain barrier. Once in the bloodstream, they can reach the central nervous system (CNS) and exert neurotoxic effects (Wang et al., 2023). Within the cell, AgNPs can generate reactive oxygen species (ROS), leading to oxidative stress that may damage celulular components and potentially cause inflammation, apoptosis, or necrosis (Rohde et al., 2021). Studies have shown that silver nanoparticles exert cytotoxic effects by modulating and disrupting key intracellular signaling pathways.

Dental anesthetics are divided into two basic chemical groups, esters or amides. Most sensitization reactions are attributed to ester anesthetics, as one of their degradation products is the antigenic agent p-aminobenzoic acid (Canfield et al., 1987). Eugenol, polyethers, and polysulfides, which are used in the impression-taking process in dental medicine, have also been shown to be cytotoxic. A retrospective report has also documented a case of fatal anaphylactic shock caused by an alginate impression (Gangemi et al., 2009). In pediatric dentistry, eugenol, used in combination with zinc oxide for root canal filling after pulpectomy, has been shown to cause toxicity. Its use has been associated with the induction of oxidative stress, disruption of cell membranes, and disturbance of cellular homeostasis (Roberts et al., 2014; Khan et al., 2011). Eugenol has also been reported to exhibit antiplatelet activity through the inhibition of the cyclooxygenase-2 (COX-2) enzyme in humans. It is widely

accepted that eugenol can induce cytotoxicity in pulp fibroblasts of deciduous teeth in a concentration-dependent manner (Escobar-García et al., 2016).

4. Evaluation of cytotoxicity

Before being approved for routine clinical use, dental materials must undergo a structured evaluation of their toxicity and biocompatibility. This evaluation includes in vitro testing, in vivo studies, and ultimately, clinical trials. These procedures are performed in accordance with international standards: ISO 10993 for biological evaluation of medical devices, ISO 14971 for risk management (Carden et al., 2021), and ISO 7405, which is specifically designed for the evaluation of dental materials (Murray et al., 2007). In vitro cytotoxicity tests represent the first step in the evaluation process. They are used to identify toxic effects, quantify the dose of released substances, and monitor the biological response of cells to those substances (Shahi et al., 2019). One commonly used method is the agar diffusion test, where the material is placed on an agar medium over a monolayer of cultured cells. The diffusion of toxic agents is observed through zones of decolorization or cell lysis (Murray et al. 2007). If a material passes in vitro testing, it proceeds to in vivo testing, typically conducted on animal models to assess both local and systemic reactions. These studies provide essential insights into tissue compatibility and systemic toxicity, though they are limited by ethical and legal concerns. Finally, clinical trials in human subjects are conducted to confirm safety and biocompatibility in real-life conditions. However, due to ethical constraints, these studies are limited in scope and are generally conducted only after extensive preclinical validation (Shahi et al., 2019).

Conflicts of Interest: The authors declare no conflict of interest

References:

1. Agnes A, Long A, Best S, Lobner D. Pulp Capping Materials Alter the Toxicity and Oxidative Stress Induced by Composite Resins in Dental Pulp Culture. Eur Endod J. 2017; 2:1-6. DOI: 10.5152/eej.2017.17001.
2. Canfield DW, Gage TW. A guideline to local anesthetic allergy testing. Anesth Prog. 1987; 34:157-63.
3. Carden L, Oladapo B. An Ethical Risk Management Approach for Medical Devices. Risk Manag Healthc Policy. 2021; 14:2311-2318. DOI: 10.2147/RMHP.S306698
4. Chaturvedi T. Allergy related to dental implant and its clinical significance. Clin Cosmet Investig Dent. 2013; 5:57-61. DOI: 10.2147/CCIDE.S35170
5. Eckhardt A, Gerstmayr N, Hiller KA, et al. TEGDMA-induced oxidative DNA damage and activation of ATM and MAP kinases. Biomaterials. 2009; 30:2006-2014. DOI:10.1016/j.biomaterials.2008.12.045
6. Emmler J, Seiss M, Kreppel H, Reichl FX, Hickel R, Kehe K. Cytotoxicity of the dental composite component TEGDMA and selected metabolic by-products in human pulmonary cells. Dent Mater. 2008; 24:1670-1675. DOI: 10.1016/j.dental.2008.04.001.
7. Escobar-García M, Rodríguez-Contreras K, Ruiz-Rodríguez S, Pierdant-Pérez M, et al. Eugenol Toxicity in Human Dental Pulp Fibroblasts of Primary Teeth. J Clin Pediatr Dent. 2016; 40:312- 318. DOI: 10.17796/1053-4628-40.4.312
8. Gangemi S, Spagnolo EV, Cardia G, Minciullo PL. Fatal anaphylactic shock due to a dental impression material. Int J Prosthodont. 2009; 22:33-4. PMID: 19260424.
9. Hallström U. Adverse reaction to a fissure sealant: report of case. ASDC J Dent Child. 1993; 60:143-146.
10. Jung YS, Ro ST, Kang SW, et al. Bisphenol A release from commercially available 3-dimensionally printed resins and human cell apoptosis to bisphenol A: an in-vitro study. J Clin Pediatr Dent. 2023; 47:89-95. DOI:10.22514/jocpd.2023.027
11. Karabucak B, Stoopler ET. Root canal treatment on a patient with zinc oxide allergy: a case report. Int Endod J. 2007; 40:800-807. DOI: 10.1111/j.1365-2591.2007.01275.x.
12. Khan A, Ahmad A, Akhtar F, Yousuf S, et al. Induction of oxidative stress as a possible mechanism of the antifungal action of three phenylpropanoids. FEMS Yeast Res. 2011; 11:114-122. DOI: 10.1111/j.1567-1364.2010.00697.x
13. Lygre GB, Gjerdet NR, Grønningsaeter AG, Björkman L. Reporting on adverse reactions to dental materials--intraoral observations at a clinical follow-up. Community Dent Oral Epidemiol. 2003; 31:200- 206. DOI: 10.1034/j.1600-0528.2003.00037.x.
14. Marquardt W, Seiss M, Hickel R, Reichl FX. Volatile methacrylates in dental practices. J Adhes Dent. 2009; 11:101-107.

15. McLachlan JA. Functional toxicology: a new approach to detect biologically active xenobiotics. Environ Health Perspect. 1993; 101:386-387. DOI: 10.1289/ehp.93101386.
16. Mitchell DL, Synnott SA, VanDercreek JA. Tissue reaction involving an intraoral skin graft and CP titanium abutments: a clinical report. Int J Oral Maxillofac Implants. 1990; 5:79-84.
17. Müller K, Valentine-Thon E. Hypersensitivity to titanium: clinical and laboratory evidence. Neuro Endocrinol Lett. 2006; 27 Suppl 1:31-35.
18. Murray PE, García Godoy C, García Godoy F. How is the biocompatibilty of dental biomaterials evaluated? Med Oral Patol Oral Cir Bucal. 2007; 12:E258- E266.
19. Ramnarayan B, Maligi P, Smitha T, Patil U. Amalgam contact hypersensitivity lesion: an unusual presentation-report of a rare case. Ann Med Health Sci Res. 2014; 4(Suppl 3):S320-S323. DOI: 10.4103/2141-9248.141981
20. Roberts SK, McAinsh M, Cantopher H, Sandison S. Calcium dependence of eugenol tolerance and toxicity in Saccharomyces cerevisiae. PLoS One. 2014; 9:e102712. DOI: 10.1371/journal.pone.0102712
21. Rohde MM, Snyder CM, Sloop J, Solst SR, et al. The mechanism of cell death induced by silver nanoparticles is distinct from silver cations. Part Fibre Toxicol. 2021; 18:37. DOI: 10.1186/s12989-021-00430-1
22. Sadoh DR, Sharief MK, Howard RS. Occupational exposure to methyl methacrylate monomer induces generalised neuropathy in a dental technician. Br Dent J. 1999; 186:380-381. DOI: 10.1038/sj.bdj.4800117
23. Schweikl H, Altmannberger I, Hanser N, et al. The effect of triethylene glycol dimethacrylate on the cell cycle of mammalian cells. Biomaterials. 2005; 26:4111-4118. DOI:10.1016/j.biomaterials.2004.10.026
24. Scott A, Egner W, Gawkrodger DJ, Hatton PV, et al. The national survey of adverse reactions to dental materials in the UK: a preliminary study by the UK Adverse Reactions Reporting. British dental journal. 2004; 196:471-477; discussion 465. DOI:10.1038/sj.bdj.4811176
25. Shahi S, Özcan M, Maleki Dizaj S, Sharifi S, et al. A review on potential toxicity of dental material and screening their biocompatibility. Toxicol Mech Methods. 2019; 29:368-377. DOI:10.1080/15376516.2019.1566424
26. Stanislawski L, Lefeuvre M, Bourd K, Soheili-Majd E, Goldberg M. TEGDMA-induced toxicity in human fibroblasts is associated with early membrane damage, mitochondrial dysfunction and oxidative stress. J Biomed Mater Res A. 2003; 66:476–83. DOI:10.1002/jbm.a.10564
27. Syed M, Chopra R, Sachdev V. Allergic Reactions to Dental Materials-A Systematic Review. J Clin Diagn Res. 2015; 9: ZE04-ZE9. DOI: 10.7860/JCDR/2015/15640.6589
28. Wang K, Wang S, Yin J, Yang Q, Yu Y, Chen L. Long-term application of silver nanoparticles in dental restoration materials: potential toxic injury to the CNS. J Mater Sci Mater Med. 2023; 34:52. DOI: 10.1007/s10856-023-06753-z. PMID: 37855967; PMCID: PMC10587321
29. Wiertelak-Makała K, Szymczak-Pajor I, Bociong K, Śliwińska A. Considerations about Cytotoxicity of Resin-Based Composite Dental Materials: A Systematic Review. Int J Mol Sci. 2023; 25:152. DOI:10.3390/ijms25010152