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Cytotoxicity of Metals and Metal Oxides Nanoparticles in Dentistry: A Comprehensive Review

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Dental materials are essential in dentistry for restoring and maintaining oral health. These materials include polymers, ceramics, composites, metals, and metal oxide nanoparticles (NPs). Metals and metal oxide nanoparticles are particularly valued for their unique properties. The biocompatibility of these materials is critical and depends on the release of elements, which is influenced by factors such as composition, pretreatment, and handling. However, the cytotoxicity of released metals can negatively impact both oral and systemic health. This review explores the cytotoxicity of commonly used metals in dentistry, emphasizing the complex relationship between dental materials and biological systems.

Keywords: Biocompatibility; Cytotoxicity; Metals; Metal oxides; Oxidative stress; Reactive oxygen species.

Toxicity refers to the degree to which a substance can harm an organism, encompassing all potential adverse effects at the systemic, organ, or organism level. Cytotoxicity is defined as a specific aspect of toxicity referring to the ability of a substance to damage or kill cells, often measured in vitro. Cytotoxic compounds can cause cell damage and death, often resulting in necrosis or apoptosis, demonstrating their capacity to harm cellular structures and functions. Cell toxicity can lead to organ dysfunction and serious health issues[1].

Nanomaterials (NMs) under 100 nm in size are widely used in medicine, cosmetics, and the food industry. However, their small size can present toxicological risks. Understanding their biological impacts is challenging due to inconsistent responses. Relationships between NM properties, absorption, localization, and biological effects remain unclear[2].To advance the safe development of NMs in medical, cosmetic, and food applications, detailed property data is essential. In one study, the penetration, cellular

localization, and cytotoxicity of amorphous silica nanoparticles (sizes ranging from 70 nm to 1000 nm) were evaluated. Particles at 70 nm were found to be cytotoxic when exposed to mouse skin, leading to systemic exposure and in vitro mutagenicity. Further research into NM properties and biological responses is crucial for developing safer NMs, allowing researchers to assess cytotoxicity levels to ensure patient safety. Examples of cytotoxic agents include chemotherapy drugs and venomous substances[3].

During the casting process of dental alloys, excess material forms sprue buttons upon completion. These sprue buttons can either be recycled into fresh alloy for reuse during casting or discarded altogether[4]. Metallic oxides and nanoparticles play an important role in the repair[5] or replacement[6] of diseased or damaged teeth. The cytotoxicity of biomaterials is evaluated *invitro* through either direct or indirect interactions between cells and biomaterials[7].

Metallic ions enter the oral cavity and can affect surrounding mucosal tissues[8]. A variety of adverse effects may occur, ranging from hypersensitivity responses and tissue overgrowth to cytotoxic and genotoxic effects[9,10,11]. The initial observed effect is often local cytotoxicity, seen in epithelial cells and periodontal ligament fibroblasts[12,13].

The historical progression of dental materials reflects a continuous quest for improved biocompatibility and durability. This review examines the cytotoxic aspects of metals and metal oxides used in dentistry, emphasizing the critical role of biocompatibility in ensuring patient safety. Metals such as amalgam, gold, titanium, various alloys, and metal oxide nanoparticles will be scrutinized for their cytotoxic potential.

Mechanism of action of metal-induced cytotoxicity

The primary objective is to ensure that drug compounds effectively reach their intended cellular targets. Metal complexes can penetrate cells either through passive diffusion or by engaging organic and metal transporters. Considerable emphasis is placed on methodologies that examine cellular accumulation, elucidate uptake mechanisms, and monitor potential efflux processes. Understanding these processes is essential for optimizing the therapeutic efficacy of metal-based drugs[14].

Metal complexes induce apoptosis through well-established pathways, including the overproduction of reactive oxygen species (ROS), disruption of mitochondrial membrane potential, and direct interference with the DNA helix. These apoptotic pathways involve the downregulation of Bcl-2 proteins and activation of the caspase family. Apoptosis may proceed via the death receptor pathway or the mitochondrial pathway, highlighting the multiple routes through which metal complexes exert their cytotoxic effects[15]. (Figure 1)

Cytotoxic Effects Of Various Metal and Its Alloys

Rank order of cytotoxicity of metals[16]

The following sequence shows the cytotoxicity of metal ions in a descending order : Silver (Ag^{+}) > Zinc (Zn^{2+}) > Cadmium (Cd^{2+}) > Mercury (Hg^{2+}) > Gold (Au^{3+}) > Platinum (Pt^{4+}) > Cobalt (Co^{2+}) > Copper (Cu^{2+}) > Nickel (Ni^{2+}) > Palladium (Pd^{2+}) > Manganese (Mn^{2+}) > Niobium (Nb^{5+}) > Molybdenum (Mo^{5+}) > Gallium (Ga^{3+}) > Chromium (Cr^{3+}) > Indium (In^{3+}) > Tin (Sn^{2+}).

Amalgam

High-copper amalgams demonstrate cytotoxicity levels comparable to zinc-free, low-copper amalgams, indicating that elevated copper content does not increase cytotoxic potential. This equivalence in biocompatibility is crucial, given amalgam's widespread use in restorative dentistry. Additionally, alloying indium with mercury and subjecting amalgams to aging processes do not heighten cytotoxicity, even as indium enhances physical properties such as corrosion resistance and mechanical strength. These findings affirm that both high-copper and indium-alloyed amalgams are biocompatible and safe for dental applications[17,18].

Cobalt chromium alloys

Cobalt chromium (Co-Cr) alloys have been extensively used in dentistry due to their strength and corrosion resistance. Composed mainly of cobalt and chromium, along with metals such as manganese (Mn), molybdenum (Mo), and nickel (Ni),[19] these alloys have been shown to exert cytotoxic effects on human growth factors and osteoblasts, primarily through increased reactive oxygen species (ROS) production[20]. Additionally, the cytotoxicity of Co-Cr alloys is associated with type IV hypersensitivity reactions, commonly manifesting as allergic contact dermatitis[21].

A 12-month study comparing the biocompatibility of Co-Cr, Au-Pt, Ti, and Zr crowns revealed that Ti and Zr crowns were the most favorable for periodontal health and bone metabolism. Ti crowns exhibited the highest osteoprotegerin (OPG) levels and the lowest receptor activator of nuclear factor kappa B ligand (RANKL) levels, resulting in the lowest RANKL/OPG ratios. These features support bone health and periodontal stability. In contrast, Co-Cr crowns demonstrated inferior biocompatibility, highlighting their limited capacity to support periodontal health compared to Ti and Zr crowns[22,23].

The protective oxide layers on dental alloys, such as Cr, O*f* /Fe, O*f* on stainless steel, Cr, O*f* /CoO on Co-Cr alloys, and Cr, O*f* /NiO on Ni-Cr alloys, influence cytotoxicity. Among these, chromium oxides exhibit the highest cytotoxicity. For cobalt oxides, CoO is severely cytotoxic, Co*f* O,, has moderate cytotoxic effects, and Co O*f* is non-cytotoxic. These findings underscore the importance of careful material selection in biomedical applications[24,25].

Cobalt nanoparticles can be synthesized through two primary methods: (1) heating trioctylphosphine oxide, 1,2-dichlorobenzene, and oleic acid with dicobalt octacarbonyl at 180°C, yielding particles 7–8 nm in size; or (2) heating a bis(salicylaldiminato)cobalt(II)-oleylamine complex at 100°C in an argon atmosphere, followed by adding triphenylphosphine at 220°C, producing particles 25–35 nm in size. In both methods, nanoparticles are collected by precipitation with ethanol[26,27].

Nickle chromium alloys

A study on human adipose-derived stem cells showed that 3D-printed cobalt chromium (Co-Cr) alloys exhibit better cytocompatibility than nickel chromium (Ni-Cr) alloys. Cytocompatibility rankings were as follows: C1 (Co-Cr) > C3 (Co-Cr) > N2 (Ni-Cr) > N3 (Ni-Cr) > C2 (Co-Cr) > N1 (Ni-Cr). These findings suggest that Co-Cr alloys are more suitable for applications requiring enhanced biological responses[28].

Further research revealed that recasting nickel-containing alloys with an additional 65% of metal significantly increased their cytotoxic activity. Various Ni-Cr (N1, N2, N3) and Co-Cr (C1, C2, C3) alloys were evaluated, with Co-Cr alloys demonstrating superior cell adhesion compared to Ni-Cr alloys[29]. Higher Co-Cr concentrations correlated with improved biocompatibility, while Ni-Cr alloys showed comparatively lower cytocompatibility, suggesting that Co-Cr alloys are more favorable for applications requiring strong cellular interactions and reduced cytotoxicity[30].

Another study assessing the cytotoxicity of Ni-Cr and Co-Cr alloys over seven days found both alloys to be non-cytotoxic. Cells exposed to alloy extracts showed robust growth and high confluence, indicating no adverse effects on viability or proliferation. This supports the suitability of both alloys for medical and dental applications involving prolonged cellular exposure[31,32].

Research on nickel- and titanium-induced cytotoxicity revealed that exposure to nickel concentrations of 75.5 ìg/L and titanium concentrations of 44.9 ìg/L caused significant damage to gastrointestinal cells, primarily due to oxidative stress[33]. Nickel oxide nanoparticles (NiO-NPs) at concentrations of 15–120 ìg/mL were also shown to induce oxidative stress, leading to cellular damage and potential DNA disruption. These findings highlight the need to understand the impact of metal ions from dental alloys on oxidative stress and cellular health[34-38].

Cobalt chromium molybdenum alloys

The favorable cytocompatibility of Co-Cr alloys was demonstrated in a literature assessing the cytotoxicity of direct metal laser-sintered (DMLS) and cast Co-Cr-Mo dental alloys on human MRC-5 fibroblast cells. The study found no cytotoxic effects for either DMLS or conventionally cast Co-Cr-Mo alloys, supporting their suitability for dental applications[39].

Another study evaluated the genotoxic effects of Co-Cr-Mo and Ni-Cr alloys in dental prosthetics and implants. The findings revealed that metal ions released from these alloys could induce significant DNA damage in oral mucosa cells, including DNA strand breaks and other markers of genotoxicity. This highlights the potential risks of prolonged exposure to these materials and underscores the importance of biocompatibility considerations in dental applications[40].

Silver and silver oxide nanoparticles

Silver nanoparticles (AgNPs) can be synthesized through various techniques, including physical, chemical, and biological methods, each

with its own set of advantages and challenges[41]. Among these, biological synthesis has gained considerable interest due to its eco-friendly nature. Studies have demonstrated that AgNPs are non-toxic to various cell types, such as mouse fibroblasts, normal human dermal fibroblasts (NHDFs), and human corneal epithelial cells (HCECs), indicating their potential for safe use in biomedical applications[42].

The biomolecules in plant extracts play a crucial role in reducing silver ions to AgNPs and preventing aggregation. The quality and composition of the extract significantly influence the efficiency and properties of the synthesized AgNPs, underscoring the importance of selecting high-quality extracts for optimal biosynthesis[43].

Standardizing bioassays is essential for generating reliable and reproducible data, which enables a thorough evaluation of the mechanisms underlying AgNP cytotoxicity[44]. AgNPs have shown notable cytotoxic effects in A549 lung cancer cells. In freshwater environments, AgNPs oxidize to form toxic Ag+ ions, with a substantial portion becoming immobilized as sparingly soluble salts, such as AgCl or Ag, S[45,46].

Within cells, AgNPs can generate reactive oxygen species (ROS), leading to oxidative stress that can damage cellular components and potentially cause inflammation, apoptosis, or necrosis. These risks highlight the need for stringent safety guidelines to mitigate health hazards associated with AgNP exposure[47-54].

Furthermore, AgNPs possess the potential to cross the blood-brain barrier (BBB) due to their small size and unique chemical properties. Once in the bloodstream, AgNPs can reach the central nervous system, where they may induce neurotoxic effects, resulting in neuronal damage and cell death. This ability to penetrate the BBB and its subsequent impact on neuronal cells emphasize the necessity for comprehensive safety assessments in their medical applications such as anti-cancer therapy.A recent study aimed to develop a novel water-soluble system by conjugating quercetin (QtN) with hyaluronic acid (HA)-coated silver nanoparticles (AgNPs). This innovative approach sought to enhance the anticancer efficacy of quercetin by improving its solubility and bioavailability while ensuring targeted delivery to tumor cells. The incorporation of HA facilitated selective targeting of cancer cells, exploiting its affinity for cell surface receptors, thus optimizing the therapeutic potential of quercetin in oncology[55-57].

Zinc oxide and zinc oxide nanoparticles

Recent research has compared the mechanical properties and cytocompatibility of zirconia incorporated zinc oxide eugenol (ZZrOE) with traditional ZOE[58-62].The study found that ZZrOE exhibited enhanced therapeutic effects on inflamed human dental pulp stem cells, suggesting it could be a promising alternative to traditional ZOE for dental restorative applications[63-66].

Zinc oxide nanoparticles (ZnO NPs) have demonstrated significant photocatalytic activity, accompanied by an approximately 1.5 fold increase in cytotoxic effects on T cell lymphoma cells. This increased cytotoxicity can be explained by the "Trojan Horse effect," where the acidic lysosomal environment degrades nanoparticles, converting core metals into ions and releasing toxic substances that disrupt cellular reproduction[67].

ZnO NPs are extensively used in various dental fields, including conservative dentistry, endodontics, regenerative endodontic therapy, prosthetic dentistry, orthodontics, preventive dentistry, implantology, and periodontology. While ZnO NPs are generally considered biologically safe with no evident cell toxicity, it is crucial to explore further the regulatory and safety considerations related to their prolonged use in oral care products.A recent study used liquid chromatography-mass spectrometry (LC-MS)-based metabolomics to assess the nanotoxicity of metal oxide nanoparticles (MOx NPs) in human bronchial epithelial cells. High-dose ZnO NPs caused significant cytotoxicity and metabolic disruptions, while low-dose ZnO NPs induced milder changes[68-70].

Tin and tin oxide nanoparticles

Conversely, other metal oxides, such as tin(II) oxide (SnO), tin(IV) oxide (SnO,), and mercury(II) oxide (HgO), have demonstrated non-cytotoxic properties, as they do not significantly affect cell viability. This suggests that these oxides may pose a lower risk of cellular damage, making them potentially safer alternatives for use in dental amalgams. The findings highlight the importance of careful material selection and evaluation to ensure that dental restorations are both safe and effective[71,72] .

Titanium and titanium oxide nanoparticles

Titanium alloys are gaining preference over cobalt-chromium (Co-Cr) alloys in dental implantology due to their superior properties. However, concerns persist regarding the cytotoxicity of metal powders and bulk metals like titanium, niobium, molybdenum, and silicon, which can impair cellular health. Bulk silicon and molybdenum, in particular, exhibit notable cytotoxic effects, raising concerns in biomedical engineering applications such as implants and prosthetics[73].

To mitigate cytotoxic risks, specific ion concentration thresholds have been established for these metals. For example, the safe concentration limit for molybdenum is set at 8.5 micrograms per liter, for titanium at 15.5 micrograms per liter, for niobium at 172.0 micrograms per liter, and for silicon at 37,000.0 micrograms per liter. Adhering to these limits is essential for ensuring the safe application of these metals in both biomedical contexts and in occupational or environmental settings[74].

Titanium dioxide (TiO,) nanoparticles, renowned for their antibacterial and self-cleaning properties, have been extensively studied. Various literatures on normal human fibroblasts exposed to Ti and Ti-6Al-4V alloy samples, however, revealed a decrease in cell viability, highlighting potential cytotoxic effects[75-80]. when evaluating the safety of TiO, nanoparticles, particularly in situations involving inhalation or direct lung exposure. The anatase phase and reduced particle size enhance surface absorption, thereby amplifying the cytotoxic effects. Despite their excellent mechanical properties, this limitation restricts the use of TiO, nanoparticles in restorative formulations[81-83].

Copper oxide and copper oxide nanoparticles

Copper nanoparticles (CuNPs) have garnered significant attention in dentistry for their ability to enhance the physical and chemical properties of dental materials. Incorporating CuNPs into dental amalgams improves mechanical strength and antimicrobial efficacy, increasing durability and resistance to bacterial colonization. In restorative cements, CuNPs enhance mechanical properties and biocompatibility, ensuring longer-lasting restorations. Similarly, dental adhesives and resins infused with CuNPs exhibit superior bonding strength and reduced polymerization shrinkage, resulting in more reliable and stable restorations. CuNPs also find applications in endodontics and orthodontics. In endodontic therapy, they are integrated into irrigation solutions and obturation materials, significantly boosting antimicrobial

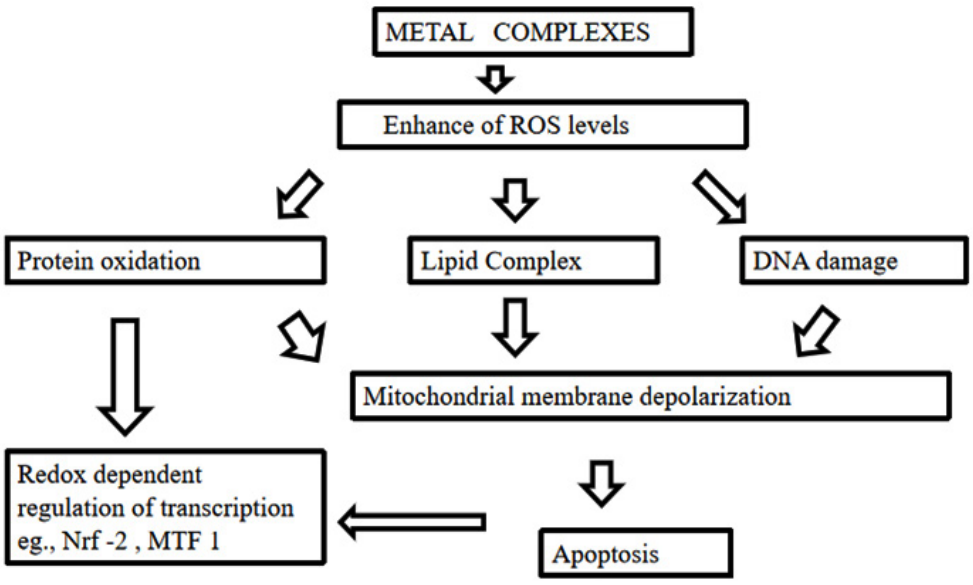

Fig. 1. Mechanism of action of cytoxicity of metals[15]

efficacy and improving root canal treatment success rates. Dental implants coated with CuNPs demonstrate enhanced osseointegration and reduced risk of peri-implantitis, ensuring better long-term outcomes. Orthodontic arch wires and brackets embedded with CuNPs offer superior mechanical properties and antimicrobial effects, minimizing infection risks and optimizing treatment efficiency. These advancements not only improve material performance but also contribute to better patient outcomes[84,85].

However, CuNPs can enter the body via inhalation, ingestion, skin absorption, or through the bloodstream[86,87]. Once in circulation, they can accumulate in various tissues and induce cytotoxic effects in human cell lines, including lung epithelial cells (A549), cardiac microvascular endothelial cells, kidney cells, and neuronal cells. CuO nanoparticles, in particular, trigger oxidative stress, inflammation, and cell death, disrupting cellular function and posing significant health risks. These findings emphasize the need for stringent regulatory oversight and careful evaluation of CuNPs in medical and consumer products due to their potential toxicity[88-90].

Zirconium oxide nanoparticles

Bioactive glass and glass ceramics have seen considerable advancement as biomaterials, with intensive research aimed at enhancing their mechanical properties through various additives. Among these, ZrO, -containing variants have shown particularly promising outcomes. Three novel compositions of bioactive glass and glass ceramics were synthesized via a melt-quenching technique, featuring the formulation 37.5 nano-SiO, –(17-X)Al, O*f* –26.5CaO–11.5CaF, –7.5P, O… –X nano-ZrO, , where X = 0.75, 1.7, and 2.7 mol%. Standard characterization methods assessed their physical, chemical, structural, and surface properties, revealing that higher nano-ZrO, content (2.7 mol%) yielded primary crystalline phases such as Fluorapatite (Ca… (PO,,)*f* F), Anorthite (Ca(Al, Si, Oˆ)), and tetragonal Zirconia (t-ZrO,)[91].

The inclusion of nano-zirconia significantly enhanced the thermal stability and microhardness of the glass ceramics. The bioactive potential of these materials was confirmed by the formation of nanometer-sized hydroxyapatite (HAp) on the glass-ceramic surfaces. Importantly, cytotoxicity evaluations demonstrated that the samples were non-toxic to living cells[92].

Aluminium oxide nanoparticles

Aluminum oxide nanoparticles (Al, O*f* NPs) are highly regarded in scientific and industrial applications due to their versatile biological and physicochemical properties. These nanoparticles can be synthesized through various methods, allowing precise control over key characteristics like particle size, shape, and surface chemistry, which are critical in optimizing their performance. Al, O*f* NPs are used in diverse fields, including catalysis, electronics, and biomedicine, where their unique properties and adaptable characteristics hold significant promise for further advancements.

In dental and medical contexts, exposure to nanoparticles like Al, O*f* and silicon dioxide (SiO,) has been shown to cause DNA damage and nuclear alterations, as observed in immunostaining genotoxicity assays. The study highlights a strong correlation between the cytotoxic and genotoxic effects of these nanoparticles. Notably, Al, O*f* and SiO, NPs often form large aggregates within cellular vesicles with limited penetration into the nucleus or cytoplasm. This morphology suggests that the low pH environment within vesicles likely promotes ionization of Al, O*f* or SiO, , contributing to cellular disruption and raising concerns about their biocompatibility in dental and medical applications[93,94]. Metal oxide nanoparticles can improve oral health, reduce healthcare costs, enhance antibacterial efficacy, prolong dental treatments, and significantly lower dental disease prevalence[95] A recent study reported that the incorporation of fluorohydroxyapatite into MTA Angelus effectively reduced its setting time while preserving an alkaline pH. Notably, cell viability remained unaffected at 1 and 7 days post-application, except in its freshly mixed state[96].

CONCLUSION

The review underscores the critical importance of advancing biocompatibility in dental materials, particularly through a nuanced understanding of metal toxicity in relation to their chemical states and compositions. Accurate assessment of biocompatibility necessitates not only analyzing the elemental components of

alloys but also their specific chemical forms and interactions within biological systems. Future research into surface properties and structural dynamics will be pivotal in designing safer and more efficacious materials for dental applications. By integrating these insights, the field can adopt a meticulous and patient-centered approach, fostering sustainable innovation in oral healthcare.

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Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials

Authors' Contribution

Ramachandran Tamilselvi – Conceptualization, writing, review and editing; Sivakumar Nandhini – Data collection and writing; Elumalai Muniyandi – Visualization and supervision; Prathibha Saravanakumar – Technical support, collection and assembly of data; Alagarsamy Venkatesh – Critical revision of article for important intellectual content; Venkatachalam Prakash – Final Drafting and approval.

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