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Antimicrobial Activity of Metal Oxide Nanoparticles

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A broad range of gram positive and gram negative bacteria have been demonstrated to be effectively suppressed in growth by Metal Oxide Nanoparticles (MONPs), suggesting that these particles may be useful in the fight against antibiotic resistance. The antibacterial characteristics of MONPs, including those of silver, zinc, titanium, zirconia, iron, copper, magnesium, and cobalt oxide are widely recognized. The use of these nanoparticles made of synthetic and natural materials in dentistry is developing quickly, has been included in a variety of dental materials and has assisted in the treatment of oral disorders as well as the removal of biofilms and smear layers. The reader will gain up-to-date knowledge on MONPs, their modes of action, and their significance in endodontics in this review.

Keywords: Antibacterial, Drug delivery, Endodontic, Metal oxides, Multidrug resistant, Nanoparticles.

Human body biology is associated with a variety of microbial symbionts and their genomes. The microbial population rapidly colonizes both the inside and outside of our bodies, developing an organ that is essential to our physiology and general health. There are about 1000 different types of bacteria in the mouth, making the populations incredibly complicated. They are second in complexity in the body, after the colon, according to estimates[1]. One of the body's most varied micro biomes is in the human mouth[2]. Viruses, protozoa, archaea, fungus, and bacteria make up the oral microbiome[3].

With an elevated risk of infection during various operations, the oral cavity is the home to a multitude of bacteria which is significant in the dental profession. Normal microflora typically comprises of a modest number of mutans microorganisms (most notably *Streptococcus mutans* and *Streptococcus sobrinus*) and non-mutans streptococci (such as *Streptococcus salivarius, Streptococcus sanguis*, etc.). Any disruption to the microbial ecosystem could make it easier for more pathogenic microbes to enter the environment, including *Escherichia coli, S. aureus, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis,* and others[4].

A significant worldwide problem is the rise in bacterial resistance to one or more antibiotics[5]. Newly, Nanomaterials have emerged as a weapon against bacteria resistant to many drugs. These nanoparticles can be employed as nanomedicines to combat resistant bacteria by working alone or in concert with other antibacterial substances. In order to improve physical and chemical characteristics and therapeutic efficacy, nanomaterials are often employed as drug delivery vehicles. A highly researched class of nanomaterials against bacteria resistant to many drugs are metal and MONPs. Metals such as gold, silver, titanium, copper, zinc and aluminum as well as metal oxides including silver, copper, magnesium, calcium and zinc oxide can be used to create these nanoparticles[6].

Materials with unique features at the nano scale, or between 1 and 100 nanometers, gave rise to the field of nanotechnology, which was first described by Norio Taniguchi in 1974 and began to take shape in the 1980s. The incorporation of nanoscale features into material components including those intended for dental use is made possible by nanotechnology[7]. Innovative methods in dentistry make use of nanoparticles that have therapeutic properties on their own or employ nanotechnology to improve the results of current treatments[8]. The primary advantages of nanomaterials in dentistry are their efficient and broad antibacterial properties, which come with a low cost of production for the nanoparticles and a minimal chance of bacterial resistance developing[9]. The current article aims to provide an overview of the antibacterial properties of metal oxide-containing nanoparticles used in dentistry.

Microbiology of Root canal Infections

Aerobes and facultative anaerobes initially predominate in the bacterial ecology of the root canal[10,11] The ecology of the root canal system changes as the condition worsens. These alterations may be connected to the oxygen tension during root canal openings for therapeutic purposes, the application of root canal irrigants, and pH variations in the root canal as a result of different materials being inserted. Due to genetic population shifts, this leads to phenotypic changes[12,13,14]. An endodontic infection may be primary or secondary. Apical periodontitis, or inflammation of the supporting tissues, is the ultimate result of microbial byproducts or microbial invasion, which typically causes pulp inflammation and root canal infection as its primary infection. Infections that return in teeth after root canal therapy can be classified as secondary infections, post-treatment infections, emergent or acquired reinfections, or persistent residual infections[15]. Polymicrobial infections cause primary endodontic infections[14,16]. The most common species among them include *Eubacterium*, *Camphylobacter*, *Fusobacterium*, *Treponema*, *Prophyromonas, Prevotella,* and *Bacteroides.*

Treatment failure is thought to be mostly caused by bacteria that continue to exist in the root canal system after treatment[17,18,19]. After root canal therapy, the diversity and number of bacterial species in primary infections can change, as well as the proportions of different bacteria. Microbial flora in secondary infections often adapt to harsh conditions such as broad pH ranges and nutrient-limited environments. The microbial phenotypes of primary infections and subsequent infections differ significantly, with gram-positive bacteria predominating in the latter[18,19,20]. Research has indicated that specific species, including *Enterococci, Streptococci, Lactobacilli, Actinomyces*, and fungus (like *Candida*), are more common in teeth that have had post-treatment infections. Specifically, it was found that a significant percentage of *Enterococcus faecalis* was present in patients with persistent apical periodontitis[21,22].

Mechanism of Action of Metal Oxide (MO) Nanoparticles

Breakdown of the cell membrane caused by electrostatic interaction

The negatively charged surfaces of bacteria interact with positively charged nanoparticles through the law of attraction between negative and positive charges, causing NPs to accumulate on the surface of bacterial cells. Because of the efficient bonding between these positively charged NPs and the cell membrane, the structure of the cell wall is disrupted, which makes the cell more permeable and makes it easier for NPs to enter the bacteria and cause cellular content escape. Those nanoparticles impact DNA replication, division, and respiration by attaching to mesosomes[23,24].

Generation of Reactive Oxygen Species (ROS)

When nanoparticles penetrate a microorganism's cell membrane, they release ROS, which puts the cell under stress due to oxidation and initiates the bacterium's attack. The attack results in reduced respiration and ATP synthesis, which damages the cell membrane. Active redox reactions and the pro-oxidant functional group on the metal oxide nanoparticles interface allow a metal oxide to produce ROS[25].

Enzyme and protein malfunction

By initiating the oxidative process of the amino acid chain, NPs promote the creation of carbonyls, which are naturally protein bound and result in protein breakdown, the deactivation of numerous enzymes, and disruption of catalytic activity[26,27].

Genotoxicity and Inhibition of Signal Transduction

Through their electrical properties, nanoparticles interact with amino acid molecules, impairing signal transduction and negatively affecting chromosomal and plasmid transcription processes[28,29,30].

Effective Physiochemical Properties of MONPs on Antimicrobial Activity

Chemical composition of MONPs

The antibacterial ability of metal oxides is impacted by the kind of metal ion particles and the composition of their molecules. Metal ions including Ca2+, Mg2+, Cu2+, Zn2+, Co2+, Fe2+, and Ni2+ are required for various metabolic processes in the majority of surviving bacterial strains, but at larger quantities, they may be toxic. Ag+ and Hg+, two unnecessary metal ions, demonstrated a significantly higher antibacterial action at remarkably low quantities[31]. The ability of metal ions to bind selectively to ligand atoms found in biomolecules and cellular constituents may contribute to their antibacterial action. Hard soft acid base theory is the foundation for the interaction between ligand atoms and metal ions (HSAB principle). According to the HSAB principle, hard acids join with hard bases, while soft acids join with soft bases[32]. An excellent affinity of divalent copper for biological molecules is shown by the Irving Williams series of ligand affinity for the essential first-row transition divalent metal ions. This shows that at higher concentrations, divalent copper may be most dangerous[33].

MONPs Size and Surface Properties

Particle size has a major impact on how MONPs interact with bacterial cells and other biological systems. Because of their increased surface to volume ratios and noticeably higher particle numbers per mass, nanoparticles demonstrated stronger antibacterial activity than microscaled (bulk) particles. Numerous reports have documented the size-dependent interaction between bacteria and MO nanostructures. Under normal lighting conditions, the Zinc Oxide Nanoparticles (ZnONPs) considerably inhibit gram-positive as well as gram-negative bacteria in comparison to the bulk particles[34,35,36]. The zeta potential of MONPs describes their surface charge characteristics. The Point of Zero Charge (PZC) is the pH at which the surface charge is neutral. MONPs can exhibit positive, negative, or neutral charges. NPs with the highest positive charge show the greatest antibacterial activity, followed by those with neutral, then negative charges. When the pH is below the PZC, the oxide surface is positively charged; when above, it is negatively charged.

Concentration of MONPs

With increasing concentration in the media, MONPs exhibit increased antibacterial activity[37]. Greater MONP concentrations may be associated with a greater surface area, this eventually promotes stronger contact with bacterial cells and increased antibacterial activity. The Minimum Inhibitory Concentration (MIC) is the most often used metric in microbiology to quantify the in vitro antibacterial activity assessment of MONP[38]. The MIC of an antimicrobial drug is the concentration at which, following overnight incubation, bacteria cannot grow visibly[39].

The shape dependent antibacterial characteristics of MONPs

Each form of nanoparticle has unique physicochemical properties, such as surface characteristics, solubility, and the potential to produce ROS in certain metal oxides, which influence their antibacterial activity. Studies show that different forms of MO-NPs exhibit varying antibacterial properties. For instance, ZnO nanopyramids have significantly greater antibacterial activity against Methicillin-Resistant Staphylococcus aureus (MRSA) compared to nanoplates and nanospheres[40]. Another study found that spherical ZnO NPs possess higher antibacterial power than rod-shaped ZnO NPs[34].

Various MONPs in Endodontics

Iron Oxide (IO NP)

The biological and medical fields find application for iron compound (FeO_2) nanoparticles. Iron oxide, a sustainable and biocompatible material that can be made on a big scale at a low cost by simple chemical synthesis techniques, is now included in FDA-approved formulations for chronic treatment.

Despite the fact the NaOCl is considered the "gold standard" for endodontic irrigants[41], it have been observed that 40% to 60% of root canals still contain live bacteria even after irrigation[42]. Similar antibacterial activity to NaOCl has also been demonstrated for chlorhexidine[43]. But only the surface layers of the dentin exhibit antibacterial activity and both irrigants have shown decreased efficiency in disinfecting dentinal tubules[44]. IO NP/H_2O_2 had strong antibacterial activity that was notably superior to chlorhexidine and sodium hypochloride in the treatment of dentinal tubule infections caused by *E. faecalis*, especially in the central and peripheral zones. Because of IO NP's innate "peroxidase-like activity," which activates H_2O_2, a high degree of disinfection can be made possible, which catalyses the production of free radicals on the spot, which quickly destroys bacteria[45].

Silver oxide

The most extensively studied antibacterial for endodontic infections is silver nanoparticles. While retaining the physicochemical characteristics of the produced sealers, it was discovered that adding 10 weight percent Ag with SiO_2 decreased the viability of *E. faecalis* during both immediate and longitudinal study[46]. It was discovered to help regulate the growth of bacteria in the intracanal environment and to be just as effective against *E. faecalis* and *Staphylococcus aureus* as 5.25% NaOCl[47]. Nevertheless, extended exposure to bacteria is necessary for their successful eradication, which has been described as a good substitute for an intracanal medication but not as an irrigant[48].

Copper oxide

Copper is a cheap, readily available metal that can be affordably manufactured into nanoparticles. Either copper metal ions or oxidized cupric ions produced from copper nanoparticles (sizes ranging from 1 to 100 nm) have antimicrobial action. It is simple to mix and link copper nanoparticles with polymers, ceramics and other metals. In certain combinations, they exhibit physiochemical stability as well[49]. Copper is a common metal in dental and medical research because of its low toxicity and antibacterial properties[50]. According to reports, Copper Oxide Nanoparticles (CuO NPs) exhibit antibacterial properties and prevent the formation of biofilms[51]. Copper nanoparticles' high surface area to volume ratio amplifies their antibacterial activity[52]. Furthermore, dose dependence is shown in the antibacterial activity of copper oxide nanoparticles[53]. The antibacterial properties of these nanoparticles have been well studied, yet it is unclear how precisely copper nanoparticles work against microorganisms[54,55,56]. These nanoparticles are effective against both gram positive and gram negative bacteria because they have the ability to enter bacterial cell membranes and damage the organism's vital enzymes. They possess some antifungal properties as well[57].

In caries prevention, copper nanoparticles have the ability to inhibit *S. mutans* from growing and colonizing on the surface of tooth roots, hence preventing root caries[58]. Composites with distinct physio chemical properties can be created by simply incorporating copper oxide nanoparticles into polymers. Copper oxide nanoparticles can be used to dental adhesive to prevent early or carious white spot lesions because they are antibacterial without compromising shear bond strength[59,60]. In soft denture liners CuO NPs were added, and this resulted in a considerable reduction in oral pathogen colonization and plaque development, particularly *C. albicans* accumulation[61].

Zirconium oxide

Because of its metallic and optical characteristics that are comparable to those of teeth, zirconia has found extensive application in dentistry. Zirconium oxide (ZrO_2) has been known as a high-performance ceramic material due to its superior properties, high strength, resistance to corrosion, and toughness. Because of its insolubility in water, it has been demonstrated to eliminate bacterial colonization with little cytotoxic effects[62,63]. Because zirconia-based NPs are so effective against some infections like *E. faecalis*, they are commonly used as a type of antimicrobial in endodontics[64]. The most used agent for pulp capping, whether direct or indirect, and root end filing is mineral trioxide aggregate (MTA). Portland cement is the main ingredient in MTA. Zirconia nanoparticles (NPs) can be added to Portland cement as an effective radio opacifier without having a detrimental effect on the cement's biocompatibility. According to ISO/ADA guidelines, both groups of micro- and nano-sized zirconia oxide particles showed improved radiopacity capabilities when examined[63]. In caries-affected dentine, an investigation on aluminum zirconate nanoparticles in etch and rinse adhesive revealed that 10 weight percent of the nanoparticles in the adhesive had the lowest survival rate of *S. mutans*[65].

Titanium dioxide

Very stable particles with photocatalytic properties are titanium dioxide nanoparticles. Because ROS are produced, it results in oxidative stress. Because of its lipid peroxidation property, the cell membrane is disrupted and there is increased membrane fluidity even for the types of fungus that are resistant to fluconazole, it is also a potent antifungal[5,66,67]. Because of its excellent aesthetics and simplicity of use, Dental resin composite is a useful restoration technique for teeth that have decayed[68,69,70], its perfect finishing and enhanced resistance to wear make it an excellent choice. However, it should be mentioned that in the case of a long-term dental resin repair, the bacteria will persist in growing and forming oral biofilms on the surface of the resin, particularly in the gap that are polymerization shrinkage produced. These biofilms will then continuously deteriorate the surrounding dental tissue and the current resin composite, leading to secondary caries and treatment failure[71,72]. Photo catalytic activity of synthesized anatase-phase Strontium Nitrogen Titanium dioxide (Sr, N, TiO_2) was increased by mixing with Nano Hydroxyapatite (nHA) fillers as reinforcing fillers to create a novel multifunctional Direct Resin Composite. It possesses antimicrobial and mineralizing properties because Sr, N, TiO_2 and nHA combine. The mutans strain of *Streptococcus* (*S. mutans*) was the target of the maximum antibacterial rate of 98.96%, which helped the resin composite survive longer in the clinical environment. Consequently, they conclude that the Direct resin composite in conjunction with nHA & Sr, N, TiO_2 fillers will probably be the most effective filler for filling dental cavities[73].

Zinc oxide

Although most living things require zinc as a trace metal for numerous metabolic functions, excessive amounts of zinc can be toxic. Because ZnO NPs are less harmful to humans than CuO NPs and AgO NPs and can be synthesized at a cheap cost, they are frequently utilized in cosmetic items, medicine and wound healing to treat fungal infections and acne. They also display a broad spectrum of antibacterial activity[74].It has been discovered that ZnO NPs possess antibacterial qualities. Modest amounts of ZnO NPs had no effect on the mechanical properties of dental resin composites, but they did prevent *S. mutans* from growing and adhering to the material[75]. When ZnO NPs were added into Glass Ionomer Cement, it considerably increase antibacterial activities against *S. mutans* without affecting mechanical properties[76]. According to the study, ZnO NP doped with magnesium and silver has a stronger antibacterial effect than ZnO NP against *S. mutans*. Synthesized NP exhibits antibacterial activity against bacteria when its cell walls are compromised. These results in the distortion of constructional proteins, inactivation of enzymes, disruption of electron transport chains, deformation of nucleic acids, and facilitation of oxidative stress caused by reactive oxygen species. According to a number of studies, adding ZnO NPs to dental adhesive systems greatly enhanced their antimicrobial capabilities without having a negative impact on the bond strength[77,78,79].

One method that is most frequently utilized to treat endodontic infections is endodontic treatment[80,81]. Complete eradication of dental infections is not possible because to the polymicrobial character of endodontic infections, which involve a variety of bacteria and germs such as *E. faecalis, S. mutans* and *S. anginosus, F. nucleatum*, and *S. aureus*[82,83]. An excellent filling material for root canals should not shrink, fill the channels easily, stick to the walls easily, or harm the periapical tissue or permanent tooth germ. Additionally, two important considerations while selecting the best among them are their antibacterial and biocompatible qualities[84,85]. The micro leakage and antibacterial properties of zinc oxide eugenol (ZOE), epoxy resin sealer (AH26), silver ZnO nano powders, and ZnO nanopowders were investigated. Shayani Rad reports that the nano ZnO sealer demonstrated better antibacterial properties against *E. faecalis*, *E. coli*, *C. albicans*, *S. mutans*, and *S. aureus* than two widely used endodontic sealers, AH26 (resin-based) and Pulpdent (ZnO based)[86,87]. These nanoparticles enhance alkalinization and antibacterial activity against Escherichia faecalis when coupled with calcium hydroxide nanoparticles and chlorhexidine as an intracanal medication. After being coated with ZnO NPs and pre-treated with argon plasma, flawless gutta percha cones displayed antibacterial action against S. aureus and *E. faecalis*. As a result, there is less chance of reinfection and endodontic failure and an effective hermetic seal is created[88]. Calcium silicate cement containing 1 wt% ZnO NPs can decrease pro-inflammatory cytokines and increase antibacterial activity without altering its physical characteristics[89].

Magnesium oxide

The caries process includes demineralization of hydroxyapatite due to acid attack[90,91]. Alkaline nanoparticles could therefore be a substitute for preventing dental cavities. About 0.5% of enamel and 1% of dentine are made of the alkaline metal magnesium[92,93]. According to a study, having enough serum magnesium levels can slow down the onset and development of tooth caries by releasing magnesium ions[94]. Glass ionomer cement treated with magnesium oxide nanoparticles exhibited strong antibacterial and biofilm activity against cariogenic bacteria[95]. Limited studies reported the use of magnesium nanoparticles to prevent dental cavities. Tooth decay and magnesium exhibit both substantial[94] and nonsignificant relationships[96]. According to the study, ZnO NP doped with magnesium and silver has a stronger antibacterial effect than ZnO NP against *S. mutans*. Synthesized NP exhibits antibacterial activity against bacteria when its cell walls are compromised. This results in the distortion of constructional proteins, inactivation of enzymes, disruption of electron transport chains, deformation of nucleic acids, and facilitation of oxidative stress caused by reactive oxygen species[97,98].

Cobalt oxide

One of the transition metal oxides, cobalt oxide (CO), is a black powder having magnetic and antibacterial properties[99]. They were added to Pit and Fissure Sealant (PFS) in order to address important clinical issues related with PFS, including microleakage and secondary caries. An investigation on antibacterial potential of Minocycline (MNC) with CO, a pH-dependent cobalt oxide nanoparticle integrated with MNC, by characterizing and testing it against *Streptococcus sobrinus*. They discovered that 2.5% MNC with CO doped PFS demonstrated strong anti-biofilm capabilities without sacrificing mechanical characteristics[100].

CONCLUSION

Due to their excellent mechanical, chemical, biological, and physical properties, nanomaterials (NMs) have recently become more and more prominent in technological advancements. These qualities have allowed performance to rise above that of its conventional counterparts. Nanomaterials can be used to fight endodontic and caries-related bacteria, lessen the formation of biofilms, and prevent the demineralization of the tooth structure. These results have been positive enough to open the door for more clinical studies that will confirm the usefulness of nanotechnology-based materials for therapeutic purposes. Thus, by promoting better oral health and lowering healthcare costs, the use of metal oxide nanoparticles in dentistry can ultimately benefit both individual patients and society at large. It can also increase the antibacterial efficacy, lengthen the duration of dental treatments and significantly reduce the incidence of dental diseases.

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Conflict of interest

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