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Dr. Alex Immanuel Y
Post Graduate Student, Department of Conservative Dentistry and Endodontics, Goa Dental College and Hospital, Goa, India

Dr. Ida de Noronha de Ataide
Professor and HOD, Department of Conservative Dentistry and Endodontics, Goa Dental College and Hospital, Goa, India

Dr. Rajan Lambor
Professor, Department of Conservative Dentistry and Endodontics, Goa Dental College and Hospital, Goa, India

Corresponding Author:
Dr. Alex Immanuel Y
Post Graduate Student, Department of Conservative Dentistry and Endodontics, Goa Dental College and Hospital, Goa, India

Antibacterial nanoparticles in endodontics and restorative dentistry

Dr. Alex Immanuel Y, Dr. Ida de Noronha de Ataide and Dr. Rajan Lambor

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Abstract
Nano dentistry primarily focuses on the utilization of Nano biomaterials in the treatment of oral conditions, aiming to enhance overall oral health. The application of nanoparticles in various aspects of oral healthcare, such as managing dentin hypersensitivity, eliminating bacterial biofilm, diagnosing and treating oral malignancies, and bone replacement, is expanding. Nanoparticles possess unique physical and chemical properties, allowing their use in irrigation solutions, intracanal medications, sealants, and restorative materials to enhance antimicrobial efficacy. Nanoparticles exhibit multiple effects on bacteria, as demonstrated by numerous *in vitro* studies on their bactericidal properties. The complex anatomy of a root canal makes it challenging to eliminate pathogens and clean the canal effectively. Nanoparticles with inherent antibacterial properties overcome the resistance developed by various pathogens against traditional medications and irrigants used in root canal treatments. This article aims to review existing literature on the purposes, and benefits of nanoparticles in restorative dentistry and Endodontics.

Keywords: Nanoparticles, antibacterial, restorative dentistry, endodontics

Introduction
Current root canal therapy encounters a significant challenge in eliminating bacterial biofilms residing within complex anatomical structures. This limitation calls for alternative approaches to address dental infections, and nanoparticles have enhanced physicochemical properties, providing possibilities for treatment [1].
The field of Nano dentistry encompasses the science and technology of utilizing nanostructured materials for diagnosing, treating, preventing, relieving pain, preserving, and enhancing dental health. Nano dentistry finds application in various areas, including dental material production, prevention of oral diseases like dental caries and periodontal disease, therapeutic agents for dentin hypersensitivity, oral cancer, endodontic diseases, tissue engineering, and disease diagnosis, such as oral cancer detection. Currently, the use of nanomaterials in endodontics is limited to a few studies that evaluate their antimicrobial properties against endodontic pathogens in different forms [2, 3].
The history of nanoparticles traces back to Dr. Richard Feynman, who introduced the concept of nanotechnology in 1959. Dr. Sumio Lijima further expanded the field with the discovery of nanotubes in 1991. In the year 2000, Dr. Freitas Jr. coined the term "nano-dentistry" and pioneered the development of nanomaterials and Nanorobots that contribute to dental regeneration. Notably, he introduced "denti-frobots," which are robots residing in dentrifices. These concepts were once considered science fiction but are now gaining recognition among clinicians [4, 5].

Types of antibacterial nanoparticles
Nano dentistry refers to the use of nanomaterials for treatment with the aim of enhancing good outcomes and prognoses. The development of nanomaterials in the field of endodontics focuses on enhancing their biological and physicochemical properties and tissue regeneration.

Nanoparticles are available based on their composition
Inorganic: Zinc oxide, Bioactive glass, Aluminium oxide, Iron oxide Metallic: Silver, Gold, Selenium, Copper, Magnesium. Polymeric: Alginate, Chitosan.

Mechanism of action

Electrostatic interaction leading to disruption of the cell membrane: The interaction between positively and negatively charged surfaces of microorganisms results in the adhesion and penetration of nanoparticles on the microbial cell membrane. This bonding to the cell membrane leads to the rupture of the cell membrane and an increase in cell permeability. This increased permeability allows more nanoparticles to penetrate the microorganisms, leading to the release of cellular content. Additionally, the binding of nanoparticles to mesosomes affects important cellular functions such as respiration, cell division, and DNA replication [6, 7].

Metal ion homeostasis: Metabolic functions in microorganisms rely on the homeostasis of metal ions. Excessive metal nanoparticles disrupt this balance, causing irreversible harm to the microorganism, resulting in growth retardation or death [8].

Production of reactive oxygen species: Nanoparticles gain access to the cell membrane of microorganisms and trigger the release of reactive oxygen species (ROS), leading to oxidative stress within the cell. This oxidative stress hampers respiration and ATP production, ultimately resulting in membrane disruption. Metal oxide nanoparticles, through active redox cycling and pro-oxidant functional groups, contribute to the formation of ROS [9].

Protein and enzyme dysfunction: Nanoparticles catalyze the oxidative process of amino acid chains, leading to the formation of protein-bound carbonyls. This process causes protein degradation, inactivation of enzymes, and disruption of catalytic activity [10, 11].

Genotoxicity and inhibition of signal transduction
Nanoparticles penetrating the micro-organisms interact with DNA and RNA due to their electrical properties, negatively affecting the transcription and translation of chromosomal DNA, which, in turn, inhibits signal transduction [12].

Properties of Nanoparticles [13]

Size: Nanoparticle size, ranging between 10 and 100 nanometers, plays a crucial role. Particles smaller than 10 nanometers or larger than 100 nanometers lack therapeutic effectiveness. Very small particles are eliminated through the kidneys, while larger particles are eliminated by the reticuloendothelial system.

Surface charge: The surface charge of nanoparticles influences their bactericidal efficacy. Positively or negatively charged nanoparticles readily bind to the oppositely charged cell walls of microorganisms, enhancing their effectiveness. However, excessively strong charges may compromise the structural integrity of nanoparticles. Therefore, appropriate charging is essential.

Surface configuration: Nanoparticles possess a hydrophilic coating on their surface, which increases their interaction with cellular receptors and enhances binding to ligands on the exterior. This property is important for biomedical applications. Superparamagnetic iron oxide nanoparticles utilize this surface composition.

Protein adsorption: Coated polymers, such as polyethene glycol, prevent protein adsorption on nanoparticles, thereby extending their half-lives.

Functionalization: Raw nanoparticles lack relevant biomedical properties. Functionalization involves modifying the exterior structure of nanoparticles by combining them with other substances to enhance their functionality and application in various fields.

Synthesis of Nanoparticles
Approaches for preparing antibacterial nanoparticles include top-down and bottom-up strategies. The top-down approach involves reducing the size of the nanomaterial from bulky to Nanoscale using specialized procedures. The bottom-up strategy involves the preparation of nanoparticles through chemical reactions. Various methods, including chemical, electrochemical, sonochemical, solution-gel, green synthesis etc. are used in nanoparticle synthesis [13].

Uses of antibacterial nanoparticles in endodontics and restorative Dentistry

Chitosan Nanoparticles
Chitosan is a natural biopolymer, a DE acetylated derivative of chitin. It exhibits exceptional antibacterial, antiviral, and antifungal properties. The effectiveness of chitosan shifts depending on factors such as the organism type, the pH level, the degree of DE acetylation (DD), the molecular weight, chemical changes, and the presence of lipids and proteins. Higher DD increases amine groups thereby increasing the antibacterial activity of chitosan. Positively charged chitosan creates an electrostatic interaction with negatively charged bacterial cell membranes is proposed as the mechanism of action, leading to altered cell wall permeability, cell rupture, and release of intracellular components [14-16].
Enterococcus faecalis exhibited significantly reduced adhesion to dentin treated with nanoparticles. Chitosan nanoparticles (CS-NPs) and zinc oxide were evaluated further for their ability to disinfect and disrupt E. Faecalis biofilms. These nanoparticles were able to eliminate biofilms in a concentration- and time-dependent manner, and even after being aged for 90 days, they did not lose their antibacterial capabilities. In a second *in vitro* investigation, CS-NPs were coupled with many different brands of chlorhexidine to completely eliminate E. Faecalis. This work demonstrated the potential applicability of CS-NPs in tissue regeneration employing membrane barriers in periapical surgery. The inclusion of CS-NPs resulted in a considerable reduction in the number of colony-forming units on infected collagen membranes and agar plates. There is a promise that cationic CS-NPs can improve root canal disinfection. However, obstacles such as the lengthy treatment time necessary for effective antibacterial properties [17].
Poly (lactose-co-glycolic) acid (PLGA) has a synergistic effect when combined with light and methylene blue-loaded nanoparticles for diminishing bacterial populations in the planktonic phase and root canal. The encapsulation of photoactive medications within PLGA nanoparticles has the potential to serve as an adjunct in antimicrobial endodontic therapy, offering a promising strategy for combating infections [18].

Bioactive Glass

Bioactive mesoporous calcium silicate NPs have a size of 100 nm. It possesses a larger surface area and pore volume. Bioactive glass (BAG) have osteoinductive properties and antibacterial effects, which make it appropriate for a variety of Orthopaedic and dental applications. BAG is made up of varying concentrations of SiO2, Na2O, CaO2, and P2O5, and its antibacterial effects are dependent on local physiological changes. The antibacterial activity of BAG is influenced concurrently by the following factors: High pH: Because BAG releases ions in an aqueous environment, the pH rises, which has antibacterial effects. Osmotic effects: Osmotic pressure greater than 1% inhibits the proliferation of a variety of microorganisms. Ca/P precipitation: BAG induces bacterial surface mineralization. To enhance root canal disinfection, micro- and Nano scale BAG forms have been evaluated. *In vitro* investigations on root canal disinfection revealed that BAG had a substantially weaker antibacterial effect than calcium hydroxide in inhibiting the growth of residual bacteria. Despite the Nano metric BAG's greater specific surface area, its micrometric counterpart exhibited a significantly greater alkaline capacity and effectively eliminated biofilms [19-22].

Silver Nanoparticles

Due to their effective antibacterial properties, silver nanoparticles have been widely employed in biomaterials. Multiple mechanisms, including interactions with proteins and DNA, disruption of hydrogen bonds and respiratory chains, the unravelling of DNA, and interference with cell-wall synthesis and cell division, contribute to silver's antibacterial effect. Additionally, silver nanoparticles (Ag-NPs) have the ability to destabilise bacterial membranes, resulting in increased permeability and the leakage of cellular components [23, 24].

In *in vitro* tests done by Hiraishi and colleagues, silver diamine fluoride was shown to be effective against E. faecalis biofilms, resulting in the total removal of the biofilm after 60 minutes of contact. A deposition of 3.8% silver diamine fluoride was also seen on the surface of the dentin, and it had infiltrated the dentinal tubules to a depth of 40 mm. According to the findings of another piece of research, the antibiofilm performance of Ag-NPs was significantly improved when they were taken as a medicine rather than used as an irrigants. When compared to calcium hydroxide groups and syringe irrigation with a greater concentration (0.1%) of Ag-NP solution, the use of a 0.02% Ag-NP gel as a medicine for a period of seven days proved to be the most efficient method for dismantling E. Faecalis biofilms. It has been hypothesized that one of the significant factors in medication application is the prolonged interaction that takes place between positively charged Ag-NPs and negatively charged biofilm bacteria and structures. The combination of calcium hydroxide and Ag-NP solution resulted in a considerable decrease of E. Faecalis in root canal dentin [25, 26].

There is a potential for dentin to become discoloured, and Ag-NPs are known to be harmful to mammalian cells. These are the two principal issues related to Ag-NPs. Experiments were carried out by Gomes-Filho and colleagues utilizing Wistar rat models and two separate doses of silver nanoparticles (Ag-NPs). They found that the concentration had an effect on the severity of the chronic inflammatory response that was detected *in vivo*. After 15 days, the tissue reaction to 47 parts per billion of silver nanoparticles was equivalent to that of 2.5% sodium hypochlorite. The range of silver ion concentrations that are hazardous to eukaryotic cells is roughly 1-10 mg/L for Ag-NPs and 10-100 mg/L for silver ions [27, 28].

Nanoparticles Incorporated Sealers

The main purpose of a root canal filling material is to fully fill and seal the prepared canal space, preventing bacterial reinfection of the canal. This is accomplished by a process known as capping. It is essential for a root canal sealer to have antibacterial properties in order to remove the microbial load that are already present in the root canal system and to stop the spread of bacteria in the event that there is any leakage [29].

The antibacterial capabilities of zinc oxide-based and resin-based root canal sealers were improved by the incorporation of CS-NP (chitosan nanoparticles) and zinc oxide nanoparticles, respectively. This enabled the sealers to more effectively release antibacterial components. It is important to note that the incorporation of nanoparticles did not result in any unfavorable changes to the flow properties of the root canal sealers. An *in vitro* investigation was carried out by DaSilva, *et al.*, in which treated bovine root canals were filled with a zinc oxide-eugenol sealer that included CS-NP inclusion. The modified sealer was successful in inhibiting the production of biofilm, as shown by a much decreased amount of biofilm covering the contact between the sealer and the dentin [30].

In another study, the incorporation of CS-NPs into an epoxy resin sealer known as Therma Seal led to an increase in the material's antibacterial activity in both direct-contact and membrane-restricted studies. Even after a period of ageing for 4 weeks, the addition of CS- NPs significantly boosted the antibacterial efficiency of different root canal sealers. Additionally, the incorporation of CS-NPs prevented the production of biofilm at the interface between the sealer and the dentin, independent of the surface treatment [30].

It has been advised that BAG-NP, which stands for bioactive glass nanoparticles, be used to encourage the closing of interfacial gaps between the root canal walls and the core infill materials. An interface of hydroxyapatite may be produced by combining polyisoprene or polycaprolactone with BAG nanoparticles. This can possibly eliminate the need for an endodontic sealer to be used. As a consequence of the addition of BAG additives to the polymers that were under investigation, the resulting composite materials became bioactive and significantly improved their ability to provide rapid sealing. Polyisoprene and polycaprolactone composites containing BAG have shown potential for use as single root canal filler materials, as evidenced by their positive results [31, 32].

Nanoparticles in restorative materials

Nanoparticles in restorative materials can be classified as

1. Organic nanoparticles- Polymeric nanoparticles, Liquid based nano-materials.
2. Inorganic nano-materials- Metal nano-materials, Metal oxides nano-materials, Ceramic nano-materials, Semiconductor based nano-materials.
3. Carbon-based nano-materials- Fullerness, Graphene, Carbon nanotubes.

Researchers have found that adding 1 wt % quaternized copolymer functionalized nanodiamonds to resin composites stops biofilm from forming without hurting the structure of the tooth [33]. Not only do nanomaterials work well with composites, but they also work well with GIC (34). GIC naturally gives off fluoride ions, which help stop tooth decay from happening again. When copper is added to GIC, it makes it better at killing germs and slows down the rate at which collagen breaks down. Renné, *et al.* showed that adding Nano hydroxyapatite to GIC makes it better at letting fluoride ions out [35]. Another study found that hydroxyapatite nanoparticles can make GIC stronger and make it less likely that bacteria will grow on it [36].

Researchers have used quaternary ammonium polyethylenimine nanoparticles (QPEI-NPs) to make many root canal sealers and temporary therapeutic materials more effective at killing germs. In a study by Barros *et al.*, adding QPEI-NPs to AH Plus and Pulp Canal Sealer EWT made both sealers more wet and gave them a higher surface charge. But after 7 days, neither the sealer with QPEI-NPs nor the one without showed any antibiotic action. In comparison, another study showed that combining AH Plus with QPEI-NPs made it much more effective at killing germs. By putting QPEI-NPs into a 2-paste epoxy-amine resin sealer, Beyth *et al.* and Kesler Shvero *et al.* proved that E. faecalis was pulled to the cationic surface. This destabilized the bacterial membrane and stopped bacterial growth totally. By stopping nanoparticles from getting into eukaryotic cells, binding them to the surface of the sealer made them less harmful. QPEI-NPs kill bacteria by attaching to and penetrating the bacterial cell wall, interacting with proteins and the lipid layer in the cell membrane, stopping critical ion exchange, destabilising the cell membrane, and finally killing the cell [3].

Bioactive multifunctional composite

Xiao, *et al.* conducted a study of a bioactive multifunctional composite (BMC) by incorporating nanoparticles of NACP, MPC, DMAHDM and AgNP. Poly (amido amine) on the BMC was investigated, with a specific focus on its application for root caries. The results revealed remarkable remineralization of root dentin, indicating the effectiveness of the developed BMC for Class V restorations. Alongside its remineralization properties, the BMC exhibited resistance to proteins and displayed antibacterial activity. The findings suggest that BMC + PAMAM provides protective benefits for tooth root structures, making it a promising option for various cavity restorations, including Class I and Class II restorations [37].

Silver nanoparticles

In their research, Paiva and colleagues attempted to generate polyacid formulations in a single step by photo-reducing silver (Ag) nanoparticles that were suspended in glass ionomer cement (GIC). The antibacterial activity of the modified cement was supposed to be preserved, and at the same time, its mechanical and manipulative qualities were supposed to be compared to those of regular GIC. S. mutans served as the test organism for determining how efficient the antibacterial properties of the GIC with added Ag were. According to the findings, the GIC that included Ag nanoparticles showed an impressive level of antibacterial activity. The idea of diffusion was the foundation upon which the modified restoration operated. This allowed for the liberation of silver ions from the cement matrix through an oxidative process. According to the findings of Pava *et al.* (2018), this led to the conclusion that GIC that contains silver nanoparticles has the capacity to prevent cavities and inhibit the development of biofilm on the surface of the repair [34].

Cao *et al* were able to effectively manufacture a nano silver (Ag)-filled resin throughout the course of their study. They used AgBr/BHPVP Nanocomposites to protect the flexural strength and modulus of the resin from any potential adverse effects that may have been caused by other factors. In addition to this, the hardness of the resin discs as measured by Vicker's was greatly improved. There was no discernible change in the rate of release of Ag^+ ions from the resin despite the presence of an anaerobic environment. This nanoscale silver-filled resin has shown promise as a potential weapon against anaerobic cariogenic bacteria. Particularly noteworthy was the fact that it had a potent antibacterial activity against.

S. Mutans, which was accomplished by the consistent release of Ag^+ ions. According to the findings of the research, the ideal percentage of AgBr/BHPVP to include in Bis-GMA/TEGDMA is 1.0 weight percent [38].

Titanium oxide nanoparticles

Ghahremani and colleagues evaluated the tensile and impact strengths of a color-modified heat-cure resin that has titanium oxide (TiO_2) nanoparticles added to improve its properties. An *in vitro* experiment was carried out by the researchers, in which TiO_2 nanoparticles were mixed into a triplex high heat-cure resin. The comparison between the reinforced group and the control group revealed a difference that was statistically significant (p=0.001) with the reinforced group demonstrating a tensile strength that was 7 MPa greater than the control group. Because of this, we were able to arrive at the conclusion that including 1% by weight of titanium dioxide in the color-modified acrylic resin resulted in an increase in both the tensile and impact strengths. On the other hand, it is essential to point out that this research did not evaluate whether or not this strategy may have any potentially negative consequences on the restorative resin or its restorative capabilities [39].

Zinc oxide nanoparticles

Al-Mosawi investigated the incorporation of ZnO nanoparticles into a composite resin for their potential as an antibacterial agent in the buccal cavity in his study. On agar dishes, the researcher evaluated the effects of three distinct concentrations of ZnO nanoparticles: 5%, 7%, and 10%. S. mutans and Pseudomonas exhibited the greatest susceptibility to the antibacterial activity of ZnO nanoparticles among the tested bacterial species. These findings suggest that ZnO nanoparticles have the potential to prevent secondary caries, an encouraging finding for dental applications [40].

Functionalized Antimicrobial Nanoparticles

Functionalization permits the modification of a material's surface composition, charge, and structure while retaining its original bulk properties. Functionalized nanoparticles, which are enriched with ROS and offer novel antimicrobial resistance-combating opportunities. It has been observed that the combination of nanoparticles and photosensitizers increases the efficacy of antimicrobial photodynamic therapy (PDT). The functionalization of various nanoparticles has demonstrated enhanced antibacterial efficacy and rapid action. It has been recognised that nanoparticle-based photosensitizers have the potential to improve the efficacy of photodynamic therapy. Photosensitizer molecules contain effective biological properties including ultra-small diameters, a high ratio of surface area to mass, and enhanced reactivity. According to Kishen *et al.* review, the combination of nanoparticles and photosensitizers can be accomplished through a variety of methods, including supplementation, encapsulation, binding, and the use of nanoparticles as photosensitizers. These combinations have shown enhanced antimicrobial PDT efficacy.

The combination of nanoparticles and photosensitizers improves the efficacy of antimicrobial PDT for several reasons, including Increased ROS production as a result of a higher photosensitizer mass concentration. Increased targeting of microbes due to the surface charge's facilitation of increased interaction. Increased photosensitizer stability after conjugation, resulting in diminished physical quenching effects induced by photosensitizer aggregation. Possibility of controlled ROS release following photoactivation [41-43].

Conclusion

Nanoparticles have emerged as highly antibacterial agents and exhibit strong inhibitory effects on various microorganisms, making them promising tools in restorative and endodontic biomaterials. Modifying nanomaterials further enhances their efficacy and facilitates their application in different areas. It is absolutely necessary for the direction of future research to explore the antibacterial, physical, and clinical impacts of nanoparticles on dental biomaterials over the long term. Due to the outstanding properties that nanomaterials possess; they have taken on a vital role in the development of significant technological advances. These unique characteristics contribute to their superior performance. In the field of endodontics, nanoparticle-based treatment strategies hold great potential for enhancing antibacterial and antibiofilm efficacy. By modifying the surface properties of nanoparticles, it becomes possible to deliver drugs or chemicals to infection sites, selectively interacting with biofilms and bacteria. Collaborations among engineers, clinicians, and biologists are driving the development of new multifunctional nanoparticles that address specific clinical needs.

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