Nanoparticle disinfection of root canals
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ABSTRACT

The aim of this comprehensive review is to highlight the importance of bacterial biofilm in root canal and role of nanoparticles (NPs) in disinfection of the canal. The widespread recognition of microbial biofilm as the contributory factor for human infection and constant increase in antimicrobial resistance warrants the discovery of a reliable and effective antimicrobial strategy to infectious diseases. The success rate of the endodontically treated tooth highly depends on the complete removal of the bacterial biofilm from the root canal. This eventually brought about various NPs to improve the root canal disinfection. NPs with its unique properties have led research toward new prospects in treating and preventing dental infections. This review article focuses on the scientific knowledge about the various developments of NPs which is used to disinfect the root canal.

KEYWORDS: Disinfection, Endodontic treatment, Irrigation, Microbial biofilm, Nano disinfection, Nanoparticle disinfection

INTRODUCTION

The main goal of endodontic treatment is to eliminate endodontic infection and promote healing of apical periodontitis.[1,2] Although the bulk of the infecting microorganisms are removed during chemomechanical debridement, bacteria are readily detectable in approximately one-half of teeth at the time of obturation.[3] The complexity of the root canal morphology makes complete debridement of bacteria more difficult, even when conventional methods of endodontic instrumentation and irrigation are performed to the highest standards.[4]

The word “nano” originated from a Greek word which means “dwarf.”[5,6] The philosophy of nanotechnology was first illustrated in 1960 by Richard P. Feynman, a Nobel Prize winner, in his lecture “There’s Plenty of Room at the Bottom.”[7] The concept of nanotechnology has been applied in numerous scientific fields such as physics, engineering as well as in the medical field.[5]

The term nano dentistry is defined as “the science and technology of diagnosis, treating and preventing oral diseases, relieving pain, preserving and improving dental health using nanostructure material.”[5,8]

Nanoparticles (NPs) are particles with a size not >100 nm, with spherical, cubic, and needle-shaped forms. They are formed from metallic or polymeric materials, and their active surface area, chemical reactivity, and biological activity are different from larger size particles.[9,10] An inverse relationship between the size of the NPs and antimicrobial activity has been clearly demonstrated, where particles in the range of 1–10 nm in size have demonstrated greater killing activity against bacteria compared to larger particles.[9]

Nanomaterials exist in different forms and shapes. They are categorized according to their dimensions into zero dimension such as NPs, one dimension such as nanorods, two dimensions such as thin films, and three dimensions such as nanocones.[11] These materials present unique physicochemical properties, such as large surface area/mass ratio and increased chemical reactivity.[12,13] The increased number of atoms and increased surface-to-volume ratio compared with micro-/macro-structures are suggested to contribute to the distinctly different properties of nanomaterials. These advantages may be exploited to design highly specific materials and devices to interact with at the subcellular and molecular level of the human body to achieve maximal therapeutic efficacy with minimal side effects.[12,14,15]
Antibacterial NPs show a broad spectrum of antimicrobial activity. According to Vier and Figueiredo, metallic NPs of titanium, gold, zinc, and copper have attracted particular attention with different physical properties and spectra of antimicrobial effect. A study using MTT assay and confocal laser scanning microscopy demonstrated that 0.1% and 0.2% nanosilver gel is more effective on biofilm comparing camphorated phenol and chlorhexidine (CHX) gluconate. An study showed that nanosilver gel is not efficient enough against; however, triple antibiotic paste and CHX gel showed better antibacterial activity than calcium hydroxide (CH) and so can be used as an alternative medications in endodontic treatment. Zhang et al. assessed the efficacy of CH with a silver NPs to eliminate the biofilm of and showed that silver NPs with CH have a significant inhibitory effect on the biofilm of Enterococcus faecalis.

The purpose of this review is to gain knowledge about the importance of root canal disinfection and to present the current state of NPs used for the disinfection of root canals.

**ENDODONTIC BIOFILM**

Different anatomy and complexities of the canal, in addition to dentin composition, are key challenges for effective disinfection in endodontics. Antimicrobials such as sodium hypochlorite (NaOCl) are most commonly used in endodontic treatment to destroy microbial biofilms. However, the anatomical complexities and undrilled parts of the canal may compromise their efficacy in endodontic treatment. All endodontic infections are polymicrobial in nature with differences between the types of microorganisms isolated from primary and secondary root canal infections. Microbiological studies have revealed >400 microbial species from endodontic samples.

Microbial agents are the common cause of pulpal and periapical pathosis. All endodontic infections are polymicrobial in nature with differences between the types of microorganisms isolated from primary and secondary root canal infections.

Endodontic infection is eventually established as a biofilm. This form of microbial colonization inside the root canal system was initially discovered by Ramachandran Nair. Microbial biofilm is a surface-attached microbial community defined by Mohammadi et al. as “a sessile multicellular microbial community characterized by cells firmly attached to a surface and enmeshed in a self-produced matrix of extracellular polymeric substance.”

**IRRIGATION IN ROOT CANAL DISINFECTION**

Irrigation has an important role in endodontic treatment. During biomechanical preparation of root canals, the irritants facilitate removal of microorganisms, tissue remnants, and dentin chips from the root canal through a flushing mechanism. It can also help prevent packing of the hard and soft tissue in the apical region and extrusion of infected material into the periapical area. Some irrigating solutions dissolve both organic and inorganic tissues in the root canal. Many irrigating solutions have cytototoxic potential, and they may cause severe pain if they gain access into the periapical tissues. An optimal irrigant should have all or most of the positive characteristics listed in but none of the negative or harmful properties. No available irrigants can be considered as ideal. Using a combination of irritants in the correct sequence can contribute to a successful treatment outcome.

The main functions of a root canal irrigants are as follows:

- Washing action (helps remove debris)
- Reduce instrument friction during preparation (lubricant)
- Facilitate dentin removal (lubricant)
- Dissolve inorganic tissue (dentin)
- Penetrate to canal periphery
- Kill bacteria and yeasts (also in biofilm)
- Dissolve organic matter (dentin collagen, pulp tissue, and biofilm)
- Do not damage vital periapical tissue, no caustic or cytotoxic effects
- Do not weaken tooth structure.

**NaOCl**

NaOCl is the most well-liked irrigating solution. NaOCl particularizes in water into Na+ and therefore the salt ion, OCl-, establishing associate equilibrium with acid (HOCI). At acidic and neutral pH scale, Cl exists preponderantly as HOCl, whereas at high pH scale of 9 and on top of, OCI predominates. Acid is liable for the medicament activity; the OCI particle is a smaller amount effective than the unmelted HOCl. Hypochloric acid disrupts important functions of the microorganism cell, leading to necrobiosis. NaOCl is usually utilized in concentrations between 0.5% and 6%. It is a potent antimicrobial agent, killing most microorganism instantly on direct contact.

There is substantial variation within the literature concerning the medicament impact of NaOCl. In some articles, NaOCl is reported to kill the target microorganisms in seconds, even at low concentrations, though different reports have revealed significantly longer times for the killing of an equivalent species.
The major disadvantage of NaOCl embody the unpleasant style, toxicity, and its inability to get rid of the smear layer by itself because it dissolves solely organic material.\cite{3} Recently, it has been shown by \textit{in vitro} studies that long-term exposure of dentin to a high concentration bleaching agent will have a prejudicial impact on dentin snap and flexural strength. The restricted antimicrobial effectiveness of NaOCl \textit{in vivo} is additionally unsatisfactory. The poorer \textit{in vivo} performance compared with \textit{in vitro} is maybe caused by issues in penetration to the foremost peripheral components of the root canal system such as fins, anastomoses, top canal, lateral canals, and dentin canals. The presence of inactivating substances like exudate counteracts the effectiveness of NaOCl.\cite{34}

In summary, bleaching agent is that the most vital irrigating solution and therefore the only one capable of dissolving organic tissue, together with biofilm, and therefore the organic a part of the smear layer. It ought to be used throughout the instrumentation section because the final rinse following EDTA or CA apace produces severe erosion of the canal wall dentin and will in all probability be avoided.\cite{26,35}

**EDTA AND CA**

Complete cleanup of the root canal system needs the utilization of irrigants that dissolve organic and inorganic material. As salt is active solely against the previous, alternative substances should be wont to complete the removal of the smear layer and dentin scrap. EDTA and CA effectively dissolve inorganic material, as well as hydroxyapatite. Few researchers have got very little or no result on organic tissue but few reports have indicated that solutions with lower concentrations (e.g., 10%, 5%, and even 1%) take away the smear layer equally. Considering the high value of EDTA, it should be worthy to think about exploitation diluted EDTA. CA is additionally marketed and employed in varied concentrations, starting from 125 to 500, with a tenth resolution being the most common. EDTA and CA are used for 2-3 min at the end of instrumentation along with NaOCl irrigation. Removal of the smear layer by EDTA or CA improves the medicinal drug result of regionally used disinfecting agents in deeper layers of dentin.\cite{23,26} EDTA and CA are available as factory-made liquids and gels. Although there are no comparative studies regarding the effectiveness of liquid and gel product to remove dentin, it is doable that the tiny volume of the foundation canal (only a number of microliters) contributes to a fast saturation of the chemical and thereby loss of effectiveness. In such things, the utilization of liquid product and continuous irrigation ought to be counseled.

**CHX DIGLUCONATE**

CHX digluconate is widely utilized in medical care in dental medicine owing to its sensible antimicrobial activity.\cite{28,29} It is gained goodish quality in dentistry as an irrigating solution and as an intracanal medication. CHX does not possess some of the undesired characteristics of germicide (i.e., unhealthy smell and robust irritation to periapical tissues). However, CHX has no tissue-dissolving capability, and thus, it cannot replace germicide. CHX permeates the microbial cell membrane or outer membrane and attacks the microorganism cytoplasmatic or inner membrane or the yeast semi-permeable membrane. In high concentrations, CHX causes curdling of living thing elements. One among the explanations for the recognition of CHX is its substantivity (i.e., continued antimicrobial effect), as a result of CHX binds to exhausting tissue and remains antimicrobial. However, just like alternative dental medicine disinfecting agents, the activity of CHX depends on the pH and is additionally greatly reduced within the presence of organic matter.\cite{31} Many studies have compared the medication impact of NaOCl and a pair of CHX against intracanal infection and have shown very little or no distinction between their antimicrobial effectiveness.\cite{32-35,28,29} Although bacterium is also killed by CHX, the biofilm and alternative organic dust do not seem to be removed by it. Residual organic tissue could have a negative impact on the standard of the seal by the permanent root filling, necessitating the utilization of NaOCl throughout instrumentation. However, CHX doesn’t cause erosion of dentin-like NaOCl, it is a decent alternative for maximized medication impact at the tip of the chemo-mechanical preparation.\cite{30} Most of the analysis on the utilization of CHX in dentistry is applied victimization \textit{in vitro} and \textit{ex vivo} models and Gram-positive takes a look at organisms, largely \textit{E. faecalis}. It has potential that the studies have given an over positive image of the quality of CHX as an antimicrobial agent in dentistry. Additional analysis is required to spot the best irrigation program for varied sorts of dental medicine treatments. CHX is marketed as a water-based answer and as a gel (with Natrosol). Some studies have indicated that the CHX gel features a slightly higher performance than the CHX liquid; however, the explanations for potential variations do not seem to be illustrous.

**MECHANISM OF NPs**

The use of NPs as antimicrobial agents has recently attracted right good attention in the medical field as a result of their superior medicinal drug properties compared with those of various antimicrobial agents together with a low potential to manufacture organism resistance.\cite{36} The antimicrobial activity of NPs against different microorganisms differs from that of
its original bulk state and may vary according to the different types of NPs.\textsuperscript{[57]}

It involves the binding of NPs to the targeted bacterial cell membrane through electrostatic forces, causing an alteration in the membrane potential, depolarization, and eventually loss of membrane integrity.\textsuperscript{[38]} This results in the disturbance of major bacterial cell functions such as respiration, transportation of nutrients, and disturbance of energy transduction, leading subsequently to bacterial cell death. The other mechanism includes the production of oxygen free radicals such as reactive-oxygen species (ROS) that can influence survival of the bacterial cell by blocking the protein function, destroying DNA, and resulting in excess radical production.\textsuperscript{[39]}

Different types of NPs have been reported recently in different forms in \textit{in vitro} studies to evaluate their efficacy against endodontic microorganisms. The NPs used in these studies can generally be classified into three categories according to their nature: Metallic or inorganic, polymeric, and bioactive non-organic NPs.\textsuperscript{[5,6]}

**SILVER NPs**

The antimicrobial properties of silver NPs were demonstrated first by Morones \textit{et al.}\textsuperscript{[40]} Silver NPs have the ability to bind to the negatively charged half of the microorganism cell membrane, disturb its functions such as porosity and respiration, inflict leaky of the cytoplasmatic content, and eventually rupture of the microorganism cell. As a result, the NPs will infiltrate inside the cytoplasmatic content and interact with sulfur- and phosphorus-containing proteins such as DNA and RNA, causing further damage to the bacterial cell.\textsuperscript{[40]}

In addition, the silver NPs release silver ions when in contact with an aqueous media, further disturbing the bacterial functions.\textsuperscript{[40-44]}

Silver NPs have different surface charges, and because of these variations, there are differences on their antibacterial efficacy which was evaluated by Abbaszadegan \textit{et al.}\textsuperscript{[45]} The efficacy of three preparations having surface charges of neutral, negatively charged, or positively charged against planktonic cells of \textit{E. faecalis} was compared with that of NaOCl and CHX.

The use of silver NPs as an antimicrobial agent against endodontic pathogens is very effective. However, further investigation is required to evaluate any effect on the color stability of the tooth structure, the dentine surface, and possible cytotoxic actions on human cells.\textsuperscript{[5]}

**ZINC OXIDE NPs**

Zinc oxide NPs showed high antimicrobial effects in destroying microorganism cells in a very higher pH scale atmosphere.\textsuperscript{[46-48]} The antibacterial effect of zinc oxide NPs is similar to that of other NPs, causing increased permeability of the cell wall membrane, a release of cytoplasmatic content, and cell lysis. The bactericidal effect of zinc oxide NPs was shown to be related to size, and the smaller the size, the higher the antibacterial effect and the production of ROS such as hydrogenperoxide when in contact with aqueous medium.\textsuperscript{[46-52]} In addition, zinc oxide NPs can produce zinc ions inside the bacterial cell causing disturbances in its enzymatic system and the mechanism of amino acid metabolism, resulting in further damage.\textsuperscript{[53]} The antibacterial effect of zinc oxide NPs has been shown to depend on concentration, higher levels, resulting in the maximum antibacterial effect. Zinc oxide NPs were incorporated into polyethylene glycol to form a creamy mix and used as an intracanal medicament.\textsuperscript{[54]}

**CHITOSAN NPs**

Chitosan is a natural polysaccharide that is obtained by deacetylation of chitin, one of the most abundant polysaccharides in nature that forms most of the external skeleton of arthropods such as crabs and shrimps.\textsuperscript{[55,56]} Chemically, chitin is composed of (1-4)-linked 2-acetamido-2-deoxy-β-D-glucose. A modification in the structure of chitin in which the acetyl group is reduced by 40%-35% by chemical hydrolysis in alkaline solution and high temperature produces a new chemical formula that consists of a copolymer of (1-4)-2-amino-2-deoxy-β-D-glucan and (1-4)-2-acetamide-2deoxy-β-D-glucan which is known as chitosan.\textsuperscript{[57,58]}

Chitosan NPs serve as drug carrier, and this property was utilized by Shertha and Kishen by conjugating a photosensitizer material (rose bengal) to the chitosan structure and then evaluating its antimicrobial property against biofilms of \textit{E. faecalis}, \textit{Streptococcus oralis}, \textit{Prevotella intermedia}, and \textit{Actinomyces naeslundi}. They showed that such a conjugation can destroy the bacterial cell membrane of the tested bacterial species and then can penetrate deep into the biofilm structure of the tested species reducing the biofilm thickness and the number of microbial cells.\textsuperscript{[59]} Chitosan NPs were incorporated into a zinc oxide eugenol-based sealer and were assessed for their antibacterial effect.\textsuperscript{[5]}

**COPPER NPs**

Compared to traditional antibiotics, NPs are effective at concentrations 1000 times lower. However, copper is cheaper than silver, pronto miscible with polymers, and comparatively stable with chemicals and physically.\textsuperscript{[60,61]}

It has been reportable that copper NPs have higher affinity toward amines and carboxyl groups in a very higher density on the surface of \textit{Bacillus subtilis} than
silver NPs, resulting in an increased bactericidal activity. Copper oxide or cuprous oxide (CuO) is a red powder and can be produced as NPs, which has shown activity against a range of the strains of bacteria.\[60]\n
They have important limitations, including the rapid oxidation in exposure to air. Copper is oxidized to CuO and CuO turns into Cu\(^{2+}\) during preparation and storage, so it is difficult to produce copper NPs in a natural environment. And also, an excess of copper in the human body leads to the generation of the most detrimental radicals, such as the hydroxyl radical. However, there are enzymes such as copper triphosphatase adenosine (CuATPases), which play an important role within the equilibrium of copper, and also the excess is export through the viscous as excreta, liver as a product of digestive fluid, and breast organ within the milk.

Chitosan-copper NP surfaces are lined by chitosan fragments that defend against oxidation and aggregation, and the discharge time of the compound is inflated. It was shown that the size of NPs could be controlled by manipulating the concentrations of chitosan and silver and copper nitrate. The size of the particle can be increased either by decreasing the concentration of chitosan or increasing the concentration of metal ions. NPs produced had a positive surface charge and chitosan used in their synthesis contributed to the stability of suspensions of such particles and had an antimicrobial effect.\[62]\n
When the drug unremarkably employed in dentistry and cupric sulfate pentahydrate was compared, it absolutely was discovered that cupric sulfate bestowed associate antimicrobial activity superior to the lower concentrations of CHX. The antimicrobial properties of copper at nanoscale can also have uses in endodontics, since the environment in the root canal, which serves as ecological niche for microorganisms; they are established in the form of biofilm, and as copper is considered a contact agent as a replacement for the normally used dentistry disinfectants is still in tilt as there square measure variation in results obtained by completely different studies concerning their effectivity against dentistry pathogens. A lot of studies when any improvement in the synthesis of bioactive glass NP square measure required.\[5,68]\n
**MAGNESIUM-CONTAINING NPs**

Magnesium-containing NPs are either magnesium oxide NPs or magnesium halogen-containing NPs such as chlorine, bromine, and fluorine. The antimicrobial properties of magnesium-containing NPs were thought to be due to multiple mechanisms. Similar to the common antibacterial mechanisms of NPs, magnesium halogen-containing NPs penetrate inside the bacterial cell, resulting in a disturbance in the membrane potential. The penetration facilitated the DNA binding and lipid peroxidation effects of the NPs, causing more destruction of the bacterial cell. Magnesium oxide NPs were found to be bactericidal when present in an aqueous form as a result of the action of superoxide anions that formed on the bacterial cell surface.\[63-65]\n
The addition of NPs of MgO to an irrigant have remarkably increased antibacterial activity when compared with NaOCl.\[66]\n
**BIOACTIVE GLASS NPs**

Bioactive glasses are novel dental materials. Bioactive glasses are composed of calcium and phosphate which are present in a proportion that is similar to bone hydroxyapatite. These glasses bind to the tissue and are biocompatible. One of the most important properties of bioactive glasses is their ability to exhibit antibacterial activity, which creates a bacteria-free environment while healing and regenerating the damaged area. The quick dissolution with rapid change in pH of the surrounding medium enables these glasses to exhibit antibacterial properties. The antibacterial action of silica-based melt-derived bioglass was investigated against certain types of microorganisms, and the results were promising.

The antimicrobial property of bioactive glass material was shown to be through its ability to:

1. Release its ions when it came into contact with an aqueous medium
2. Increase the surrounding pH
3. Increase the osmotic pressure around the bacterial cell and inhibit bacterial growth
4. To precipitate calcium and phosphate ions in the bacterial cell membrane, disturbing its functions.\[67]\n
The use of bioactive glass NPs as associate antimicrobial agent as a replacement for the normally used dentistry disinfectants is still in tilt as there square measure variation in results obtained by completely different studies concerning their effectivity against dentistry pathogens. A lot of studies when any improvement in the synthesis of bioactive glass NP square measure required.\[5,68]\n
**CONCLUSION**

Recent advances in root canal disinfection using newer technologies may improve the ability to disinfection of root canal system. A large number of NPs are available today which provide multiple uses in medical field. The current focus is on endodontic research based on NP disinfection of root canal. Available studies show that there is a promise in the use of different types of NPs as microbial agents, especially against endodontic pathogens. Although more research on effectiveness of antimicrobial NPs in Dentistry is required, the potential of metal NPs, together with its high availability and low cost, make it worthy as a therapeutic alternative for successful treatment.


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