The Potential Translational Applications of Nanoparticles in Endodontics

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Abstract: Nanotechnology has substantially progressed in the past decades, giving rise to numerous possible applications in different biomedical fields. In particular, the use of nanoparticles in endodontics has generated significant interest due to their unique characteristics. As a result of their nanoscale dimensions, nanoparticles possess several properties that may enhance the treatment of endodontic infections, such as heightened antibacterial activity, increased reactivity and the capacity to be functionalized with other reactive compounds. Effective disinfection and sealing of the root canal system are the hallmarks for successful endodontic treatment. However, the presence of bacterial biofilms and resistance to endodontic disinfectants pose a significant challenge to this goal. This has encouraged the investigation of antibacterial nanoparticle-based irrigants and intracanal medicaments, which may improve the elimination of endodontic infections. In addition, photosynthesizer-functionalized nanoparticles could also serve as a worthy adjunct to root canal disinfection strategies. Furthermore, despite the myriad of commercially available options for endodontic obturation, the "ideal" material has yet to be conceived. This has led to the development of various experimental nanoparticle-incorporated obturation materials and sealers that exhibit a range of favourable physicochemical properties including enhanced antibacterial efficacy and bioactivity. Nanoparticle applications also show promise in the field of regenerative endodontics, such as supporting the release of bioactive molecules and enhancing the biophysical properties of scaffolds. Given the constantly growing body of research in this field, this article aims to present an overview of the current evidence pertaining to the potential translational applications of nanoparticles in endodontics.

Keywords: nanoparticles, endodontics, translational research, disinfection, obturation, regenerative procedures

Introduction

Nanomaterials are natural, incidental or synthetic materials that contain particles in unbound states, aggregates or agglomerates, and of which 50% or more of the particles possess external dimensions between 1 nm to 100 nm.[1] Nanomaterials are classified based on the dimensions, the materials used and the origin of the materials.[2] Typically, nanoparticles are comprised of three layers: the core, which is the inner portion and the main material of which the nanoparticle is made of; the shell layer, which is the intermediate layer and is chemically different from the core; and the surface layer, which is the outer layer and can be functionalized with other particles via surface interactions.[3] Nanomaterials have distinct advantages in that they exhibit unique physicochemical properties when compared to their bulk counterparts due to their nanoscale sizes and high surface-area-to-volume ratio.[2,3]

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This includes increased reactivity, greater solubility, biomimetic features and the ability to be functionalized with other materials such as drugs, bioactive molecules and photosynthesizers.[3–5] Furthermore, antimicrobial nanoparticles can better infiltrate biofilms, are potent at smaller doses and may help mitigate the increasing use of antibiotics.[6] Many antimicrobial nanoparticles possess common mechanisms of action. Nanoparticles can penetrate into biofilms and interact electrostatically with bacterial cell walls, which leads to cell membrane damage, increased cell permeability, generation of reactive oxygen species, interference with cellular functions, destruction of proteins, DNA damage and, ultimately, cell death (Figure 1).[6–8]

Given their many inherent benefits, the potential utilization of nanoparticles in the fields of medicine and dentistry has garnered substantial enthusiasm.[5] The term "nanodentistry" was first introduced at the turn of the
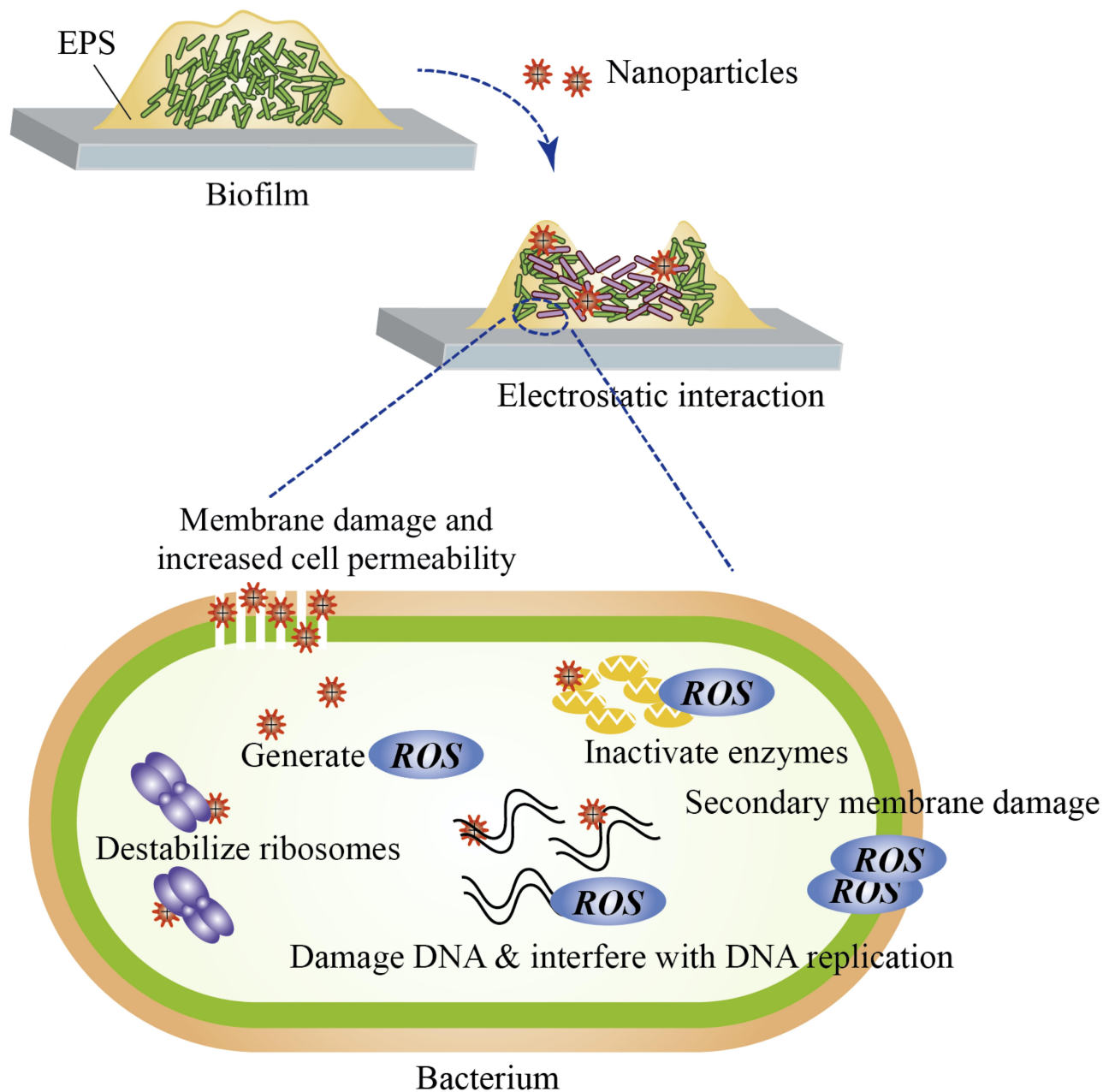
Figure 1 Proposed mechanisms of action of antibacterial nanoparticles. Nanoparticles penetrate biofilm and electrostatically interact with bacterial cell walls. The resultant cell wall and membrane disruption leads to increased cell permeability and leakage of the cellular constituents. Nanoparticles are also able to interfere with cellular functions, denature proteins and destabilize ribosomes. Reactive oxygen species (ROS) are produced from the membrane-nanoparticle interactions. ROS may interfere with DNA replication, cause DNA destruction, enzyme inactivation and secondary membrane damage.
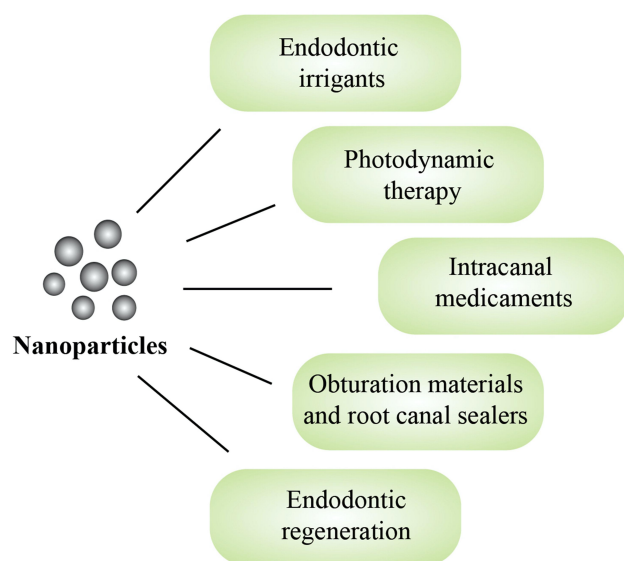
Figure 2 The potential translational applications of nanoparticles in endodontics.

21st century.[9] Innovative endeavours have since led to nanotechnology being incorporated into a myriad of areas in clinical dentistry, such as direct restorative materials, materials used for dental prostheses, periodontal treatment, guided tissue regeneration, modifications of implant surfaces and endodontics.[7,10]

Over the last decade, the use of nanoparticles in endodontics has attracted significant attention from researchers. Since its introduction, our collective understanding of this field has grown substantially, with new studies constantly adding to the existing knowledge. Hence, the aim of this review was to summarize, compile and integrate the current literature on the potential translational applications of nanoparticles in endodontics. The scope of this article encompasses an overview of the different nanomaterials used in endodontic irrigation strategies, photodynamic therapy, intracanal medicaments, obturation materials and regenerative procedures (Figure 2), as well as a summary of their advantages and limitations in the given applications.

Irrigation Strategies

The current understanding of endodontic infections suggests that microbial biofilms play a crucial role in the development and pathogenesis of apical periodontitis.[11,12] Thus, effective chemo-mechanical debridement is essential in order to eliminate the root canal infection. However, biofilm bacteria benefit from a variety of factors that help enhance their survival. For example, bacteria within biofilms are protected by a self-produced matrix of extra-polymeric substance (EPS), which dampens the infiltration of root canal disinfectants and reduces their efficacy.[13,14] Furthermore, anatomically minute regions in the root canal system may be inadequately debrided, resulting in residual infection.[15] In addition, irrigants are often unable to reach the depths of dentinal tubules,[16] permitting the persistence of bacteria, in particular *Enterococcus faecalis* (*E. faecalis*), which is commonly associated with post-treatment failure.[17,18] The most commonly used endodontic irrigant is sodium hypochlorite (NaOCl), often at concentrations ranging between 0.5% to 5.25%.[19,20] Classic studies have regularly touted its tissue dissolving and antimicrobial properties.[21,22] However, use of NaOCl can have several unfavourable outcomes such as disintegration and weakening of the organic dentine matrix,[23] damage to the periapical tissues[24] and formation of persister bacteria.[25] Chlorhexidine has been suggested to be a less caustic endodontic disinfectant and is generally used at a concentration of 2%,[19] however its main drawbacks include the inability to degrade necrotic tissue[26] and its reduced efficacy against Gram-negative microbes.[27] Ethylenediaminetetraacetic acid (EDTA) is a chelating agent, often employed to remove the smear layer.[28] *E. faecalis* biofilm was found to be more susceptible to 2.5% NaOCl used in conjunction with 17% EDTA.[29] However, excessive use can lead to dentine demineralization and erosion, especially when combined with NaOCl.[30] Owing to the aforementioned limitations of current irrigation practices, it is no surprise that there has been an increasing interest in nanoparticle-based irrigants, particularly silver nanoparticles (AgNPs), which are the most studied to date in this field.[31]

Silver Nanoparticles

AgNPs have been widely investigated in dentistry.[10] AgNPs exhibit both antimicrobial and antifungal properties as they possess a multi-level mode of action. By electrostatically interacting with cell membranes and binding to thiol groups of proteins, AgNPs are able to disrupt cell walls and metabolic processes, inactivate bacterial enzymes, increase cell permeability and generate reactive oxygen species.[7,32] More recently, it has been shown that AgNPs could not only improve the antifungal effects of antifungal agents, but also enhance antibacterial effects of antibiotics against a range of bacteria, including antibiotic resistant strains.[33,34] The synergistic and multimode antibacterial actions substantially decrease the need for high

doses of antibiotics, therefore minimizing the potential of antibiotic resistance and toxicity to the neighbouring tissues.[33,34] On the other hand, bacteria such as Gramnegative *Escherichia coli* and *Pseudomonas aeruginosa* could quickly develop resistance to AgNPs after repetitive exposure by producing flagellin, which triggers the aggregation of the nanoparticles.[35]

In regard to endodontic infections, AgNPs were shown to possess antimicrobial and antibiofilm efficacy against *E. faecalis*.[36–38] When used as an irrigating solution, poly (vinyl alcohol) (PVA) coated AgNPs were efficacious against *Pseudomonas aeruginosa, Candida albicans* and *E. faecalis*.[39] Positively charged AgNPs bactericidal efficacy was not inhibited by dentine powder after 24 hours,[36] whilst the antibiofilm and antimicrobial properties were found to be enhanced by ultrasonic activation[40] and Nd: YAG laser irradiation respectively.[41] Surface charge and contact time were important factors in determining its antimicrobial properties, as positively charged AgNPs had the lowest minimum inhibitory concentration and took the shortest amount of time to inhibit planktonic *E. faecalis* growth compared to neutral and negatively charged AgNPs.[36] A study reported that AgNPs antimicrobial activity was on par with conventional endodontic irrigants such as 2% chlorhexidine, 1% NaOCl and 5% NaOCl.[42] Halkai et al reported that biosynthesized AgNPs had antibacterial activity against *E. faecalis* similar to that of ampicillin and 2% chlorhexidine.[43] On the other hand, some studies have questioned the efficacy of AgNPs-based irrigants in comparison to conventional endodontic irrigants. AgNPs solutions were less effective at reducing viable *E. faecalis* in biofilm than 2% chlorhexidine after 5 minutes of irrigation and only equally effective after 15 minutes.[44] Furthermore, NaOCl also exhibited superior biofilm dissolving and antibacterial properties compared to both chlorhexidine and AgNPs solutions.[44] Wu et al suggested the use of AgNPs may be more appropriate as an intracanal medicament due to their contact and time-dependent nature, reporting that syringe irrigation of AgNPs was less effective than gel application in eliminating biofilms.[37] Studies have suggested that AgNPs used in conjunction with graphene oxide may increase the stability, prevent aggregation and promote synergistic antimicrobial properties.[40,45] Furthermore, nanoscale graphene oxide also has inherent antibacterial properties against common dental pathogens.[46] A AgNPs-graphene oxide system was reported to successfully disrupt biofilm in an ex vivo model, however 2.5% NaOCl was still superior in

reducing biofilm volume and microbial cell counts.[40] It is possible that the varying results of different studies could be attributed to the different formulations, characteristics and concentrations of AgNPs used, as well as dissimilar experimental conditions.

Irrigating with AgNPs solutions may influence the physical and structural properties of root dentine. Using a AgNPs-based irrigant as a final rinse almost doubled the fracture resistance of endodontically treated teeth compared to when NaOCl was used.[47] An imidazolium-based AgNPs solution was found to increase dentine roughness, which may have implications regarding the adhesion of obturation and restorative materials to root canal walls.[48] AgNPs-based irrigants did not negatively interfere with the hardness and elastic modulus of dentine[49] or the bond strength and interface permeability of resin-bonded glass-fibre posts.[50] However, AgNPs may be deleterious to the aesthetic outcome of endodontic treatment given their potential to stain dentinal walls and induce discolouration.[51]

Concerns have been raised regarding the cytotoxicity of AgNPs and their tendency to aggregate. The cytotoxic effects could be due to the production of reactive oxygen species that initiate pro-inflammatory host responses, the extent of which depends on the concentration, dimensions and aggregation of AgNPs.[52] Furthermore, aggregation of AgNPs may also affect the release of Ag ions and hence decrease the antimicrobial efficacy.[10] Stabilizing agents such as imidazole can prevent the aggregation of AgNPs and dampen the cytotoxic effects.[53] Abbaszadegan et al reported that imidazolium-based ionic liquid-protected AgNPs exhibited minimal cytotoxicity.[36] Gomes-Filho et al studied the tissue reactions to different concentrations of AgNPs dispersions of rats that were implanted with fibrin-filled polyethylene tubes and found that lower concentrations of AgNPs were more biocompatible.[54] Another in vivo study suggested the tissue reactions of rats injected with either imidazolium-based ionic liquid-protected AgNPs, 2.5% NaOCl or 2% chlorhexidine were all comparable.[55] PVA has been similarly used to stabilize AgNPs, and the resultant irrigant showed promising biocompatibility and no genotoxic effects to fibroblast cells.[56] Encapsulation of AgNPs using a silica coating was found to improve the stability and prolong the antimicrobial activity of an experimental irrigant, resulting in minimal biofilm regrowth at 7 days compared to bare AgNPs which were associated with biofilm regrowth at 2 days.[57] Nevertheless, before advancing to clinical studies, careful investigation into the appropriate use of AgNPs in

endodontic therapies, as well as the potential hazards to human health and the environment, is essential.[10,31]

As demonstrated, numerous studies have been dedicated to investigating the translational potential of AgNPs in endodontic irrigation strategies. In order to provide sufficient context for interpretation, allow for comparison between papers and aid the assessment of potential risks, complete physicochemical characterization is important.[58,59] This includes, but is not limited to, the average diameter, zeta potential, surface plasmon resonance peak and concentration.[32,35,36,57] A full list of the key physicochemical properties of nanomaterials can be found in Table 1.[59] For example, Abbaszadegan et al provided a comprehensive characterization of the synthesized AgNPs with negative, neutral and positive surface charges, reporting the plasmon resonance peak was 400 nm, 425 nm and 407 nm, average size was 7.5 nm, 10.1 nm and 9.0 nm, concentration was 9.7×10^{-8} mol L^{-1}, 4.0×10^{-8} mol L^{-1} and 5.7×10^{-8} mol L^{-1} and zeta potential was -38.0 mV, 0.0 mV and $+50.0$ mV respectively.[36] Overall, it appeared that a number of studies investigating AgNPs in the context of root canal irrigation provided partial characterization and mainly stated the particle size, concentration and, if the nanoparticles were outsourced, the manufacturing companies.[37,39–42,44,47,49–51,54,56] Regardless of the source of nanoparticles, whether they may be purchased from third parties or synthesized by the researchers, declaring the relevant physicochemical properties can help strengthen the scientific value of future studies on nanoparticle applications in endodontics.[7]

Table 1 Important Physicochemical Properties of Nanomaterials

Physical Properties	Chemical Properties
• Size and size distribution • Shape and specific surface area • Agglomeration/ aggregation • Surface modifications and topography • Crystalline structure • Solubility	• Chemical composition and concentration • Surface chemistry • Zeta potential/surface charge • Reactivity (photocatalytic activity, radical formation potential, redox potential) • Hydrophilicity/hydrophobicity

Notes: Data from the Scientific Committee on Emerging and Newly Identified Health Risks of the European Commission.[59]

Chitosan Nanoparticles

Chitosan is a natural, organic biopolymer derived from chitin, which can be sourced from the shells of crabs and shrimps. As chitosan is a cationic compound, it exerts its broad spectrum antimicrobial action by interacting with negatively charged bacterial cell membranes, which increases its permeability and leads to leakage of the intracellular constituents and ultimately cell death.[60] Apart from its antimicrobial properties, chitosan is also biodegradable, biocompatible and has chelating abilities, making it an attractive alternative to modern root canal irrigants.[61]

Chitosan nanoparticle solutions were found to possess antibacterial properties against *E. faecalis* and were able to inhibit biofilm growth.[62,63] However, another study found its antibacterial efficacy may depend on the state of the bacteria, as planktonic bacteria were totally eliminated whilst their biofilm counterparts persisted after 72 hours.[64] Chitosan nanoparticles were able to retain their antimicrobial properties after aging for 90 days.[64] Furthermore, its bactericidal effects are time, concentration and contact-dependent.[64,65] Antibacterial efficacy may be hampered by the presence of inhibitors such as pulpal remnants and bovine serum albumin, but was not affected by dentine, the dentine matrix or lipopolysaccharides (LPS).[66] Conditioning the root canal surface with carboxymethyl chitosan was reported to improve disinfection and prevent bacterial adhesion to dentine prior to obturation.[67] Another study found that although the antibiofilm effects were not enhanced by surface treatment with chitosan nanoparticle formulations per se, it may still provide additional structural benefits via collagen cross-linking.[68] Several methods have been suggested in order to enhance the distribution and the effects of chitosan nanoparticles within the root canal system including electrophoresis,[63] diode laser application,[69] high-intensity focused ultrasound[70] and manual dynamic activation, which involves pumping a well-fitted gutta-percha cone in a prepared root canal to produce microbubbles and enhance fluid dynamics.[71]

Several studies have demonstrated chitosan's ability to act as a chelating agent and potentially improve the wettability of dentine.[62,72,73] At the same time, chitosan nanoparticles showed the potential to stabilize dentine collagen by providing resistance to bacterial collagenase degradation.[74] It has been suggested that conventional chelating agents remain superior in promoting sealer

penetration, as a final rinse of Qmix® or 17% EDTA resulted in roughly double the sealer penetration depth into dentinal tubules at 5 mm from the apex compared to a final rinse of chitosan nanoparticles only.[75] On the other hand, a recent study found that conditioning dentine with a chitosan-hydroxyapatite precursor nanocomplex prior to tricalcium silicate sealer application resulted in significantly improved mean sealer penetration into tubules.[73] Coupled with its antimicrobial properties, chitosan nanoparticle solutions appear to be promising contenders for novel irrigants,[62,76] although it has been suggested that the prolonged treatment time and contact-dependent nature of chitosan nanoparticle-based irrigants are limitations that warrant further research to overcome.[64]

Other Metal and Metal Oxide Nanoparticles

Metal oxide nanoparticles have also been investigated as potential endodontic irrigants. Zinc oxide nanoparticles (ZnONPs) have been touted for their bactericidal properties, with a mechanism of action similar to that of AgNPs.[6] A ZnONPs-based irrigant was found to eliminate planktonic *E. faecalis* and disrupt the biofilm matrix, whilst retaining its antibacterial activity after 90 days of aging.[64] However, its antibacterial efficacy was less pronounced against biofilm bacteria compared to their planktonic equivalents.[64] A combination of AgNPs and ZnONPs in a polymeric solution showed superior antimicrobial activity against *E. faecalis* compared to when each was used independently, although 2.5% NaOCl was still more effective in reducing colony forming units (CFU).[31] A study reported that a ZnONPs-based solution exhibited weaker antimicrobial efficacy against *E. faecalis* compared to 2% chlorhexidine and 5% NaOCl, however the result was not statistically significant.[42] Its use as a final irrigant resulted in an approximately 400N greater mean fracture resistance of root canal treated teeth compared to when NaOCl was used.[47] On the other hand, another study reported the push-out bond strength of endodontic sealers was negatively affected following irrigation with a polymeric suspension containing AgNPs and ZnONPs, possibly due to the deposition of nanoparticles on the dentine surface which decreased sealer adhesion.[77]

Magnesium oxide, titanium dioxide and iron oxide likewise exhibit antimicrobial properties,[6,78] albeit the body of research on these compounds as potential endodontic irrigants is comparably less. Monzavi et al found that a nano-magnesium oxide solution had long-term antimicrobial efficacy in both in vitro and ex vivo environments against *E. faecalis*.[78] Using a titanium dioxide nanoparticle solution as a final rinse was found to result in double the mean fracture resistance of endodontically treated teeth compared to when NaOCl was used.[47] Iron oxide nanoparticles exhibited peroxidase-like activity when synthesized into an irrigating solution with hydrogen peroxide, resulting in antibiofilm and bactericidal activity against *E. faecalis*.[79] However, like AgNPs, metal and metal oxide nanoparticles may also possess a degree of cytotoxicity, hence risk assessment and biocompatibility studies are paramount before proceeding to in vivo research.[6]

Lastly, gold nanoparticles have been reported to be a promising nanomaterial with substantial biomedical applications.[80] However, they have not been as commonly investigated for their use in endodontics, possibly due to reservations regarding their antimicrobial efficacy.[6,31,41] Kushwaha et al evaluated the effect of AgNPs and gold nanoparticle-based irrigants on microbial eradication in teeth inoculated with *E. faecalis*, with or without Nd: YAG laser activation. Whilst Nd:YAG laser activation did improve the antimicrobial activity of gold nanoparticles, the use of AgNPs still resulted in significantly lower mean CFU.[41] However, gold nanoparticles have been shown to exhibit antibacterial properties in other contexts such as in burn wound infections,[81] possibly due to differences in the microbiological composition and the presence of more susceptible pathogens compared to that of root canal infections.

Overall, research into nanoparticle-based irrigants may pave the way for novel and innovative endodontic disinfection strategies. Further studies are needed to fully elucidate the potential of various nanomaterials to be utilized as endodontic irrigants. Furthermore, studies should aim to investigate the methods of nanoparticle incorporation into irrigation solutions, their long-term antibacterial action and in vivo efficacy, whilst minimizing any potential negative or adverse effects.[7]

Photodynamic Therapy

Photodynamic therapy involves the combination of light and photosynthesizers to induce photochemical reactions. Light of specific wavelengths is used to activate the photosensitizers which produce reactive oxygen species and cause cytotoxic effects to the target cells.[82] More recently, studies have been dedicated to exploring disinfection

strategies employing functionalized nanoparticles in conjunction with photodynamic therapy in order to enhance the antibacterial efficacy, encourage irrigant delivery and improve the physical properties of dentine. The antimicrobial properties of nanoparticles combined with the oxidative abilities of photosynthesizers, such as methylene blue, rose bengal and indocyanine green, could give rise to synergistic effects.[83–85] Although several limitations exist, such as the potential of aggregate formation of these compounds and possible difficulties in infiltrating into the complex anatomical spaces,[86,87] further research in this area may help optimize the potential of these novel disinfection modalities.

Rose bengal-conjugated chitosan has been investigated for its antimicrobial potential in several studies by Shrestha and co-workers with promising results. It was found to not only exhibit antibiofilm properties but also low levels of cytotoxicity.[85] Photoactivated rose bengal-conjugated chitosan nanoparticles were also able to inactivate endotoxins.[88,89] In the presence of tissue inhibitors, these functionalized nanoparticles showed a 50% - 65% reduction in planktonic *E. faecalis*, whereas when coupled with photoactivation, it resulted in complete elimination of the microorganisms after 24 hours.[90] Chitosan has also been used in conjunction with methylene blue. Photodynamic activation of chitosan with methylene blue showed superior antibacterial action against *E. faecalis* in infected root canals compared to chitosan or methylene blue alone, although the differences were not statistically significant.[84] Indocyanine green, a photosynthesizer that has recently gained more attention, was investigated in conjunction with AgNPs and laser activation. This combination was able to produce a 99.12% reduction in CFU counts of *E. faecalis*, which was higher than the CFU reduction for diode laser alone and AgNPs alone, albeit not statistically significant.[83] Another study reported that nano-graphene oxide was able to act as a photosynthesizer carrier and enhance the bioavailability and stability of indocyanine green.[91] Photoactivation of indocyanine green incorporated into nano-graphene oxide resulted in 1.3 times greater antibiofilm efficacy compared to photoactivation of indocyanine green alone.[91]

Another benefit of the application of nanoparticles with photosynthesizers is the potential to improve the physical properties of root dentine. Much of the attention has been placed on chitosan nanoparticles decorated with rose bengal as multiple studies have shown its potential to enhance

dentine properties.[92–94] Photodynamic activation of rose bengal was able to enhance collagen cross-linking and simultaneously facilitate chitosan nanoparticle binding to collagenase, which inhibited collagenolytic activity.[94] Its application has been found to enhance the dentine fatigue resistance,[92] nanohardness and modulus of elasticity.[93] Hence, these synergistic actions may help reinforce the structural integrity of weakened dentine.[94]

Intracanal Medicaments

An intracanal medicament is an antibacterial dressing that is placed during multiple-visit endodontic treatment to aid the disinfection of the root canal system. Calcium hydroxide is a frequently used intracanal medicament in modern endodontics due to its proven antibacterial properties.[95,96] However, the evidence has since called into question the efficacy of calcium hydroxide against recalcitrant endodontic infections.[18,65,97] Prolonged placement may also result in weakening of the root structure.[98] Other medicaments such as triple antibiotic pastes have emerged as an alternative, particularly for regenerative endodontic procedures, although some guidelines challenge the evidence supporting its routine use, especially given the risks of antibiotic resistance.[99] All in all, the quest for the ideal intracanal dressing continues with researchers turning towards nanoparticles as a potential area of further innovation and development.

Calcium Hydroxide Nanoparticles

Calcium hydroxide nanoparticles may possess several advantages over their conventional counterparts, such as improved depth of penetration, increased surface area contact with pathogens, superior solubility and greater antimicrobial activity.[100–102] Several studies have found that nano-calcium hydroxide showed deeper penetration into dentinal tubules and had superior antibacterial activity against *E. faecalis* compared to conventional calcium hydroxide.[100–103] Furthermore, nano-calcium hydroxide resulted in less reduction of dentine microhardness compared to conventional calcium hydroxide dressing.[104] Conventional calcium hydroxide also resulted in a greater decrease in fracture resistance compared to the application of their nanosized counterparts.[101] Cytotoxicity, on the other hand, was found to be greater for nano-calcium hydroxide compared to conventional calcium hydroxide, although this finding was not statistically significant.[105]

Antimicrobial Nanoparticles

Apart from calcium hydroxide, other types of antimicrobial nanoparticles, such as AgNPs and chitosan, have been incorporated or formulated into novel intracanal medicaments. It has been reported that the delivery method and duration of placement of nanoparticles were important factors that affected antimicrobial potential.[37] For example, AgNPs exhibited better antibiofilm properties as medicaments rather than irrigants.[37] Longer periods of dressing may lead to better effects, as a 4-week placement resulted in approximately 25% greater proportion of dead cells in *E. faecalis* biofilm compared to placement for 2 weeks.[106] Application of a chitosan nanoparticle-based medicament resulted in appreciable antimicrobial properties and was less detrimental to dentine strength compared to calcium hydroxide, likely due to the promotion of collagen cross-linking and the neutralizing effect on matrix metalloproteinases.[101] However, the same study found that the depth of sealer penetration into dentinal tubules may be compromised due to chitosan's tendency to agglomerate.[101] A study compared the antimicrobial efficacy of nanoparticulate chitosan and poly(lactic-co-glycolic) acid (PLGA) employed as potential intracanal antibiotic delivery agents, with the latter exhibiting better sustained antimicrobial effects over 2 weeks.[107]

Intracanal dressings require certain physical and chemical properties to allow them to remain in the root canal system whilst ideally maintaining a certain level of antimicrobial activity. These properties can be affected by the choice of vehicle.[108] Hydroxyethylcellulose, polyethyleneglycol and carbomer were evaluated as vehicles for AgNPs, and whilst all resulted in stable formulations, hydroxyethylcellulose had the most promising properties such as homogeneity, fluidity and antimicrobial efficacy.[109] On the other hand, a AgNPs-methylcellulose gel was found to have limited antifungal effects compared to calcium hydroxide and 2% chlorhexidine gel. The authors suggested potential reactions between the carrier and AgNPs may have been responsible.[110] Another study also implicated the limitations of viscous carriers in allowing diffusivity of nanoparticles, as ZnONPs gels, with or without AgNPs, eliminated significantly less *E. faecalis* compared to when calcium hydroxide and chlorhexidine were used.[111] The study called for further research to evaluate the different formulations of nanoparticle-based intracanal medicaments to optimize their disinfecting abilities and minimize interferences between the materials.[111]

Combination of Antimicrobial Nanoparticles and Calcium Hydroxide

Studies have explored the concept of incorporating nanoparticles to improve the properties of existing medicaments, particularly calcium hydroxide. It has been suggested that combining calcium hydroxide and antimicrobial nanoparticles together may encourage synergistic effects, collectively resulting in enhanced antimicrobial properties.[91] When AgNPs were added to calcium hydroxide, the antimicrobial activity of this combination was more effective than calcium hydroxide, with or without chlorhexidine, and AgNPs alone,[112–114] but not significantly different to that of triple antibiotic paste.[106] Balto et al remarked on the time-dependent nature of these novel intracanal medicaments, suggesting long-term contact improved the antibiofilm efficacy.[106] Furthermore, no significant colour change of dentine was observed in several studies.[106,115] It has also been reported that the combination of calcium hydroxide and ZnONPs exhibited higher antimicrobial efficacy compared to ZnONPs alone.[116] Another study found that the antimicrobial properties of this pairing was further enhanced by the addition of chlorhexidine.[117]

Porous Calcium Silicate and Bioactive Glass Nanoparticles

The interest in nanoparticulate calcium silicate compounds with internal porous structures stems from their bioactive, biocompatible and osteogenic properties, as well as their potential to act as drug carriers.[118] Porous calcium silicate nanospheres were able to infiltrate dentinal tubules and enhance mineralization, setting up a promising foundation for the development of novel intracanal dressings.[118] With the addition of AgNPs, mesoporous calcium silicate nanoparticles showed sustained release of Ag ions and inhibited *E. faecalis* colonization.[119,120] Other studies found the combination of AgNPs and nano-zinc with mesoporous calcium silicate nanoparticles exhibited good antibiofilm efficacy, minimal cytotoxicity,[121] sustained ion release, dentinal tubule infiltration and negligible changes to the mechanical properties of dentine.[122] The substantivity of these compounds may be attributed to the mesoporous structure which allows these compounds to carry antimicrobial nanoparticles and support their sustained release.[120,123] Bioactive glasses also possess antibacterial properties due to their ability to transform the alkalinity of the environment.[124] Waltimo et al found that

nanoparticulate bioactive glasses released more alkaline species and hence had greater antibacterial activity compared to their micron-sized equivalents.[124] However, this alkalinity may have an impact on the physical properties of dentine as a study reported the application of nanoparticle bioactive glasses resulted in a 20% decrease in flexural strength compared to the saline control, although not statistically significant.[125] Given that radiopacity is an important characteristic of endodontic sealers, nanosized bioactive glasses were modified with bismuth oxide to improve radiopacity whilst maintaining bioactive capacity.[126] Apart from their potential use as intracanal disinfectants, several authors have also advocated the development of calcium silicate nanoparticles into novel root canal sealers due to the aforementioned benefits.[121,122,126]

Obturation Materials and Root Canal Sealers

Subsequent to adequate cleaning and shaping, it is important to seal the root canal system in order to prevent the ingress of bacteria and reduce the risk of re contamination.[127] Therefore, an ideal endodontic obturation material should not only possess adequate physicochemical properties, such as dimensional stability, radiopacity, moisture resistance and non-toxicity, but it should also exhibit some antimicrobial properties against potential surviving bacteria in the root canal.[128,129] Furthermore, bioactivity and remineralization potential may provide additional benefits for root canal obturation materials by strengthening root dentine and promoting formation of hard tissue.[130] However, the most commonly used obturation material, gutta-percha, is characteristically inert, whilst commercially available endodontic sealers also present with a range of drawbacks, including provisional antimicrobial activity and lack of adhesion to the root dentine.[128,131,132] In order to overcome the various limitations, researchers have turned to nanoparticles as a means to improve existing sealers as well as develop new ones.

Quaternary Ammonium Compounds

Quaternary ammonium compounds have been explored as antibacterial compounds in restorative dental materials and also as components of root canal sealers.[133,134] In particular, quaternary ammonium polyethyleneimine (QPEI) is a polycationic disinfectant that has demonstrated broad spectrum antimicrobial and antibiofilm properties via electrostatic interaction with bacterial cell membranes, leading to cell damage and leakage of cellular constituents.[135] QPEI nanoparticles are also unique in that they are able to induce intracellular signals that lead to programmed cell death.[6] Furthermore, these compounds are capable of offering long-term antimicrobial effects due to their insoluble nature.[133] When incorporated into epoxy resin-based sealers, the addition of QPEI nanoparticles was found to enhance the sealer's antibacterial activity.[134,136–138] It has been suggested that QPEI nanoparticles improve the bactericidal action of sealers by not only directly disrupting membrane integrity, but also indirectly acting on distant regions of biofilms, although the exact mechanism has yet to be elucidated.[137,139] The addition of QPEI nanoparticles into AH Plus™, an epoxy resin-based sealer, and Pulp Canal Sealer™, a zinc oxide eugenol-based sealer, was also found to modulate osteoblast and osteoclast growth and differentiation by regulating intracellular signalling pathways, with the degree of impact depending on the concentration, bone cell type and sealer used.[140] Furthermore, studies have shown the possibility of combining commercially available sealers and QPEI nanoparticles without having detrimental effects on sealer cytotoxicity and the physicochemical properties, such as solubility, flow, compressive strength and dimensional stability.[134,140,141] However, it has been reported that the incorporation of QPEI nanoparticles led to no significant improvement in the antibacterial efficacy of AH Plus™.[141] Another study reported that the antibiofilm effects against *E. faecalis* of QPEI nanoparticle-modified AH Plus™ may be strain-dependent.[142] In contrast, the addition of QPEI nanoparticles to Pulp Canal Sealer™ improved the antibacterial and antibiofilm efficacy against *E. faecalis*.[141,142] Contradicting results between studies may be attributed to differences in experimental protocols[143] or as a consequence of interferences with the base sealer constituents.[141,142] Furthermore, although the addition of QPEI nanoparticles to existing root canal sealers may provide a host of different benefits, it is also important to consider the potential drawbacks of this nanomaterial, such as polymerization shrinkage, solvent sorption, altered mechanical properties and cytotoxicity.[144]

Several studies have been dedicated to formulating novel endodontic sealers based on quaternary ammonium methacrylates. Dimethylaminohexadecyl methacrylate (DMAHDM) is a long-chain chemical variant that can be immobilized in a resin matrix upon the formation of bonds via free radical polymerization, hence leading to prolonged

antibacterial potency via penetration and disruption of bacterial membranes upon contact.[145] With the addition of other antimicrobial nanoparticles, synergistic effects may be able to enhance the antibacterial activity and remineralization potential of these novel root canal sealers. A study found the combination of AgNPs and DMAHDM in a novel endodontic sealer showed promising antibiofilm efficacy against *E. faecalis*, as the novel sealer reduced the biofilm CFU by 6 orders of magnitude more than AH Plus[TM].[146] It has been reported that an experimental sealer containing amorphous calcium phosphate nanoparticles and DMAHDM exhibited antibiofilm activity and high levels of calcium and phosphate ion release, suggesting a potential to encourage remineralization and strengthen compromised root structures.[147–149] Apart from the development of experimental sealers, another study modified an existing epoxy resin-based sealer using DMAHDM and AgNPs which resulted in improved and prolonged antimicrobial properties. Whilst AH Plus[TM] lost its antibacterial efficacy by day 7, the modified sealer retained its antibacterial properties for up to 14 days.[150]

Nanostructured Silver Vanadate with AgNPs

As previously discussed, AgNPs possess potent antimicrobial properties, however concerns have been raised regarding their tendency to aggregate. Hence, to maximize their potential application as endodontic sealers, nanostructured silver vanadate has been suggested as a means to stabilize AgNPs.[151,152] The incorporation of nanostructured silver vanadate with AgNPs into endodontic sealers appeared to have no dire effects on the physicochemical properties.[153] However, conflicting results have been published regarding its effects on the antibacterial activity of sealers. One study reported that there was no additional antibacterial benefit gained when the sealers were in a freshly mixed state,[154] whilst another reported enhanced antibacterial properties in both freshly mixed and set states.[155] Furthermore, the extent of benefit from the inclusion of silver vanadate nanowires decorated with AgNPs may depend on the type of commercial sealer and the concentration used.[156] It has been suggested that only higher concentrations of these compounds enhance the antimicrobial activity of sealers, thus more clinically relevant experiments should be carried out to ascertain the extent of the benefits against the costs, such as cytotoxicity and tooth discolouration.[143]

Zinc Oxide Nanoparticles

ZnONPs have likewise been used to formulate novel endodontic sealers or modify existing zinc oxide eugenol sealers in order to improve physicochemical and antibacterial properties.[157,158] One of the first studies to highlight the potential uses of nanoparticles in endodontics incorporated ZnONPs, with or without chitosan nanoparticles, into a zinc oxide eugenol-based sealer, which resulted in improved antibacterial properties.[158] Obturation with gutta-percha and nano-zinc oxide eugenol sealer resulted in less apical microleakage compared to AH 26[TM] and micro-zinc oxide eugenol sealer.[157] The cytotoxic effects of nano-zinc oxide eugenol sealer were reported to be not more than that of other commercially available sealers, such as AH 26[TM] and Pulpdent[TM].[159] When incorporated into polyethylene tubes implanted in rats, nano-zinc oxide eugenol sealer instigated similar tissue reactions compared to Pulp Canal Sealer[TM] and AH 26[TM].[160] Versiani et al modified a zinc oxide eugenol sealer with different amounts of ZnONPs and found that replacing 25% of zinc oxide powder with ZnONPs resulted in improved physicochemical properties, such as dimensional stability, flowability, radiopacity and solubility.[161] A recent study combined ZnONPs and AgNPs with an experimental urethane acrylate composite sealer which resulted in better antibacterial activity at lower concentrations compared to when either of the nanoparticles were added independently.[162]

Chitosan Nanoparticles

As prior mentioned, given that the antibacterial properties of chitosan nanoparticles are time and contact-dependent, these nano-biopolymers certainly have potential to be formulated into novel antibacterial endodontic sealers.[64,65,68] Several studies have explored the possibility of using chitosan nanoparticles to modify existing zinc oxide eugenol sealers which improved their antibacterial and antibiofilm efficacy.[68,158] A combination of chitosan nanoparticles and ZnONPs improved the antibiofilm efficacy of a calcium hydroxide-based sealer, Apexit Plus[TM], however only the sealer modified with ZnONPs was effective against the endodontic isolate strain of *E. faecalis*.[163] It has been reported that the incorporation of chitosan nanoparticles into ThermaSeal Plus[TM], an epoxy resin-based sealer, promoted its antibacterial efficacy.[67] However, when added to MTA fillapex[TM], a calcium silicate-based sealer, the same extent of improvements was not observed.[67] A possible

explanation for the varying results is the dissimilar physicochemical properties of different sealers which may interfere with or dampen any additional antibacterial benefit from chitosan nanoparticles.[67]

Other Nanoparticle-Based Modifications of Obturation Materials and Sealers

Polymeric nanoparticle carriers have also been used to modify endodontic obturation materials and sealers so as to improve the sustained and temporal release of antimicrobial compounds whilst reducing the toxicity of these compounds in their free form.[164] For example, an experimental root canal sealer that incorporated propolis-loaded PLGA nanoparticles resulted in antimicrobial effects against *E. faecalis, Streptococcus mutans* and *Candida albicans*.[165] Recently, the application of doxycycline-functionalized PolymP-*n* Active nanoparticles was found to occlude dentinal tubules and exert antibiofilm effects against *E. faecalis*.[166]

Apart from antibacterial efficacy, nanoparticle-based modifications have been used to enhance other physicochemical properties of endodontic sealers, including bioactivity and radiopacity. One study found the inclusion of bioactive glass and hydroxyapatite nanoparticles enhanced the bioactivity of an epoxy resin-based sealer.[167] Another study reported mesoporous calcium silicate nanoparticles could provide multiple functions as a potential root filling material including drug delivery, bioactivity and osteostimulation.[168] In order to enhance the radiopacity of Portland cement-based sealers, Viapiana et al incorporated either niobium oxide or zirconium dioxide in micro- or nanoparticle forms. However, the modified sealers were not sufficiently radiopaque according to the specifications set by the International Organization for Standardization.[169,170]

Lastly, several studies have investigated nanoparticulate modifications to gutta-percha. An in vitro study reported that nanodiamond-embedded gutta-percha functionalized with amoxicillin introduced antibacterial properties to this originally inert obturation material.[171] Following that, a clinical study used nanodiamond-embedded gutta-percha to obturate the middle third of root canals and observed no adverse effects and no difference in healing outcome compared to the control up to a period of 6 months.[172] As with most of these innovations, further research is required to determine the optimal concentration and tailor the synthesis of nanoparticle-based obturation materials so as to deliver adequate

antimicrobial efficacy without compromising the physicochemical properties.

Regenerative Endodontic Strategies

Conventional endodontic treatment concerns the cleaning, shaping and obturation of root canal systems. However, since the pioneering report by Banchs and Trope, more researchers have shifted their focus towards regenerative therapies.[173] These strategies aim to restore the form and function of a tooth by eliminating infection, promoting the development and closure of immature root apices and re-establishing pulpal vitality.[99,173] Tissue engineering and biological procedures that involve stem cells, bioactive molecules and scaffolds form the basis of regenerative endodontics.[174]

Nanoparticle-based carrier systems have been proposed as a method for the sustained release of bioactive molecules,[175,176] which are a crucial component of regenerative endodontics as they modulate cellular activity, such as proliferation, migration and differentiation.[177] As nanoparticles possess enhanced solubility, high surface-area-to-volume ratio and minute dimensions, nanoparticle-based carrier systems may improve the dissolution and absorption of bioactive molecules and drugs.[176] Several polymeric nanocarriers have been investigated in the context of conventional root canal treatment, such as those previously discussed including chitosan, PLGA and PolymP-*n* Active nanoparticles,[107,165,166] as well as regenerative therapies.[178–182] Bovine serum albumin-loaded chitosan nanoparticles were found to improve the viability of stem cells from the apical papilla (SCAP) and enhance alkaline phosphatase activity.[180] Dexamethasone-loaded chitosan nanoparticles were able to improve the odontogenic differentiation of SCAP.[181] Dentine conditioning with either chitosan nanoparticles or dexamethasone-modified chitosan nanoparticles also had the potential to mitigate the deleterious impacts of NaOCl and LPS, whilst stimulating SCAP adherence, viability and differentiation.[179,182] The sustained release of dexamethasone encapsulated by poly (ε-caprolactone)-forsterite nanocomposite fibrous membranes has been reported to promote osteogenic differentiation and proliferation of stem cells from human exfoliated deciduous teeth.[178]

Nanoparticles have also been customized to develop various forms of scaffolds, which are another key component of regenerative endodontic therapies. Scaffolds are temporary structures that mimic the extra-cellular matrix to support the growth and differentiation of stem cells and

aid the controlled release of drugs and bioactive molecules.[183] They can also be combined with nanocarriers to allow multiple bioactive molecule releasing mechanisms.[175] By taking advantage of chitosan's ability to adapt and swell to fit the configuration of different sites and its readiness to be conjugated with other molecules,[184] Bellamy et al reported that a carboxymethyl chitosan-based scaffold with transforming growth factor-β1-loaded chitosan nanoparticles was able enhance the viability, differentiation and migration of SCAP.[185] Similarly, it has been demonstrated that odontogenic differentiation of human dental pulp cells can also be stimulated by the incorporation of dexamethasone and bioactive glass nanoparticles into a nanofiber scaffold system.[186] The addition of bioactive glass nanoparticles may improve the mechanical properties of scaffolds whilst promoting bioactivity and mineralization via the release and deposition of calcium.[187] Another study used cellulose nanocrystals to reinforce hydrogel scaffolds, resulting in increased stiffness and stability. The reinforced hydrogel was also laced with platelet lysate, which is rich in proangiogenic and chemotactic factors and has the potential to enhance the revascularization and regeneration of pulpal tissue.[188]

Lastly, nanoparticles have also been used in novel methods for the assessment of regenerative outcomes. Biz et al complexed gold nanoparticles and a biodegradable organic plastic, L-lysine, to create compounds that would be readily internalized by stem cells. The study found that the resultant increase in cell radiopacity could allow for microtomography to identity the presence of viable cells after regenerative procedures without apparent deleterious cytotoxic effects.[189]

Potential Adverse Effects of Nanoparticles

When investigating the potential translational applications of nanoparticles, attention must be given to the possible adverse effects.[31] Nanotoxicology has hence emerged as a field of study focused on evaluating the hazards associated with nanomaterial exposure.[190] The characteristics responsible for nanoparticles' unique properties are also responsible for their potential toxicity to oral tissues, systemic health and the environment.[191] The extent of toxicity depends on a myriad of factors, such as the material, concentration, duration of exposure, aggregation, particle size, geometry and surface charge.[2]

Effects on Oral Tissues

Nanoparticle-based endodontic therapies are not without disadvantages, such as the potential for cytotoxic effects to periapical and pulpal tissues. As previously mentioned, the application of AgNPs has been met with reservations due to potential cytotoxicity.[192] The cytotoxicity of AgNPs-based irrigants on rat tissue was reported to be concentration-dependent, as more persistent inflammation was associated with the application of a 47 ppm AgNPs dispersion when compared to 23 ppm.[54] These findings were echoed by another study that reported AgNPs in low concentrations were minimally cytotoxic to L929 murine fibroblasts.[36] AgNPs-based irrigation solutions were reported to be less cytotoxic than conventional endodontic irrigants, such as 3% NaOCl and 17% EDTA, to human gingival fibroblasts.[57] The extent of cytotoxicity indeed depends on the material, as more favourable interactions have been associated with chitosan nanoparticles.[193] Shrestha et al found that these naturally occurring biopolymers exhibited no cytotoxicity to both macrophages[88] and fibroblasts.[85] Photoactivated rose bengal-conjugated chitosan likewise showed low levels of cytotoxicity.[85,88] Furthermore, special efforts should be made to ensure the biocompatibility of novel nanoparticle-based endodontic obturation materials and intracanal medicaments, given the possibility of extrusion beyond the apex and direct contact with the periapical tissues.[20,128] For instance, the widely used intracanal medicament calcium hydroxide is known for being cytotoxic to periapical tissues,[194] however its nanoparticle equivalents were not found to be significantly less cytotoxic.[100] A novel endodontic sealer incorporating QPEI nanoparticles was reported to have favourable biocompatibility, with no observed lysis of L929 fibroblasts.[134] Similarly, a mesoporous calcium silicate nanoparticle-based root filling material had no cytotoxic effects to periodontal ligament cells and even encouraged osteogenic properties by modulating gene expression.[168]

Effects on Systemic Health

There are multiple routes that may allow the entry of nanoparticles into the human body, including the lungs, skin, gastrointestinal tract and systemic administration.[191] Given that nanoparticles have similar dimensions to biological molecules, they are readily absorbed by various organs and tissues, and have been found to accumulate in the lungs, liver and reticuloendothelial system.[195–197]

Toxic concentrations can cause damage by both reactive oxygen species-dependent and independent mechanisms.[198] Oxidative stress may have a significant role in causing tissue damage by instigating DNA mutations, cytokine release, protein denaturation, lipid peroxidation and cell apoptosis.[190,191] For example, AgNPs have been reported to induce inflammation in the lungs and damage alveoli.[199] When exposed to peripheral blood mononuclear cells, AgNPs at high concentrations were found to be potentially cytotoxic and could modulate cytokine expression.[200] The organic biopolymer chitosan, on the other hand, is generally considered to be non-toxic and biocompatible.[61] However, it has been suggested that greater cytotoxicity is associated with smaller particle sizes and higher concentrations.[201] QPEI nanoparticles, another frequently investigated organic nanomaterial in dentistry, were incorporated into restorative materials and reported to exert no adverse effects on macrophage viability.[202] These examples highlight the importance of a full understanding of the potential hazardous health effects of nanoparticle applications in endodontics before clinical research is undertaken.

Environmental Effects

There also exists environmental concerns associated with the use of nanoparticles.[58] Nanoparticles may act as pollutants and accumulate in the environment and, given that the toxic effects are often concentration-dependent, bioaccumulation could result in subsequent systemic toxicity to exposed living organisms.[203] A level of uncertainty exists regarding the ideal policies for proper recycling and safe disposal of nanoparticles, as the extent of the harmful effects from their biopersistence has yet to be fully elucidated.[2]

Overall, the potential adverse effects of the application of nanoparticles in endodontics must not be overlooked as they could impact treatment success, patient health and the environment.[31] It is important to thoroughly investigate the extent of potential harm and ascertain safe methods of application to minimize the biological and environmental risk whilst maximizing the therapeutic benefit.

Conclusion

The advances in nanotechnology may lead to a new era of translational applications of nanoparticles in endodontic treatment. The current literature suggests that nanoparticles may be developed for a variety of purposes in endodontics, such as disinfection strategies, photodynamic therapy, obturation materials and regenerative procedures. Nonetheless, nanoparticles should not be applied with a "one size fits all" approach, given that different materials, formulations and combinations will generate different properties, both beneficial and adverse. Thus, nanoparticle applications in endodontics have a lot of potential but there is still some way to go before the basic research translates to clinical studies.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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