Antibacterial Nanoparticles in Endodontics: A Review

Annie Shrestha, BDS, MSc, PbD, and Anil Kishen, BDS, MDS, PbD

Abstract

Introduction: A major challenge in root canal treatment is the inability of the current cleaning and shaping procedures to eliminate bacterial biofilms surviving within the anatomic complexities and uninstrumented portions of the root canal system. Methods: Nanoparticles with their enhanced and unique physicochemical properties, such as ultrasmall sizes, large surface area/mass ratio, and increased chemical reactivity, have led research toward new prospects of treating and preventing dental infections. This article presents a comprehensive review on the scientific knowledge that is available on the application of antibacterial nanoparticles in endodontics. Results: The application of nanoparticles in the form of solutions for irrigation, medication, and as an additive within sealers/restorative materials has been evaluated to primarily improve the antibiofilm efficacy in root canal and restorative treatments. In addition, antibiotic or photosensitizer functionalized nanoparticles have been proposed recently to provide more potent antibacterial efficacy. Conclusions: The increasing interest in this field warrants sound research based on scientific and clinical collaborations to emphasize the near future potential of nanoparticles in clinical endodontics. (J Endod 2016;42:1417–1426)

Key Words
Antibacterial, chitosan, functionalized, nanoparticles, silver

Nanomaterial denotes a natural, incidental, or manufactured material containing particles in an unbound state or as an aggregate or agglomerate in which 50% or more of the particles in number, size, distribution, or 1 or more external dimensions is in the size range of 1–100 nm (1). Nanomaterials offer unique physicochemical properties, such as ultrasmall sizes, large surface area/mass ratio, and increased chemical reactivity, compared with their bulk counterparts (2, 3). The increased surface to volume ratio and increased number of atoms that are present near the surface compared with micro-/macrostructures 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 in order to achieve maximal therapeutic efficacy with minimal side effects (4, 5).

Nanotechnology has progressed rapidly in science and technology, creating a myriad of biomedical applications such as drug delivery, tissue regeneration, antimicrobial application, gene transfection, and imaging (2, 3, 5, 6). The term nanodenistry implies the application of nanomaterials and dental nanorobots toward diagnosis and treatment, with the goal of improving comprehensive oral health. The scope of such strategies includes a wide variety of oral health–related issues such as treatment of dentin hypersensitivity, biofilm elimination, diagnosis and treatment of oral cancers, bone replacement materials, and so on. In the field of endodontics, the development of nanomaterials is focused on steps that would improve antimicrobial efficacy, mechanical integrity of previously diseased dentin matrix, and tissue regeneration. Currently, newer technologies are being tested in endodontics, mainly toward overcoming the microbial challenge (7, 8).

Based on the composition, nanoparticles are generally classified as either naturally occurring or synthetic (Table 1). They are further categorized as organic or inorganic in nature. Based on the shape, they are classified as particles, spheres, tubes, rods, plates, and so on. Functionalized nanoparticles are those that have a core of 1 material and additional molecules or proteins bonded on its surface or encapsulated within. Depending on the specific applications, nanoparticles can be functionalized with peptides, drugs, photosensitizers, and so on (9, 10). The core nanoparticles can be used as a convenient surface for molecular assembly and may be composed of inorganic or organic materials. An additional layer of linker molecules is required to proceed with functionalization wherein the linker molecules have reactive groups at both ends that bind various moieties like biocompatables (dextran), antibodies, fluorophores, and so on onto the core nanoparticle.

This review aimed to provide comprehensive information on the scientific knowledge that is available for the use of nanotechnology toward antibacterial applications in endodontics.

Antibacterial Nanoparticles

Bacterial biofilms are considered the major cause of both primary and secondary root canal infection (11, 12). Conventionally, chemical antimicrobials are used...
within the root canals in combination with mechanical instrumentation to achieve effective microbial reduction before filling the root canal with an inert filling material (7, 8, 13). The current level of evidence showed that despite the advancements in treatment strategies the rate of treatment failure has not decreased below 18%–26% for the past 4 to 5 decades (14–16). This could be mainly attributed to the inability or limitations of current technologies to deal with the disease process as a whole (17). Other than this, the conservative management of infections involving topical or systemic antibiotics has been shown to be ineffective because of several challenging factors (Table 2) (19, 20). Furthermore, the use of antibiotics is highly debatable, as “In the ongoing war against antibiotics, the bacteria seem to be winning, and the drug pipeline is verging on empty” (21).

Because of the shortcomings of current antibiofilm strategies in root canal treatment, advanced disinfection strategies are being developed and tested. We review newer antibacterial nanoparticles that have been introduced at the laboratory levels with significant potential for eliminating endodontic biofilms.

### Chitosan Nanoparticles

Chitosan (poly[1,4-β-D-glucopyranosamine]), a deacetylated derivative of chitin, is the second most abundant natural biopolymer. Nanoparticles of chitosan could be synthesized or assembled using different methods depending on the end application or the physical characteristics required in the nanoparticles (22). Chitosan has received significant interest in biomedicine (22–24) because of its versatility in various forms such as powder (micro- and nanoparticles), capsules, films, scaffolds, hydrogels, beads, and bandages (22). Chitosan has a structure similar to extracellular matrix components and is therefore used to reinforce the collagen constructs (25). This hydrophilic polymer with a large number of hydroxyl and free amino groups can be subjected to numerous chemical modifications and grafting, resulting in functionalization, which is discussed in a separate section (26–28). Nanoparticles of chitosan have been developed mainly for antibacterial and drug/gene delivery applications.

Chitosan has excellent antibacterial, antiviral, and antifungal properties (29). In case of bacteria, gram-positive bacteria were more susceptible than gram-negative ones. The minimum inhibitory concentrations ranged from 18–5000 ppm depending on the organism, pH, degree of deacetylation (DD), molecular weight, chemical modifications, and presence of lipids and proteins (29, 30). DD is known to influence the antibacterial activity. With higher DD, the number of amine groups increases per glucosamine unit, and, thus, chitosan showed higher antibacterial efficacy (31). Chitosan nanoparticles (CS-NPs) by virtue of their charge and size are expected to possess enhanced antibacterial activity.

#### Mechanism of Action

The proposed mechanism of action is contact-mediated killing that involves the electrostatic attraction of positively charged chitosan with negatively charged bacterial cell membranes (Fig. 1). This might lead to altered cell wall permeability, eventually resulting in the rupture of cells and leakage of the proteinaceous and other intracellular components (29, 32). Under transmission electron microscopy, the bacterial cells were noted to be completely enveloped in the chitosan, forming an impermeable layer (35). This could result in the prevention of transport of essential solutes leading to cell death. In case of fungi, chitosan was hypothesized to enter the cell and reach the nucleus, bind with DNA, and inhibit RNA and protein synthesis.

#### Current Applications

For the first time, Kishen et al (34) looked into the efficacy of various cationic nanoparticles to improve root canal disinfection. Dentin treated with nanoparticles resulted in significantly reduced adherence of Enterococcus faecalis. The antibacterial efficacy of CS-NPs and zinc oxide in disinfecting and disrupting E. faecalis (ATCC and OG1RF) biofilms was evaluated later on (35). These nanoparticles eliminated biofilms on a concentration- and time-dependent manner and also retained their antibacterial properties after aging for 90 days (35). CS-NPs can be delivered within the anatomic complexities and dentinal tubules of an infected root canal to enhance root canal disinfection (36). Biofilm bacteria are known to express efflux pumps as a resistance mechanism to antimicrobials (37). When tested along with known efflux pump inhibitors, the antibacterial efficacy of CS-NPs was not affected against bacterial biofilms compared with cationic photosensitizers (38). Another challenge in using antibacterial agents inside the root canal space is the neutralizing effect of different tissue inhibitors (39). Similarly, tissue inhibitors such as pulp and serum albumin inhibited the antibacterial effect of CS-NPs significantly (40); whereas dentin, the dentin matrix, and lipopolysaccharides did not affect the efficacy of CS-NPs. In another in vitro study, CS-NPs were used in combination with different brands of chlorhexidine to eliminate E. faecalis with potential application toward tissue regeneration using membrane barriers in periapical surgery (41).
of CS-NPs provided a significantly greater reduction of colony-forming units in agar plates as well as infected collagen membranes.

**Clinical Significance**

Initial studies highlighted that cationic CS-NPs hold significant potential to achieve improved root canal disinfection. However, the prolonged treatment time required to achieve effective bacterial elimination and the effect of tissue inhibitors presented as major setbacks to CS-NPs. This warranted methods to overcome these shortcomings in future research toward the application of CS-NPs.

**Bioactive Glass**

Bioactive glass (BAG) received considerable interest mainly because of its osteoinductive effect and antibacterial properties toward various orthopedic and dental applications. The antibacterial activity of BAGs has been investigated using 3 different approaches (Fig. 2A–C) (42–45). BAG consists of SiO₂, Na₂O, CaO₂, and P₂O₅ at different concentrations and depends on the local physiological changes for its antibacterial effects (45).

**Mechanism of Action**

The antibacterial activity of BAG depends on the following factors acting simultaneously (45):

1. **High pH**: An increase in pH because of release of ions in an aqueous environment
2. **Osmotic effects**: An increase in osmotic pressure above 1% is inhibitory for many bacteria
3. **Ca/P precipitation**: Induces mineralization on the bacterial surface

Furthermore, the release of Ca²⁺, Na⁺, PO₄³⁻, and Si⁴⁺ could lead to the formation of bonds with the mineralized hard tissues.
Current Applications

BAGs in micro- and nanoforms have been tested to improve root canal disinfection (46–48). The nanometric BAG used by Zehnder et al (47) was amorphous in nature, ranging from 20–60 nm in size. In vitro root canal disinfection studies showed a significantly less antibacterial effect of BAG compared with calcium hydroxide in preventing residual bacterial growth (47). Waltimo et al (44) suggested that an ideal preparation of 45S5 BAG suspensions/slurries for root canal disinfection should combine a high pH induction with a capacity for continuing release of alkaline species. Despite the higher specific surface area of nanometric BAG, the micrometric counterpart had a considerably higher alkaline capacity and eliminated biofilms significantly better (44). Planktonic bacteria were killed significantly better compared with biofilm bacteria (49, 50). The reduced antibacterial efficacy of nano-BAG compared with micro-BAG has been mainly contributed to the 10-fold increase in silica release and solution pH elevation by more than 3 units by the latter (49).

Clinical Significance

The conflicting evidence on BAG microparticles and nanoparticles to generate a significant antibacterial effect warrants further research before clinical application.

Silver Nanoparticles

Silver compounds and nanoparticles have been widely used in biomedicine, mainly because of their antibacterial property (51, 52). In case of dental application, silver and its nanoparticles have been tested for application as dental restorative material, endodontic retrograde filling material, dental implants, and caries inhibitory solution (53).

Mechanism of Action

Silver is known to produce an antibacterial effect by acting on multiple targets starting from interaction with the sulfhydryl groups of proteins and DNA, alter the hydrogen bonding/respiratory chain, unwind DNA, and interfere with cell-wall synthesis/cell division (54, 55). Silver nanoparticles (Ag-NPs) are known to further destabilize the bacterial membrane and increase permeability, leading to leakage of cell constituents (51, 56).

Current Applications

Hiraishi et al (57) tested silver diamine fluoride against *E. faecalis* biofilms in vitro and showed complete elimination of 48-hour biofilms after 60 minutes of interaction; 3.8% silver diamine fluoride was also found deposited on the surface of dentin and penetrated up...
to 40 μm into dentinal tubules. Another study showed that the antibiofilm efficacy of Ag-NPs was significant when applied as a medicament and not as an irrigant (58); 0.02% Ag-NP gel as medicament for 7 days was found to be significantly better in *E. faecalis* biofilm disruption compared with calcium hydroxide groups and syringe irrigation with higher concentration Ag-NP (0.1%) solution. They suggested that when used as medicament there is a prolonged interaction between positively charged Ag-NPs and negatively charged biofilm bacteria/structure resulting in this difference. Ag-NP suspension when combined with calcium hydroxide showed significantly reduced bacteria/structure resulting in this difference. Ag-NP suspension when combined with calcium hydroxide showed significantly reduced *E. faecalis* from root canal dentin (59). Another recent study showed variation of antibacterial efficacy of Ag-NPs with charge and in the presence of dentin as a tissue inhibitor (60). Ag-NPs of 9 nm size and positive charge had the smallest minimal inhibitory concentration against planktonic *E. faecalis* when compared with sodium hypochlorite, chlorhexidine, and the other tested Ag-NPs. Positively charged Ag-NPs completely prevented the growth of planktonic *E. faecalis* in as short as 5 minutes of contact time and retained the antibacterial efficacy even in the presence of dentin. In addition, these Ag-NPs were found to be cytocompatible to fibroblast cells. Ag-NP–loaded mesoporous bioactive glass (Ag-MBG) was tested against 4-week-old *E. faecalis* biofilms in root canals. A significant disruption of the biofilm structure was observed compared with mesoporous bioactive glass and calcium hydroxide, which has been mainly contributed to sustained Ag ions released from the mesoporous structure (42). All these positive results with *in vitro* studies warrant further experiments in clinically relevant *in vitro* models/*in vivo* situations to confirm the advantages of using Ag-NPs for root canal disinfection.

Two main issues associated with Ag-NPs are the potential discoloration of dentin and toxicity toward mammalian cells. Gomes-Filho et al (61) tested 2 different concentrations of Ag-NPs (90 μm) in Wistar rat models. The *in vitro* tissue reaction of a mild to moderate chronic inflammatory response was observed and was concentration dependent. After 15 days, 47 ppm Ag-NPs showed a reaction that was comparable with 2.5% sodium hypochlorite. The toxic concentrations of silver ions are approximately 1–10 mg/L, and Ag-NPs are 10–100 mg/L for eukaryotic cells (62). Research has been directed toward modifying and developing Ag-NPs with specific antibacterial activity and lower cytotoxicity to host cells (63). Other than the size and structure of Ag-NPs, the presence of proteins in a biological medium has been shown to greatly influence the interaction and toxicity of these nanoparticles toward cells.

**Clinical Significance**

Ag-NPs with significant antibacterial activity could be used for root canal disinfection. However, the prolonged interaction time required by Ag-NPs for effective bacterial killing needs to be considered, and its use ideally should be limited to medicament rather than as an irrigant. In addition, toxicity associated with silver ions should not be ignored with careful selection of nontoxic concentration for use *in vivo*.

**Nanoparticles Incorporated Sealers and Restorative Materials**

Root canal filling material is expected to occupy and seal the prepared canal space to prevent bacterial recontamination. One of the desired properties of root canal sealer is antibacterial efficacy, which can eliminate bacteria remaining in the root canal system and prevent bacterial penetration in case of leakage. Commonly used sealers are known to possess antibacterial activity for a maximum period of 1 week, with most of them showing a significant decrease in antibacterial properties immediately after its setting (64–66). The addition of NPs in sealers or restorative materials has been tried mainly to achieve reduced bacterial penetration, increased antibacterial activity within dentinal tubules, increased substantivity of sealers, and increased diffusion of antibacterial activity.

**Current Applications**

The incorporation of CS-NP and zinc oxide nanoparticles in a zinc oxide–based root canal sealer and resin-based root canal sealer improved the antibacterial property and ability to diffuse the antibacterial component (34). The addition of nanoparticles did not deteriorate the flow characteristics of the root canal sealer. DaSilva et al (67) also used CS-NP incorporated zinc oxide–eugenol sealer for filling treated bovine root canals *in vitro*. The nanoparticle modified sealer inhibited biofilm formation with a significantly lower percentage of biofilm covering the sealer-dentin interface. Another recent study added CS-NPs to epoxy resin sealer (ThermaSeal, York, PA), resulting in enhanced antibacterial ability by direct-contact and membrane-restricted tests (68). The CS-NP incorporation significantly increased the antibacterial efficacy of these different root canal sealers even after a 4-week aging time with lesser biofilm formation at the sealer-dentin interface with and without surface treatment. BAG-NP has been recommended to promote closure of the interfactual gap between the root canal walls and core filling materials (69).

The combination of polysiloxane or polycaprolactone and BAG nanoparticles that could create a hydroxyapatite interface and thus ultimately make the use of an endodontic sealer unnecessary has been suggested. The incorporation of BAG fillers into the polymers under investigation made the resulting composite materials bioactive and improved their immediate sealing ability. It was concluded that polysiloxane and polycaprolactone composites with BAG showed promising results as single root canal filling materials (69).

Quaternary ammonium polyethyleneimine nanoparticles (QPEI-NPs) have been used to improve the antibacterial efficacy of various root canal sealers and temporary restorative materials (70–73). Barros et al (70) incorporated QPEI-NPs into AH Plus (Dentsply International, York, PA) and Pulp Canal Sealer EWT (SybronEndo, Orange, CA), which increased the wettability and surface charge of both AH Plus and Pulp Canal Sealer EWT. However, antibacterial efficacy of both the sealers was lost after 7 days of setting with and without the QPEI-NPs. In contrast to the previous finding, another study displayed significantly increased antibacterial efficacy of AH Plus and QPEI-NPs (72). Similarly, Beyth et al (73) and Kessler Silvero et al (74) showed that the incorporation of QPEI-NPs in 2-paste epoxy-amine resin sealer resulted in the attraction of *E. faecalis* to the cationic surface and caused membrane destabilization and total inhibition of bacterial growth. These nanoparticles when bound to the sealer surface presented with the advantage of lower toxicity because of the impediment of nanoparticle penetration into eukaryotic cells (71, 73). QPEI-NPs exerted bacterial effects mainly by adsorption and penetration through the bacterial cell wall, interaction with the proteins and fat layer in the cell membrane, blocking the exchange of essential ions, destabilization of the cell membrane, and cell death (75).

**Clinical Significance**

Most of the nanoparticles tested for root canal disinfection depend on contact-mediated and time-dependent antibacterial activity. Thus, the incorporation of various nanoparticles into sealers or root filling materials significantly improved the antibacterial efficacy by inhibition of bacterial biofilm formation on the surface as well as the resin-dentin interface. The limited *in vitro* studies on nanoparticle-
Figure 3. (A and B) Transmission electron microscopic images for planktonic E. faecalis after treatment with CSRB-NPs for 15 minutes. Aggregates of CSRB-NPs could be seen surrounding the bacterial cell. (C) Nanoparticles were found attached to the bacterial cell surface and forming an envelope. The cells did not show any disruption of morphology. (D) After PDT of the sensitized bacteria, various stages of membrane damage as well as release of cell constituents were evident. (E) Scanning electron microscopic images of 3-week-old multispecies biofilms presented as a uniformly thick matlike structure covering the entire dentin surface. (Inset) Three specific bacterial morphologies are evident in higher magnification. The surface showed an abundant polymeric matrix (magnified area shown by the open arrow). (F) CSRB-NP treatment rendered the dentin surface clean of the biofilm with open dentinal tubules. (G) RB treatment showed cleaner areas of dentin along with dense bacterial aggregates (inset: magnified area shown by the arrowhead). (A–D, Reprinted from Nanomedicine: Nanotechnology, Biology and Medicine, volume 10, Shrestha A, Hamblin MR, Kishen A. Photoactivated rose bengal functionalized chitosan nanoparticles produce antibacterial/biofilm activity and stabilize dentin-collagen, pages 491–501, Copyright 2014, with permission from Elsevier. E–G, Reprinted from Journal of Endodontics, volume 40, Shrestha A, Kishen A, Antibiofilm efficacy of photosensitizer-functionalized bioactive nanoparticles on multispecies biofilm, pages 1604–10, Copyright 2014, with permission from Elsevier.)
incorporated root filling materials emphasizes thorough and standardized studies toward possible aspects of clinical application.

**Functionalized Antimicrobial Nanoparticles**

The word *functionalize* from Merriam-Webster dictionary means to organize (as work or management) into units performing specialized tasks. Functionalization could alter the surface composition, charge, and structure of the material wherein the original bulk material properties are left intact (76, 77). In a functionalized nanoparticle, the inorganic or polymeric materials usually form the core substrate. Functionalized nanoparticles containing various reactive molecules and decorated with peptides or other ligands have led to new possibilities of combating antimicrobial resistance (9, 10) (Figs. 3A–G and 4).

**Current Applications**

Nanoparticle-based photosensitizers have been considered to potentiate photodynamic therapy efficacy (78, 79). Functionalized nanoparticles with photosensitizer molecules offer unique physicochemical properties of nanoparticles, such as ultrasmall sizes, large surface area/mass ratio, and increased physical/chemical reactivity. As mentioned in the review by Kishen (13), the combination of nanoparticles with photosensitizers could be achieved by

1. Photosensitizers supplemented with nanoparticles
2. Photosensitizers encapsulated within nanoparticles
3. Photosensitizers bound or loaded to nanoparticles
4. Nanoparticles themselves serving as photosensitizers (13)

The combinations of nanoparticles with photosensitizer have been found to enhance antimicrobial PDT (78, 80) (Table 3).

Methylene blue–loaded poly(lactic-co-glycolic) acid (MB-PLGA) nanoparticles have been tested in vitro on *E. faecalis* biofilm and human dental plaque bacteria in combination with PDT (81). The cationic MB-PLGA nanoparticles exhibited significantly higher bacterial phototoxicity in both planktonic and biofilm phases. It was concluded that cationic MB-PLGA nanoparticles have the potential to be used as carriers of photosensitizer photodynamic therapy (PDT) within root canals. Similarly, photosensitizer-bound polystyrene beads with rose bengal (RB) as a photosensitizer were used by Bezman et al.

![Figure 4](image-url) Representative field emission scanning electron microscopic images showing colonization of *E. faecalis* on root canal walls. (A) Blank control group, (B) calcium hydroxide group, (C) MCSNs group, (D) nanosilver-incorporated MCSNs prepared by the adsorption method group, (E and F) dead bacteria in direct contact with Ag-MCSNs contained perforated membranes (arrows), (F and I) a bacterium in direct contact with Ag-MCSNs showing deformed cell membrane (arrows), and (G) nanosilver-incorporated MCSNs prepared by the template method group. (Reproduced with permission of *International Journal of Nanomedicine* [Dove Medical Press Ltd.], from Effects of adsorbed and templated nanosilver in mesoporous calcium-silicate nanoparticles on inhibition of bacteria colonization of dentin, Fan W, Wu D, Tay FR, et al, volume 9, pages 5217–30, copyright 2014; permission conveyed through Copyright Clearance Center, Inc.)
TABLE 3. Combinations of Nanoparticles with Photosensitizer Have Been Found to Enhance Antimicrobial PDT Efficacy because of Several Factors

| Higher concentration of photosensitizer per mass with resultant production of ROS |
| Reduced efflux of photosensitizer from the target cell, thereby decreasing the possibility of drug resistance |
| Possibility of targeting the bacteria because of greater interaction associated with the surface charge |
| Greater stability of photosensitizers after conjugation |
| Less physical quenching effect because of photosensitizer aggregation |
| Controlled release of ROS after photoactivation possible |


(82) after activation with light, improving bacterial elimination with reactive oxygen species. However, the binding of RB with silica nanoparticles resulted in a slower yield of reactive oxygen species compared with the free RB (80). The slower decay has been suggested to be advantageous in certain applications when light/dose fraction and/or deeper tissue or bacterial penetration is required (80).

The application of naturally occurring biopolymers such as chitosan could circumvent such issues of biocompatibility (83, 84) and favor intimate contact between the photosensitizer and the aqueous suspension of microorganisms. In RB photosensitizer functionalized CS-NPs, singlet oxygen released by the photoactivation of the photosensitizer potentiated the antibacterial activity of CS-NPs (85, 86). Shrestha et al (85) used RB to functionalize CS-NPs (CSRB-NPs), which possessed the combined properties of chitosan and RB. These functionalized CSRB-NPs interacted greatly with bacteria and biofilms, leading to membrane damage and leakage of cellular constituents (Fig. 5). The increased uptake into biofilms of CSRB-NPs compared with the photosensitizer alone resulted in significant elimination of biofilm bacteria and disruption of the structure after photoactivation. Multispecies biofilms of Strepptococcus oralis, Prevotella intermedia, and Actinomyces naeslundii grown on dentin sections were completely disrupted with a reduction in viable bacteria and biofilm thickness after CSRB-NP and PDT treatment (Fig. 3) (86). The CSRB-NPs were found to maintain the antibacterial efficacy in the presence of bovine serum albumin, which is a strong tissue inhibitor (87). The higher affinity of cationic CS-NPs to bacterial cell surfaces and singlet oxygen release after photoactivation of RB provided a synergistic antibacterial mechanism for CSRB-NPs. Furthermore, CSRB-NPs could be used for the treatment of infected dentin because it showed a collagen cross-linking ability that could prevent degradation of the dentin matrix. Lipopolysaccharide, the toxic component of gram-negative bacteria, was inactivated by photoactivated CSRB-NPs as shown by reduced inflammatory markers in macrophage cells (88). CSRB-NPs provided a single-step treatment of infected root dentin by combining the properties of chitosan and that of the photosensitizer to eliminate bacterial biofilms.

Fan et al (42, 89) used Ag-MBG powders to eliminate E. faecalis biofilms of 4 weeks inside root canals. Ag-MBG showed Ag-NPs incorporated in the mesopores that resulted in sustained release of Ag ions. Compared with unmodified mesoporous bioactive glass, Ag-MBGs showed a significant structural disruption of biofilms. The same group of investigators have also used mesoporous calcium silicate nanoparticles (MCNSNs) with nanosilver incorporation (Ag-MCNSNs) (90). These modifications were prepared using both the adsorption and template methods. Ag-MCNSNs exhibited a sustained release of Ag ions over time. Planktonic E. faecalis was killed by Ag-MCNSNs and showed significantly better antibacterial effects when compared with unmodified MCNSNs. When dentin surfaces were treated with Ag-MCNSNs, they aggregated on the dentin surface of the root canal walls and infiltrated into dentinal tubules after ultrasound activation (89, 90). This dentin surface treatment resulted in the significant inhibition of adherence and colonization of E. faecalis on dentin. The results of the present study indicated that nanosilver could be incorporated into MCNSNs using the template method. The templated nanosilver released silver ions, inhibited the growth and colonization of E. faecalis both in the planktonic form and as biofilms on dentin surfaces, and showed lower cytotoxicity compared with Ag-MCNSNs (adsorbed nanosilver). PLGA polymer solution containing zinc oxide, Ag, and zinc oxide/Ag nanoparticles have been tested to decontaminate root canals with E. faecalis (91). The minimum colony-forming unit counts were observed in the sodium hypochlorite groups followed by zinc oxide/Ag and Ag groups. Despite the not so strong bacterial model, the nanoparticles containing polymer solutions could not achieve total bacterial elimination.

Clinical Significance
Functionalization of various nanoparticles showed an overall increase in efficacy along with rapid action against bacteria. The photosensitizer functionalized nanoparticles could be used as a final root canal disinfection strategy, whereas functionalized BAG and Ag-NPs still need to be considered only as long-term root canal medicament. Optimization of the concentration and application method of these functionalized nanoparticles is under constant progress.

Conclusion
The therapeutic efficacy of antibacterial nanoparticles necessitate optimization of their physical, chemical, and biological characteristics, keeping in mind the tissue-specific factors at the site of infection and the method to deliver the nanoparticles effectively in the target tissue. Nanoparticle-based treatment strategies have the potential to improve antibacterial/antibiofilm efficacy in endodontics. Functionalized nanoparticles via surface modifications would provide the opportunity to deliver drugs/chemicals to the site of infection in order to selectively interact with biofilm and bacteria. Newer multifunctional nanoparticles are being developed based on the clinical requirements in collaboration with engineers, clinicians, and biologists. In terms of toxicity of nanoparticles, safe usage guidelines should be developed that could be expanded toward growing areas of newer antibacterial nanoparticles. This whole concept of nanoparticles in health care and endodontics should be accepted with positive zeal and caution in future developments.

Acknowledgments
The authors deny any conflicts of interest related to this study.

References

Review Article

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