Silver nanoparticles in endodontics: recent developments and applications

Aysenur Oncu 1, Yan Huang 2, Gulin Amasya 3, Fatma Semra Sevimay 3, Kaan Orhan 4, Berkan Celikten 1*

1Department of Endodontics, Ankara University Faculty of Dentistry, Ankara, Turkey
2Department of Dental Hygiene Research & Development in Health & Care, Artevelde University of Applied Sciences, Ghent, Belgium
3Department of Pharmaceutical Technology, Ankara University Faculty of Pharmacy, Ankara, Turkey
4Department of Dentomaxillofacial Radiology, Ankara University Faculty of Dentistry, Ankara, Turkey

ABSTRACT

The elimination of endodontic biofilms and the maintenance of a leak-proof canal filling are key aspects of successful root canal treatment. Several materials have been introduced to treat endodontic disease, although treatment success is limited by the features of the biomaterials used. Silver nanoparticles (AgNPs) have been increasingly considered in dental applications, especially endodontics, due to their high antimicrobial activity. For the present study, an electronic search was conducted using MEDLINE (PubMed), the Cochrane Central Register of Controlled Trials (CENTRAL), Google Scholar, and EMBASE. This review provides insights into the unique characteristics of AgNPs, including their chemical, physical, and antimicrobial properties; limitations; and potential uses. Various studies involving different application methods of AgNPs were carefully examined. Based on previous clinical studies, the synthesis, means of obtaining, usage conditions, and potential cytotoxicity of AgNPs were evaluated. The findings indicate that AgNPs are effective antimicrobial agents for the elimination of endodontic biofilms.

Keywords: Antimicrobial agents; Endodontic biofilm; Silver nanoparticles

INTRODUCTION

A main cause of failure and secondary infection in root canal treatment (RCT) is the presence of biofilms [1], which are organized polymeric structures formed by microorganisms. The extracellular matrix of a biofilm is secreted by bacteria and is composed of metabolic-output polymers that adhere strongly to surfaces [2,3]. Biofilms develop in stages: initial adherence of microbes to a surface or bacterial cell, generation of microcolonies, maturation, and finally, the expansion of the biofilm [4,5]. The main purpose of endodontic treatment is to eliminate the complex, resistant polymeric biofilm structure. Therefore, in RCT, chemical irrigation agents and medicaments are used in addition to mechanical preparation. During the irrigation process, sodium hypochlorite (NaOCl), EDTA, and chlorhexidine (CHX) solutions are applied at different concentrations to eradicate the smear layer [6]. A number of hydrogel-based pharmaceuticals have also been produced for placement in the root canal
between treatments. However, no approach to treating stubborn infections has been entirely successful. Clinical research has shown variable success rates for non-surgical endodontic therapies, ranging from 73.5% to 92.3% [7].

Nanotechnology, which emerged in the 21st century, led to a paradigm shift in dentistry (Figure 1). Nanoparticles (1–100 nm) form the foundation of this technology [8]. Many nanomaterials occur naturally or arise via chemical synthesis. Because of their antibacterial properties and high surface-area-to-volume ratio [9], nanoparticles have attracted considerable attention from endodontic researchers and clinicians.

Metallic nanoparticles that disrupt bacterial cell membranes have long been available [10]. To treat persistent infections [11], nanoparticles of silver, gold, copper, or zinc—all of which have unique physical properties and mechanisms underlying their antimicrobial activities—have been used [12,13]. Silver nanoparticles (AgNPs) are among the most well-studied due to their wide range of antimicrobial properties against various bacteria, viruses, and fungi [11,14]. In endodontics, AgNPs have been tested for use as endodontic retrofill materials, canal sealers, root canal pharmaceuticals, and irrigation solutions [15]. In this study, recent evidence from in vitro and in vivo studies was reviewed regarding the chemical, physical, and antimicrobial properties of AgNPs, as well as their dosage and cytotoxicity.

**REVIEW**

**Biofilm in endodontic diseases**

Biofilms are highly organized, surface-adherent structures of microcolonies [16] (Figure 2). The main component of biofilms is an exopolymERIC matrix consisting of polysaccharides, proteins, enzymes, and bacterial metabolites [17,18]. Exopolysaccharides are synthesized both intracellularly and extracellularly, and have skeletal functions [19] (Figure 3). Moreover, biofilms are in contact with other compounds that play roles in bacterial adhesion and resistance [20]. Another component of biofilms is protein, which facilitates stabilization and binding to dentin. The glucan-binding proteins of *Streptococcus mutans*, a major cause of dental caries, are well-studied components that are critical to biofilm formation [21,22]. The maturing biofilm varies depending on environmental and nutritional factors as well
as fluid movement [23]. The bacterial cells in these structures communicate via their own signals or those of other microbial cells [24]. Based on extracellular signal generation and detection, quorum sensing (a cell-to-cell communication process) increases with population density [25,26]. Microbial biofilm infections can reappear after long periods of inactivity [27]. Secondary endodontic infections can become acute due to asymptomatic processes. The main known cause of recurrent apical periodontitis after RCT is Enterococcus faecalis [28], a Gram-positive facultative anaerobic bacteria species that is among the most commonly isolated from root canal systems [29]. The removal of these bacteria from the canal is a major obstacle, since they can remain alive under a wide variety of acidic and basic conditions, as well as under conditions of long-term nutritional deprivation [28].

The biofilm of E. faecalis contains extracellular DNA (eDNA), which is released from the cell in various ways, as well as exopolysaccharides, proteins, and lipids [30]. A previous report showed that eDNA is produced during an early stage of E. faecalis biofilm formation [31]. This species

![Figure 2. Cycle of biofilm formation.](https://rde.ac)

![Figure 3. Structural elements of the exopolymeric matrix.](https://rde.ac)
also has a peptidoglycan cell wall, which improves bacterial survival [32]. Scanning electron microscopy has shown that *E. faecalis* adheres to collagen structures, colonizes dentin surfaces, and can progress along dentin tubules of the root canal, resulting in organized biofilms [33].

Chemical irrigation solutions, intracanal preparations, and local antibiotics have been used for years to eliminate biofilms. However, microorganisms may develop resistance to these antimicrobial agents over time. Therefore, research has focused on new antibiofilm strategies [34,35].

**Antimicrobial nanoparticles**

Nanotechnology, or the examination and application of certain objects of extremely small size, can be adapted in different scientific fields, such as chemistry, biology, physics, materials science, and engineering, as well as the health sciences. A nanoparticle is defined as a nano-object, approximately 1 to 100 nm, with 3 external nanoscale dimensions.

Nanoparticles have gained many new applications in dentistry due to their remarkable physical properties, such as small size, large surface area, surface charge, and shape. To eliminate root canal infections, chemical and mechanical instrumentation is performed before filling. The use of nanoparticles in the disinfection process is a new strategy to reduce the treatment failure rate [36,37]. Previous studies have reported that particle size is an important factor in the antimicrobial activity of nanoparticles [38]. In addition, the high charge density and large surface areas of nanoparticles allow bacterial cells to interact more with the negatively charged surface [39]. Metallic and organic nanoparticles with different morphologies have been used in dentistry to combat drug-resistant bacteria. In addition, nanoparticles obtained from natural biopolymers such as chitosan or nanoparticle-incorporated biomaterials have been found to exhibit superior antimicrobial properties [37,40]. Metallic nanoparticles, such as copper, gold, titanium, cerium, magnesium, iron, and zinc, exhibit antimicrobial activity upon contact with bacterial cells. After attachment to the cell membrane and entrance into the cell, nanoparticles interact with vital cell components such as DNA and RNA and alter the cell membrane permeability, genetic material, ribosomes, and proteins. These effects mainly depend on the capacity of metallic nanoparticles to produce reactive oxygen species (ROS), which can alter the metabolic activity of bacteria [13]. Major ROS that can cause oxidative cellular damage include superoxide, hydrogen peroxide, and hydroxyl radicals. Additionally, after metal ions are released from metal oxides and attach to the cell membrane, they can bind to functional groups of proteins and adversely impact normal cellular functions.

Although these nanoparticles are a potential technology for endodontic disinfection, their long contact time and toxicity can be significant disadvantages [41]. Among the metallic nanoparticles, AgNPs stand out due to their surface properties, particle reactivity in solution, and ion release.

**Synthesis of AgNPs**

Various physical, chemical, and biological methods have been adopted for the synthesis of AgNPs. A reliable and environmentally friendly methodology for the synthesis of metal nanoparticles is a key goal in nanotechnology [42]. Physical and chemical syntheses tend to be more difficult, expensive, and dangerous than the biosynthesis of AgNPs [43,44]. Biological procedures for the synthesis of AgNPs, which involve microorganisms and plants, have enormous advantages over physical and chemical methods due to the use of nontoxic
and biocompatible substrates and relatively easier synthesis in environmental terms [45].

In recent years, the development of bio-inspired green synthesis of AgNPs has been a focus in medical science and disease treatment [46]. Biomolecules in plant extracts, which are involved in the reduction of metal ions to nanoparticles, provide a single-stage and environmentally friendly synthesis. In addition to serving as reducing agents in the green synthesis of AgNPs and gold nanoparticles, these biomolecules act as capping or stabilizing agents [47]. Previous studies have suggested that the antimicrobial properties of AgNPs are affected by factors such as shape, size, and concentration. In a study by Hong et al., [48] 3 types of AgNPs were synthesized, and their antimicrobial effects were compared against *Escherichia coli*. According to the results, nanocubes and nanospheres exhibited stronger antibacterial effects than nanowires. This can be attributed to the fact that nanocups and nanospheres interact faster and more frequently with the cell membrane, as they have larger surface areas and greater reactivity. Most such research has focused on various plant sources for synthesis, diverse characterization techniques for identification, and antimicrobial activity against pathogens [42]. Information about the physical appearance and the characterization of AgNPs can be revealed using ultraviolet-visible spectroscopy, electron microscopy, and energy-dispersing spectroscopy [49].

**Mechanisms of action of AgNPs**

Many studies have shown that the antimicrobial effects of AgNPs are associated with oxidative dissolution and silver ion release. Silver ions have high affinity for electron-donating groups (such as sulfhydryl, amino, imidazole, phosphate, and carbonyl groups), which are densely located on membranes or proteins [50]. Thus, they can act on diverse components of bacterial cells (Figure 4). These ions can adhere to the cell wall and cytoplasmic membrane via electrostatic attraction, and they can also adhere to sulfur-rich proteins, thereby increasing the permeability of the membrane and damaging these structures [51]. This can also result in the uptake of free silver ions into the cell, disrupting ATP molecules, thus preventing DNA replication or resulting in the formation of ROS via

![Figure 4. Possible antibacterial mechanisms of AgNPs. AgNPs can: 1) bind to the cell membrane, membrane proteins, and DNA bases, leading to the disruption of normal function; 2) release silver ions, affecting the membrane, DNA, and proteins; and 3) generate ROS, which may also affect DNA, the cell membrane, and membrane proteins. AgNP, silver nanoparticle; ROS, reactive oxidative species.](https://rde.ac)
AgNPs [52]. In Gram-negative bacteria, pores in the outer membrane also promote the uptake of AgNPs [53]. In addition, AgNPs modify the effects of phosphotyrosine, impairing communication between organelles [52,54]. All of these mechanisms result in oxidative stress in the cell and increased quantities of free oxygen radicals, as well as cell lysis due to protein denaturation [50,55]. Another important property of nanoparticles is their large surface area. AgNPs with a larger surface area have greater silver ion density [56,57]. Additionally, AgNPs further destabilize bacterial membranes, increase permeability, and cause leakage of cell components [37]. The response of microbial cells to silver ions can differ, so the properties of AgNPs and their relationships with cells should be elucidated to better understand antibiofilm activity [58].

**AgNP characterization and analysis**

Many techniques have been introduced and applied in laboratory research (Table 1), including scanning electron microscopy [33,59], transmission electron microscopy [44], scanning electrochemical microscopy [60], atomic force microscopy [61], dynamic light scattering [62], ultraviolet-visible spectroscopy [63], and confocal laser scanning microscopy analysis [64].

**Endodontic applications of AgNPs**

The success of RCTs depends on the removal of endodontic biofilms from canal walls, the elimination of microorganisms, and leak-proof canal filling. For the endodontic treatment of teeth with complex root canal anatomy, chemomechanical canal preparation should be performed during instrumentation. Nanoparticles have also been used for disinfection in endodontics. Metallic nanoparticles are attractive due to their clinically effective antimicrobial properties. Many studies have involved the use of AgNPs to eliminate biofilm layers, which are the main cause of secondary infections [9,15,34]. Lotfi et al. compared the effectiveness of AgNPs against *E. faecalis* with that of NaOCl in an irrigation solution; 5.25% NaOCl and low-concentration AgNPs showed similar bactericidal effects [65]. Hiraishi et al. reported that biofilms were completely eliminated 60 minutes after administration of 3.8% sodium diamine fluoride [66]. Another study found that an AgNP solution destroyed fewer bacteria than a CHX solution, but dissolved more biofilm [67]. Wu et al. suggested that antimicrobial efficacy varies by application technique [68]. Treatment with 0.02% AgNP medicament gel was significantly more successful in disrupting biofilm structure than

**Table 1. Current analysis methods used for silver nanoparticle characterization**

| Methods                        | Abbreviation | Uniqueness of the method                                                                 | Reference                        |
|--------------------------------|--------------|----------------------------------------------------------------------------------------|----------------------------------|
| Scanning electron microscopy   | SEM          | SEM can be used to completely differentiate particle sizes, size distributions, nanomaterial shapes, and surface morphologies of synthesized particles in microscale and nanoscale. Additionally, a histogram can be derived from the images by manually measuring and counting particles or using specific software. | Fissan et al., 2014 [59]         |
| Transmission electron microscopy | TEM         | While TEM has advantages that include good spatial resolution and additional analytical measurements, sample preparation is time-consuming. | Zhang et al., 2016 [44]          |
| Scanning electrochemical microscopy | SEC M     | SEC M is a noninvasive method developed to measure load/mass transport rates across surface film using electrodes. | Blanchard et al., 2016 [60]      |
| Atomic force microscopy        | AFM          | AFM can also be used to characterize the real-time interaction of nanomaterials with supported lipid layers. However, a major disadvantage is that the lateral dimensions of the samples are overestimated. | Zhang et al., 2016 [44] and Eaton and Batziou, 2019 [61] |
| Dynamic light scattering       | DLS          | DLS, a method that depends on the interaction of light with particles, is used to characterize the particle size and dimension distribution in aqueous or physiological solutions. | Leung et al., 2006 [62]          |
| Ultraviolet-visible spectroscopy | UVS         | UV-Vis spectroscopy is quick, simple, precise, and selective for nanoparticles. Additionally, it requires only a short time for measurement, and calibration is not required for particle characterization of colloidal suspensions. | Zhang et al., 2016 [44] and Das et al., 2009 [63] |
| Confocal laser scanning microscopy | CLSM       | Rapid visualization of dynamic processes in fixed and live cells enables the detailed morphological analysis of tissues and automatic collection of 3-dimensional data. | Paddock and Eliceiri, 2014 [64]  |
Applications of silver nanoparticles in endodontics

Table 2. Recent applications of silver nanoparticles in endodontics

| Related area                  | Application procedure                                                                                                                                                                                                 | Main results                                                                                                                                                                                                 | Reference                                                                 |
|-------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Irrigation solution           | The antimicrobial effects of 6 solutions were compared: 0.85% saline (control), 2% CHX, 5% NaOCl, 1% NaOCl, 1% AgNP, and 26% ZnONP.                                                                                     | The 1% AgNP and 26% ZnONP solutions were similarly effective against *E. faecalis* biofilm relative to conventional endodontic irrigants.                 | De Almeida et al., 2018 [74]                                             |
| Irrigation solution           | Equal amounts of 2% CHX and 15 µg/mL AgNPs were mixed homogeneously and compared with the solutions used individually.                                                                                        | The CHX-AgNP combined solution exhibited higher efficacy than the individual solutions.                                                                                                                     | Charannya et al., 2018 [69]                                             |
| Root canal medicaments        | The antibacterial efficacy of silver nanoparticles as an irrigant (0.7% AgNP) or medicament (0.02% and 0.001% AgNP) against *E. faecalis* biofilms was evaluated. | As a medication, 0.02% AgNP gel significantly impaired the structural integrity of the biofilm and resulted in the fewest viable *E. faecalis* cells remaining after treatment. | Wu et al., 2014 [68]                                                   |
| Development of bioactive material | The antibacterial activities of NanoAg and NanoAg-MTA against 4 types of anaerobic pathogens were tested in vitro. Each gram of MTA powder was mixed with 350 µL of 25 ppm, 12.5 ppm, and 6.25 ppm preparations of NanoAg solution on sterile glass slabs using a sterile spatula. | AgNPs can effectively enhance the antibacterial activity of MTA against anaerobic periodontal/endodontic pathogens.                                                                                       | Bahador et al., 2015 [72]                                              |
| Root canal sealer             | Methacrylate-resin dual-cured root canal sealer contained 5% dimethylaminohexadecyl methacrylate (DMAHDM), 0.15% AgNP, and nanoparticles of amorphous calcium phosphate (NACP) at 10%, 20%, and 30% mass fractions. Antimicrobial properties against *E. faecalis* were measured. | The novel therapeutic root canal sealer with triple bioactive agents of DMAHDM, AgNP, and NACP neutralized acid and raised the pH, regenerated dentin minerals, and increased root dentin hardness. | Baras et al., 2019 [73]                                                |
| Regenerative endodontic        | In regenerative endodontics, the antibacterial effectiveness of double antibiotic paste (1 mg/mL DAP), silver nanoparticle (0.02% AgNP) gel, and tailored amorphous multiporous bioactive glass (100 mg/mL TAMP-8G) against 3 weeks of *E. faecalis* biofilms were evaluated. | These medicaments can function as potent intracanal drugs for regenerative endodontic procedures. However, complete elimination of *E. faecalis* biofilms occurred only at recommended concentrations and was made possible with AgNPs. | Athanassiads et al., 2007 [76]                                         |
| Fiber post cementation        | The effect of AgNP solution on the mechanical properties of resin cements used for fiber post bonding was investigated.                                                                                      | The results indicate that the AgNP solution can be used as an irrigation protocol before glass fiber post cementation.                                                                                  | Suzuki et al., 2019 [77]                                                |

CHX, chlorhexidine; NaOCl, sodium hypochlorite; AgNP, silver nanoparticle; ZnONP, zinc oxide nanoparticle; MTA, mineral trioxide aggregate; E. faecalis, *Enterococcus faecalis*.

treatment with 0.01% AgNP gel, 0.01% AgNP irrigation solution, and calcium hydroxide. When AgNPs are employed as a medicament, an extended interaction occurs between positively-charged AgNPs and negatively-charged biofilm bacteria. In another in vitro study, researchers investigated the effectiveness of AgNPs against *E. faecalis*, *Klebsiella pneumoniae*, and *Candida albicans*, and they found that the greatest antimicrobial effect was achieved with a combination of 15 µg/mL AgNP and 2% CHX solution [69].

Recently, Yousefshahi et al. applied calcium hydroxide with silver, copper, zinc, or magnesium; the combination of 1% AgNP and calcium hydroxide was more effective against biofilms than calcium hydroxide paste alone, but a mixture of 1% copper and calcium hydroxide paste was the most effective [70]. Additionally, less leakage has been found to occur when using nanosilver-coated gutta-percha points [71]. In another study, calcium-disilicate-based mineral trioxide aggregate (MTA), which has known antibacterial properties, was combined with AgNPs; the AgNP-MTA formulation inhibited the growth of *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, and *Prevotella intermedia* [72]. Additionally, Baras and Melo et al. combined 5% dimethylaminohexadecyl methacrylate sealer with 0.15% AgNPs and reported a strong antibiofilm effect with no reduction in sealing ability [73].

In summary, nanotechnology has been used in a wide range of endodontic applications (Table 2). Clinicians should be aware of the latest developments and information on how best to use nanoparticles.

**Efficacy of AgNPs against *E. faecalis***

Endodontic treatments aim to annihilate microorganisms and their biofilm architecture and thereby minimize the treatment failure rate. In many studies, AgNPs have been applied through various methods for this purpose. Almedia et al. reported that a solution of 1%
AgNPs had effects against *E. faecalis* similar to those of conventional irrigation solutions [74]. Likewise, Halkai *et al.* found that biosynthesized AgNPs greatly inhibited *E. faecalis* [75]. Calcium hydroxide paste has been used as a medication for RCT for many years [76]. However, this material has also been shown to fail to provide adequate disinfection and biofilm elimination. Afkhami *et al.* used AgNPs as carriers for calcium hydroxide and found that this treatment had the potential to remove *E. faecalis* from root dentin [78]. In another study, Wu *et al.* noted that the effectiveness of AgNPs depends on the method of application; 0.02% AgNP medicament gel significantly altered biofilm structures and resulted in fewer post-treatment *E. faecalis* cells than treatment with 0.01% AgNP gel and calcium hydroxide [68]. In another study, it was found that Ag–Ca–Si mesoporous nanoparticles had an enhanced ability to prevent the growth of *E. faecalis* on the dentin surface. Findings also included high pH and the continuous release of Ag, Ca\(^{2+}\), and SiO\(_3\)\(^{2-}\) ions, although this method did not completely eliminate *E. faecalis* [79]. Laboratory research on this subject is still ongoing.

**Potential toxicity of AgNPs**

Over the years, numerous *in vitro* and *in vivo* experiments have been conducted to investigate the toxic effects of AgNPs on living tissues and organisms [80]. The factors that affect the toxicity of AgNPs include particle shape, size, and surface chemistry; crystallinity; capping agents; ionic strength; pH; and the presence of ligands, divalent cations, and macromolecules [81]. Due to the exposed and complex nature of AgNPs, uncertainty (and to some degree controversy) remains regarding the extent to which each constituent ion, ion-protein complex, and particle contributes to cellular toxicity [82]. In some *in vitro* studies, it has even been reported that AgNPs cause oxidative stress and disrupt the mitochondrial function of human cells [83]. Panáxc4x8dek *et al.* used low concentrations of AgNPs against multiresistant bacteria and noted that low concentrations were not cytotoxic for potential medical applications in mammalian cells [84]. It has also been suggested that the green synthesis of AgNPs does not affect human dermal fibroblasts when administered at a concentration of less than 32 \(\mu\)g/mL [85]. This underscores the importance of the method of synthesis and the concentration density. Importantly, the toxicity is always related to the dose and duration of contact. Direct contact with the oral cavity, teeth, and surrounding tissues is an important consideration due to the potentially harmful effects of AgNP treatment in endodontic applications.

Over the past decades, the application of AgNPs in endodontics has attracted increasing attention. Using the published literature, we reviewed recent findings regarding the unique chemical, physical, and antimicrobial properties of AgNPs, as well as dosage and cytotoxicity. Although several studies have revealed promising clinical treatment results, the potential cytotoxicity of AgNPs should also be taken into consideration, even given the superior antimicrobial properties of these particles in endodontic disinfection.

**CONCLUSIONS**

Biofilms are an important factor that should be eliminated in the treatment of primary and recurring endodontic infections. Nanoparticles present new opportunities for endodontic disinfection. Based on their superior antimicrobial properties, AgNPs have attracted attention in this field, and many studies have been conducted. Based on these studies, it can be concluded that treatment with AgNPs is an effective method to eliminate endodontic biofilms. In light of this finding, studies on the applications of nanoparticles in endodontics should be continued.
REFERENCES

1. Vestby LK, Grønseth T, Simm R, Nesse LL. Bacterial biofilm and its role in the pathogenesis of disease. Antibiotics (Basel) 2020;9:59.
2. Neelakantan P, Romero M, Vera J, Daooud U, Khan AU, Yan A, Cheung GS. Biofilms in endodontics-current status and future directions. Int J Mol Sci 2017;18:1748.
3. Mensi M, Scotti E, Sordillo A, Agosti R, Calza S. Plaque disclosing agent as a guide for professional biofilm removal: a randomized controlled clinical trial. Int J Dent Hyg 2020;18:285-294.
4. Tolker-Nielsen T. Biofilm development. Microbiol Spectr 2015;3:MB-0001-MB-2014.
5. Abusrewil S, Alshanta OA, Albashaireh K, Alqahtani S, Nile CJ, McLean W. Detection, treatment and prevention of endodontic biofilm infections: what’s new in 2020? Crit Rev Microbiol 2020;46:194-212.
6. Haapasalo M, Shen Y, Wang Z, Gao Y. Irrigation in endodontics. Br Dent J 2014;216:299-303.
7. García-Guerrero C, Delgado-Rodríquez CE, Molano-González N, Pineda-Velandia GA, Marin-Zuluaga DJ, Leal-Fernandez MC, Gutmann JL. Predicting the outcome of initial non-surgical endodontic procedures by periapical status and quality of root canal filling: a cohort study. Odontology 2020;108:697-703.
8. Schmaltz G, Hickel R, van Landuyt KL, Reichl FX. Nanoparticles in dentistry. Dent Mater 2017;33:1298-1314.
9. Kaur P, Luthra R. Silver nanoparticles in dentistry: an emerging trend. SRMJ Res Dent Sci 2016;7:162.
10. Abdul Dayem A, Hossain MK, Lee SB, Kim K, Saha SK, Yang GM, Choi HY, Cho SG. The role of reactive oxygen species (ROS) in the biological activities of metallic nanoparticles. Int J Mol Sci 2017;18:120.
11. Tang S, Zheng J. Antibacterial activity of silver nanoparticles: structural effects. Adv Healthc Mater 2018;7:e1701503.
12. Prabhu S, Poulose EK. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int Nano Lett 2012;2:32.
13. Stohs SJ, Bagchi D. Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med 1995;18:321-336.
14. Samiei M, Farjami A, Dizaj SM, Lotfipour F. Nanoparticles for antimicrobial purposes in endodontics: a systematic review of in vitro studies. Mater Sci Eng C 2016;58:1269-1278.
15. Salata O. Applications of nanoparticles in biology and medicine. J Nanobiotechnology 2004;2:3.
16. Du Q, Fu M, Zhou Y, Cao Y, Guo T, Zhou Z, Li M, Peng X, Zheng X, Li Y, Xu X, He J, Zhou X. Sucrose promotes caries progression by disrupting the microecological balance in oral biofilms: an in vitro study. Sci Rep 2020;10:2961.
17. Pamp SJ, Gjermansen M, Tolker-Nielsen T. The biofilm mode of life: mechanisms and adaptation. Biosci Horiz 2007;10:37-69.
18. Teves A, Blanco D, Casarotto M, Torres J, Alvarado D, Jaramillo DE. Effectiveness of different disinfection techniques of the root canal in the elimination of a multi-species biofilm. J Clin Exp Dent 2019;11:e978-e983.
19. Nwodo UU, Green E, Okoh AI. Bacterial exopolysaccharides: functionality and prospects. Int J Mol Sci 2012;13:14002-14015.
20. Sutherland I. Biofilm exopolysaccharides: a strong and sticky framework. Microbiology (Reading) 2001;147:3-9.
21. Lynch DJ, Fountain TL, Mazurkiewicz JE, Banas JA. Glucan-binding proteins are essential for shaping *Streptococcus mutans* biofilm architecture. FEMS Microbiol Lett 2007;268:158-165.

22. Lemos J, Palmer S, Zeng L, Wen Z, Kajfasz J, Freires I, Abranches J, Brady L. The biology of *Streptococcus mutans*. 3rd ed. Gram-Positive Pathogens 2019:435-448.

23. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 2002;15:167-193.

24. Ihajharia K, Parolia A, Shetty KV, Mehta LK. Biofilm in endodontics: a review. J Int Soc Prev Community Dent 2015;5:1-12.

25. Abisado RG, Benomar S, Klaus JR, Dandekar AA, Chandler JR. Bacterial quorum sensing and microbial community interactions. MBio 2018;9:e02331-17.

26. Schluter J, Schoech AP, Foster KR, Mitri S. The evolution of quorum sensing as a mechanism to infer kinship. PLOS Comput Biol 2016;12:e1004848.

27. Koo H, Allan RN, Howlin RP, Stoodley P, Hall-Stoodley L. Targeting microbial biofilms: current and prospective therapeutic strategies. Nat Rev Microbiol 2017;15:740-755.

28. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: its role in root canal treatment failure and current concepts in retreatment. J Endod 2006;32:93-98.

29. Saatchi M, Shokraneh A, Navazi H, Maracy MR, Shojaei H. Antibacterial effect of calcium hydroxide combined with chlorhexidine on *Enterococcus faecalis*: a systematic review and meta-analysis. J Appl Oral Sci 2014;22:356-365.

30. Yu MK, Kim MA, Rosa V, Hwang YC, Del Fabbro M, Sohn WJ, Min KS. Role of extracellular DNA in *Enterococcus faecalis* biofilm formation and its susceptibility to sodium hypochlorite. J Appl Oral Sci 2019;27:e2018069.

31. Barnes AM, Ballering KS, Leibman RS, Wells CL, Dunny GM. *Enterococcus faecalis* produces abundant extracellular structures containing DNA in the absence of cell lysis during early biofilm formation. MBio 2012;3:e00193-e12.

32. Chang JD, Wallace AG, Foster EE, Kim SJ. Peptidoglycan compositional analysis of *Enterococcus faecalis* biofilm by stable isotope labeling by amino acids in a bacterial culture. Biochemistry 2018;57:1274-1283.

33. Bulacio ML, Galván LR, Gaudioso C, Cangemi R, Erimbaue MI. *Enterococcus Faecalis* biofilm. Formation and development in vitro observed by scanning electron microscopy. Acta Odontol Latinoam 2015;28:210-214.

34. Kuang X, Chen V, Xu X. Novel approaches to the control of oral microbial biofilms. BioMed Res Int 2018;2018:6408932.

35. Rabin N, Zheng Y, Opoku-Temeng C, Du Y, Bonsu E, Sintim HO. Agents that inhibit bacterial biofilm formation. Future Med Chem 2015;7:647-671.

36. Veerapandian M, Yun K. Functionalization of biomolecules on nanoparticles: specialized for antibacterial applications. Appl Microbiol Biotechnol 2011;90:1655-1667.

37. Shrestha A, Kishen A. Antibacterial nanoparticles in endodontics: a review. J Endod 2016;42:1417-1426.

38. Khezerlou A, Alizadeh-Sani M, Azizi-Lalabadi M, Ehsani A. Nanoparticles and their antimicrobial properties against pathogens including bacteria, fungi, parasites and viruses. Microb Pathog 2018;123:505-526.

39. Cao W, Zhang Y, Wang X, Li Q, Xiao Y, Li P, Wang L, Ye Z, Xing X. Novel resin-based dental material with anti-biofilm activity and improved mechanical property by incorporating hydrophilic cationic copolymer functionalized nanodiamond. J Mater Sci Mater Med 2018;29:162.
40. Saafan A, Zaazou MH, Sallam MK, Mosallam O, El Danaf HA. Assessment of photodynamic therapy and nanoparticles effects on caries models. Open Access Maced J Med Sci 2018;6:1289-1295.

41. Bukhari S, Kim D, Liu Y, Karabucak B, Koo H. Novel endodontic disinfection approach using catalytic nanoparticles. J Endod 2018;44:806-812.

42. Rajeshkumar S, Bharath LV. Mechanism of plant-mediated synthesis of silver nanoparticles - A review on biomolecules involved, characterisation and antibacterial activity. Chem Biol Interact 2017;273:219-227.

43. Lee SH, Jun BH. Silver nanoparticles: synthesis and application for nanomedicine. Int J Mol Sci 2019;20:865.

44. Zhang XF, Liu ZG, Shen W, Gurunathan S. Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. Int J Mol Sci 2016;17:1534.

45. Singh R, Shedbalkar UU, Wadhani SA, Chopade BA. Bacteriogenic silver nanoparticles: synthesis, mechanism, and applications. Appl Microbiol Biotechnol 2015;99:4579-4593.

46. Mousavi SM, Hashemi SA, Ghasemi Y, Atapour A, Amani AM, Savar Dashtaki A, Babapoorn A, Arjmand O. Green synthesis of silver nanoparticles toward bio and medical applications: review study. Artif Cells Nanomed Biotechnol 2018;46 suppl 3:S855-S872.

47. Patil MP, Kim GD. Eco-friendly approach for nanoparticles synthesis and mechanism behind antibacterial activity of silver and anticancer activity of gold nanoparticles. Appl Microbiol Biotechnol 2017;101:79-92.

48. Hong X, Wen J, Xiong X, Hu Y. Shape effect on the antibacterial activity of silver nanoparticles synthesized via a microwave-assisted method. Environ Sci Pollut Res Int 2016;23:4489-4497.

49. Mie R, Samsudin MW, Din LB, Ahmad A, Ibrahim N, Adnan SN. Synthesis of silver nanoparticles with antibacterial activity using the lichen Parmotrema prasorediosum. Int J Nanomedicine 2014;9:1214-1227.

50. Tang S, Zheng J. Antibacterial activity of silver nanoparticles: structural effects. Adv Healthc Mater 2018;7:e170503.

51. Raffi M, Hussain F, Bhatti TM, Akhter JI, Hameed A, Hasan MM. Antibacterial characterization of silver nanoparticles against E. coli ATCC-15224. J Mater Sci Technol 2008;24:192-106.

52. Bapat RA, Chaubal TV, Joshi CP, Bapat PR, Choudhury H, Pandey M, Gorain B, Kesharwani P. An overview of application of silver nanoparticles for biomaterials in dentistry. Mater Sci Eng C 2018;91:881-898.

53. Radzig MA, Nadtochenko VA, Koksharova OA, Kiwi I, Lipasova VA, Khmel IA. Antibacterial effects of silver nanoparticles on gram-negative bacteria: influence on the growth and biofilms formation, mechanisms of action. Colloids Surf B Biointerfaces 2013;102:300-306.

54. Shrivastava S, Bera T, Singh SK, Singh G, Ramachandrarao P, Dash D. Characterization of antiplatelet properties of silver nanoparticles. ACS Nano 2009;3:1357-1364.

55. Manikprabhu D, Lingappa K. Antibacterial activity of silver nanoparticles against methicillin-resistant Staphylococcus aureus synthesized using model Streptomyces sp. pigment by photo-irradiation method. J Pharm Res 2013;6:255-260.

56. Zawadzka K, Kądziola K, Feczak A, Wróńska N, Pioński I, Ksiecieńska A, Lisowska K. Surface area or diameter—which factor really determines the antibacterial activity of silver nanoparticles grown on TiO2 coatings? New J Chem 2014;38:3275-3281.

57. Qing Y, Cheng L, Li R, Liu G, Zhang Y, Tang X, Wang J, Liu H, Qin Y. Potential antibacterial mechanism of silver nanoparticles and the optimization of orthopedic implants by advanced modification technologies. Int J Nanomedicine 2018;13:3311-3327.

58. Markowska K, Grudniak AM, Wolska KI. Silver nanoparticles as an alternative strategy against bacterial biofilms. Acta Biochim Pol 2013;60:523-530.
59. Fissan H, Ristig S, Kaminski H, Asbach C, Epple M. Comparison of different characterization methods for nanoparticle dispersions before and after aerosolization. Anal Methods 2014;6:7324-7334.

60. Blanchard PY, Sun T, Yu Y, Wei Z, Matsui H, Mirkin MV. Scanning electrochemical microscopy study of permeability of a thiolated aryl multilayer and imaging of single nanocubes anchored to it. Langmuir 2016;32:2500-2508.

61. Eaton P, Batziou K. Artifacts and practical issues in atomic force microscopy. Methods Mol Biol 2019;1886:3-28.

62. Leung AB, Suh KI, Ansari RR. Particle-size and velocity measurements in flowing conditions using dynamic light scattering. Appl Opt 2006;45:2186-2190.

63. Das R, Nath S, Chakdar D, Gope G, Bhattacharjee R. Preparation of silver nanoparticles and their characterization. J Nanotechnol 2009;5:1-6.

64. Paddock SW, Eliceiri KW. Laser scanning confocal microscopy: history, applications, and related optical sectioning techniques. Methods Mol Biol 2014;1075:9-47.

65. Lotfi M, Vosoughhosseini S, Ranjkesh B, Khani S, Saghiri M, Zand V. Antimicrobial efficacy of nanosilver, sodium hypochlorite and chlorhexidine gluconate against Enterococcus faecalis. Afr J Biotechnol 2011;10:6799-6803.

66. Hiraishi N, Yiu CK, King NM, Tagami J, Tay FR. Antimicrobial efficacy of 3.8% silver diamine fluoride and its effect on root dentin. J Endod 2010;36:1026-1029.

67. Rodrigues CT, de Andrade FB, de Vasconcelos LR, Midena RZ, Pereira TC, Kuga MC, Duarte MA, Bernardini L. Antibacterial properties of silver nanoparticles as a root canal irrigant against Enterococcus faecalis biofilm and infected dentinal tubules. Int Endod J 2018;51:901-911.

68. Wu D, Fan W, Kishen A, Gutmann JL, Fan B. Evaluation of the antibacterial efficacy of silver nanoparticles against Enterococcus faecalis biofilm. J Endod 2014;40:285-290.

69. Charannya S, Duraivel D, Padmeeke K, Poorni S, Nishanthine C, Srinivasan MR. Comparative evaluation of antimicrobial efficacy of silver nanoparticles and 2% chlorhexidine gluconate when used alone and in combination assessed using agar diffusion method: an in vitro study. Contemp Clin Dent 2018;9 Supplement 2:S204-S209.

70. Yousefshahi H, Aminosobhani M, Shokri M, Shahbazi R. Anti-bacterial properties of calcium hydroxide in combination with silver, copper, zinc oxide or magnesium oxide. Eur J Transl Myol 2018;28:7545.

71. Shantiaae Y, Dianat O, Mohammadkhani H, Akbarzadeh BA. Cytotoxicity comparison of nanosilver coated gutta-percha with Gutaflow and normal gutta-percha on L929 fibroblast with MTT assay. Shahid Beheshti Univ Dent J 2011;29:62-68.

72. Bahador A, Pourakbari B, Bolhari B, Hashemi FB. In vitro evaluation of the antimicrobial activity of nanosilver-mineral trioxide aggregate against frequent anaerobic oral pathogens by a membrane-enclosed immersion test. Biomed J 2015;38:77-83.

73. Baras BH, Melo MA, Sun J, Oates TW, Weir MD, Xie X, Bai Y, Xu HH. Novel endodontic sealer with dual strategies of dimethylaminohexadecyl methacrylate and nanoparticles of silver to inhibit root canal biofilms. Dent Mater 2019;35:1117-1129.

74. de Almeida J, Cechella BC, Bernardi AV, de Lima Pimenta A, Felipe WT. Effectiveness of nanoparticles solutions and conventional endodontic irrigants against Enterococcus faecalis biofilm. Indian J Dent Res 2018;29:347-351.

75. Halkai KR, Mudda JA, Shivanna V, Rathod V, Halkai R. Evaluation of antibacterial efficacy of fungal-derived silver nanoparticles against Enterococcus faecalis. Contemp Clin Dent 2018;9:45-48.

76. Athanasiadis B, Abbott PV, Walsh LJ. The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics. Aust Dent J 2007;52 Supplement:S64-S82.
77. Suzuki TY, Gallego I, Assunção WG, Briso AL, Dos Santos PH. Influence of silver nanoparticle solution on the mechanical properties of resin cements and intrarradicular dentin. PLoS One 2019;14:e0217750.

78. Afkhami F, Pourhashemi SJ, Sadegh M, Salehi Y, Fard MI. Antibiofilm efficacy of silver nanoparticles as a vehicle for calcium hydroxide medicament against Enterococcus faecalis. J Dent 2015;43:1573-1579.

79. Fan W, Wu Y, Ma T, Li Y, Fan B. Substantivity of Ag-Ca-Si mesoporous nanoparticles on dentin and its ability to inhibit Enterococcus faecalis. J Mater Sci Mater Med 2016;27:16.

80. Marin S, Vlasceanu GM, Tiplea RE, Bucur IR, Lemnaru M, Marin MM, Grumezescu AM. Applications and toxicity of silver nanoparticles: a recent review. Curr Top Med Chem 2015;15:1596-1604.

81. Mathur P, Jha S, Ramteke S, Jain NK. Pharmaceutical aspects of silver nanoparticles. Artif Cells Nanomed Biotechnol 2018;46 suppl:115-126.

82. Reidy B, Haase A, Luch A, Dawson KA, Lynch I. Mechanisms of silver nanoparticle release, transformation and toxicity: a critical review of current knowledge and recommendations for future studies and applications. Materials (Basel) 2013;6:2295-2350.

83. Palacios-Hernandez T, Diaz-Diestra DM, Nguyen AK, Skoog SA, Vijaya Chikkaveeraiah B, Tang X, Wu Y, Petrochenko PE, Sussman EM, Goering PL. Cytotoxicity, cellular uptake and apoptotic responses in human coronary artery endothelial cells exposed to ultrasmall superparamagnetic iron oxide nanoparticles. J Appl Toxicol 2020;40:918-930.

84. Panáček A, Smékalová M, Večeřová R, Bogdanová K, Růžerová M, Kolář M, Kilianová M, Hradilová Š, Froning JP, Havrdová M, Prucek R, Zbořil R, Kvítek L. Silver nanoparticles strongly enhance and restore bactericidal activity of inactive antibiotics against multiresistant Entrobacteriaceae. Colloids Surf B Biointerfaces 2016;142:392-399.

85. Chowdhury NR, MacGregor-Ramiasa M, Zilim P, Majewski P, Vasilev K. 'Chocolate’ silver nanoparticles: Synthesis, antibacterial activity and cytotoxicity. J Colloid Interface Sci 2016;482:154-158.