CURRENT USE OF NANOPARTICLES IN ENDODONTICS: A SYTEMATIC REVIEW

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ABSTRACT

Nowadays, technology evolves very fast and we are witnesses of major changes in dentistry. Nanotechnology improved many fields of dentistry, including endodontics.

In this paper, we compared different irrigants and sealers which are currently used in endodontic treatments, their advantages and disadvantages and their limitations. In the context of emerging trends of nanotechnology in all fields of dentistry, we aimed to highlight the importance of developing new irrigants and sealers improved with nanoparticles, with superior properties compared to traditional ones. As resulted from our research, the most used irrigants in endodontics (NaOCl, EDTA, CHX) possess different kinds of advantages, but none is flawless, also having some limitations. Also, every current sealer available on the market has one or more disadvantages. For this reason, nanotechnology is very welcomed in this field and different kinds of nanoparticles were proposed for their particularities in order to improve the performances of endodontic materials. We present in this work a review of the literature regarding different types of nanoparticles, their effects on endodontic microbiota and also, their particularities.

Key words: Endodontics, Nanoparticles, Sealer, Irrigant.

INTRODUCTION

Endodontic therapy has a big role in preservation of teeth. As described by Karamifar et al. and the entire modern literature, there are three main steps of an endodontic treatment: a) the diagnostic phase, which includes finding the source of the problem and establishing the treatment plan; b) the preparatory phase, which includes cleaning and shaping the root canal; c) the obturation phase, in which the root canal system is sealed with an inert material [1]. At this moment, there are numerous techniques of preparation and irrigation and a proper treatment is required in order to reduce or eliminate the microorganisms from the root canal. However, complete elimination of bacteria is very unlikely to be obtained because of the complex anatomy of root canals.

In the context of emerging trends of nanotechnology in all fields of dentistry, we aimed to highlight the importance of developing new irrigants and sealers improved with nanoparticles, with superior properties, compared to traditional ones. As resulted from our research, it is obvious that the most used irrigants in endodontics (NaOCl, EDTA, CHX) possess different kinds of advantages, but none is flawless, also having some limitations. Also, every current sealer available on the market has one or more disadvantages.

For this reason, nanotechnology is very welcomed in this field and different kinds of nanoparticles were proposed for their particularities in order to improve endodontic materials.

We present in this work a review of the literature regarding different types of nanoparticles, their effects on endodontic microbiota and also, their particularities.

MATERIAL AND METHODS

This review was performed using the PRISMA 2020 flow chart based on the suggestion of Page et al., 2021 [2].

The steps and selection criteria, followed by the number of studies used for our review, are shown in **Figure 1**.

Databases such as PubMed, Scopus, Science Direct, Elsevier, Google Scholar, Google Patents have been accessed to search the literature. The keywords of the medical subject titles included in the search were: ″Endodontics″, ″Nanoparticles″, ″Sealer″, ″Irigant″, ″Oral desease″, etc.

All the information systematized in the tables was obtained from research articles. Studies published in languages other than English and Romanian were excluded. A total of 97 studies were selected and included in this review.

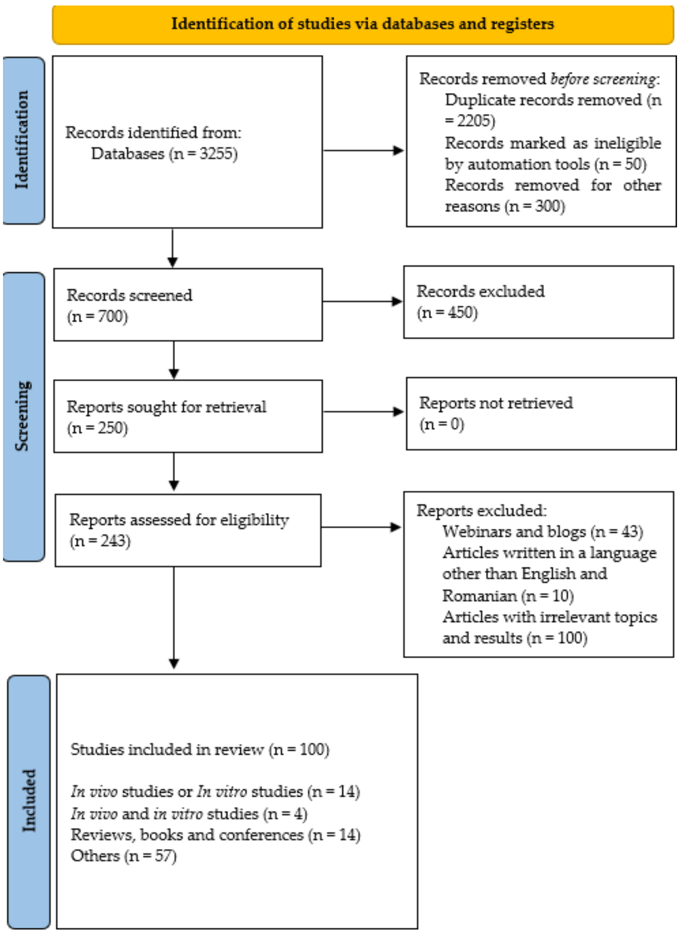

Figure 1. PRISMA 2020 flow chart for this review.

1. IRRIGANTS

Shaping the root canal systems can remove tissue remnants, biofilms and infected dentin, but large portions may remain untouched. The most effective current chemical irrigants are chlorhexidine (CHX) and sodium hypochlorite (NaOCl), but they still have limitations in eradicating bacterial infection in order to completely disinfect dentinal tubules. Both irrigants have their antibacterial activity limited to the superficial layers of the dentin [3].

Nowadays, the most used irrigants are sodium hypochlorite (NaOCl), chlorhexidine (CHX) and ethylenediaminetetraacetic acid (EDTA) [4].

According to Tonini et al. [5] every irrigant should possess the following properties: removal of the smear layer, antibacterial effect, disinfection of root canal anatomy, dentin and dentinal tubules, nontoxic, noncarcinogenic, nonantigenic, to not have toxic effects on the patient and the doctor, to not affect the sealing ability of the sealer, no discolorations, relatively cheap.

Recent studies reported that nanoparticles can efficiently improve root canal irrigant's properties and also the tissue reaction [4,6].

1.1. SODIUM HYPOCHLORITE

Sodium hypochlorite is the most used irrigant in Endodontics. NaOCl has the capacity to partially neutralize the necrotic tissues or any antigenic or microbial component from the root canal anatomy. Its properties can be improved by increasing its temperature [7–9].

It possesses lots of advantages. It has most of the properties of an ideal irrigant, it is available in concentrations between 0,5-7% and has a high pH. It dissolves the organic part of the pulp, has antibacterial properties and it actions quickly. NaOCl also has some disadvantages which include its cytotoxicity, massive reactions when extruded outside of the apical foramen, like severe pain, edema, bleeding, infection and possibly anesthesia and it has no effect on the inorganic component of the pulp.

1.2. EDTA

EDTA [ethylenediaminetetraacetic acid] is usually used in Endodontics in combination with NaOCl for its effect on the inorganic component of the pulp, it is a chelating agent [10]. Its advantages include lubricating properties, it facilitates the movements of the instruments and it is indicated in the early stages of preparation to remove soft tissue blockages [11]. EDTA also possess disadvantages life a low antibacterial effect, it isn't solvent [12].

1.3. CHLORHEXIDINE

Chlorhexidine (CHX) is also a very effective antimicrobial agent and its advantages can be attributed to its substantivity within the oral cavity [13].

It is used in concentrations of 0.12%–2% [13]. One of its most known advantages is that CHX has been proven to be the most efficacious irrigant against *E. faecalis* because of its bis-guanide nature that interacts with the negatively charged bacterial surface and because of that, it causes cellular disintegration [14]. Unlike NaOCL it has no unpleasant smell and also doesn't provoke massive reactions if extruded beyond apical foramen. As disadvantages there can be mentioned that unlike NaOCl, CHX is not solvent, doesn't dissolve the organic component of the pulp and also it interacts negatively with NaOCl and EDTA. In combination with NaOCl it forms parachlor aniline which is toxic and with EDTA it forms a white precipitate, both can affect the seal of the obturation.

1.4. *IRRIGANTS LIMITATIONS*

Taking into consideration the limits of actual irrigants, nanoparticles have been introduced in Endodontic practice in order to improve them [4].

Studies have shown that currently used irrigants are only moderately efficient and microorganisms still persist in the root canal anatomy after the endodontic treatment, this being the most common factor of failure in endodontic therapy [15].

2. SEALERS

The endodontic root canal sealer is covering the walls of the canals and also it seals canal's ports of entry and exit by filling the space between the obturation and the root [5].

During the obturation phase, the sealers are used as a lubricant material which allows the gutta-percha or other obturation materials to become fixed in the canal. Also, sealers can fill lateral canals, voids and accessory canals and if they cannot perform their role, microleakage can occur and various fluids, bacteria, molecules can cause failure of the endodontic non-surgical treatment [16].

Also, root canal sealers can serve as lubricants and they have adhesive features to dentin tubules [17].

The root canal filling has no antibacterial effect after setting and because of this, there were many attempts to create improved antimicrobial root canal sealer [16].

Unfortunately, these attempts resulted in severe cytotoxic effects when sealer leached through dentin ports of exit to periodontal tissues [18]. These effects caused nerve paresthesia, anesthesia, pain and delayed healing [18].

Examples of cytotoxic endodontic sealers include zinc-eugenol sealers, which were found to be dangerous to nerve cells and human periodontal ligament fibroblasts.

Paraformaldehyde is a component of epoxy resin-based sealer and it is also cytotoxic and mutagenic. Another cytotoxic component that is released during the setting phase is polyketone [19].

According to the literature the requirements of an ideal root canal sealer are: excellent sealing ability when set; sufficient setting time to ensure working time; dimensional stability; insolubility against tissue fluids; good adhesion to canal walls; suitable antimicrobial properties; and biocompatibility [20,21].

Root canal sealers classification can be seen in **Figure 2** [22,23].

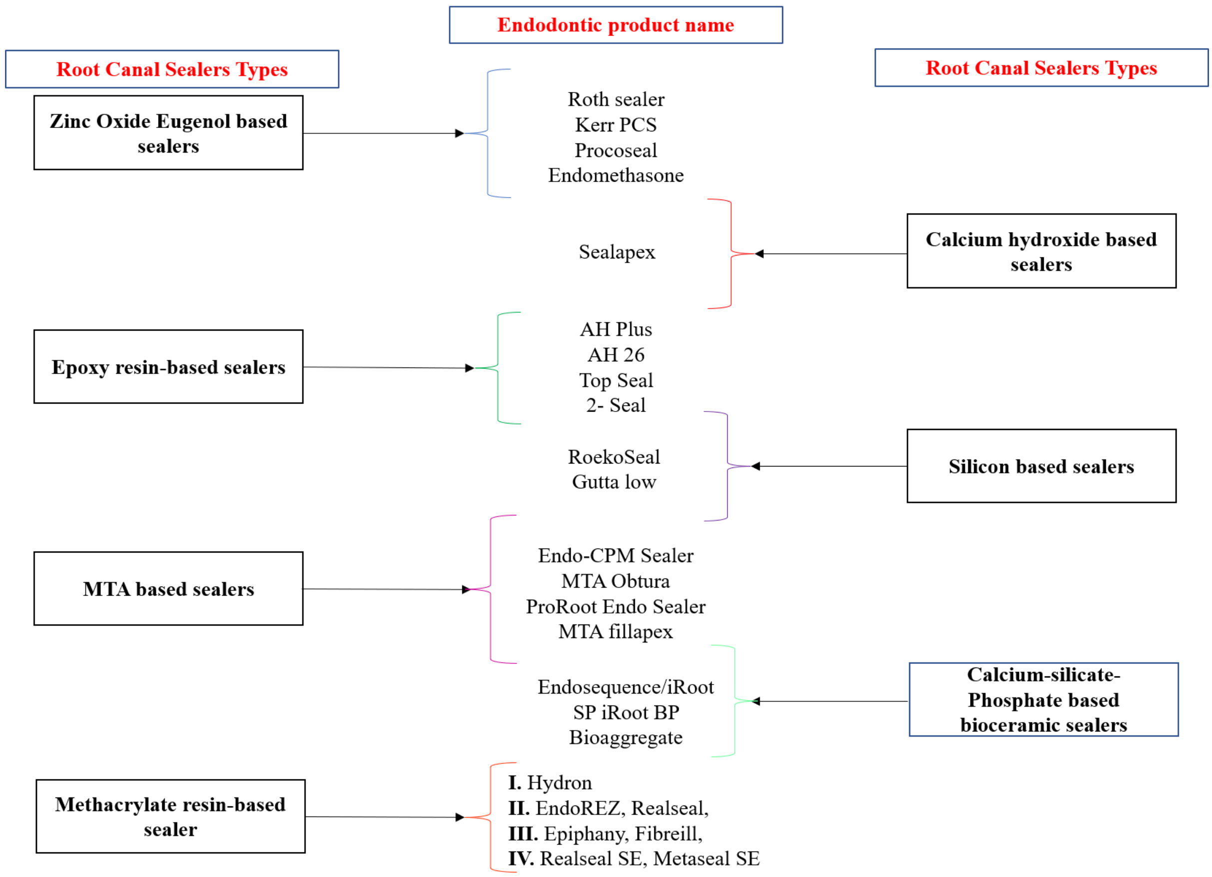

Figure 2. Root Canal Sealers Classification. MTA- Mineral trioxide aggregate, I-First generation, II-Second generation, III-Third generation, IV-Fourth generation.

Endodontic root canal sealers can be also classified by composition based on setting reaction zinc oxide-eugenol, methacrylate resin sealer systems, salicylate, glass ionomer, tricalcium silicate, silicone and epoxy resin [16].

3. ENDODONTIC SEALERS AND NANOPARTICLES

After the smear layer is removed by irrigants, the dentinal tubules are open and sealers exhibit expanded adhesive features to them. Because of their unique properties such as the same size domain as proteins (1 to 100 nm), large surface to volume ratio and high percentage of atoms/molecules on the surface, nanoparticles can easily penetrate dentinal tubules [17].

Dispersion of nanoparticles into current materials could improve the sealing ability of obturating and sealer materials and also root-repair/root-end filling materials [24]. Another important property of an obturation material is its antimicrobial activity that can be advantageous in the reduction of persisting bacteria. As stated before, nanoparticles incorporated in sealers can facilitate the penetration into dentinal tubules and allow prolonged release of antimicrobial components.

No currently available sealer possess all the desired properties, and hence, a modified one can be the best option. The addition of micro and nanoparticles are a good alternative for the prolonged release of antimicrobial agents. Currently available sealers with antimicrobial activity possess a limited effect of maximum 1 week, which decreases after complete setting; the addition of nanoparticles is an important attempt to prolong this activity [25].

Nanoparticles have unique physicochemical properties: ultrasmall sizes, large surface area/mass ratio and increased chemical reactivity. In endodontics, nanoparticles can be used in the form of solutions for irrigation, medication or as an additive for sealers/restorative materials in order to improve antibiofilm efficacy.

3.1. ORGANIC NANOPARTICLES

3.1.1. Graphene

Graphene is a form of carbon planar sheet, arranged in a honeycomb-shaped lattice [26]. It has many excellent properties like physico-mechanical ones, stability, biocompatibility, biodegradability, electrical conductivity, antimicrobial properties, good mechanical ones and chemical stability [27]. It is useful for diagnosis and detection of disease and formation of anti-bacterial surfaces [26].

They are used in regenerative dentistry procedures and also for the debridement of root canals.

Because of the disadvantages of NaOCl Graphene was incorporated into silver nanoparticles and even if the antibacterial property remained the same, the cytotoxic effects to bone and soft tissues were reduced. Also, against *S. mutans*, Graphene oxide NPs were demonstrated to be very effective [24].

3.1.2. Chitosan

Chitosan NPs are biopolymeric nanoparticles which offer various advantages like antibacterial activity, biocompatibility and also, they are able to resist aging for longer periods when compared with conventional sealers [28]. It has excellent antimicrobial, antifungal and antiviral properties.

In dentistry, Chitosan NPs are used for their properties of penetrating in the complexities of the root canal and dentinal tubules, in order to eliminate microorganisms. The combination between Chitosan Nps and CHX can remove *Enterococcus faecalis* from the canals, by formation of membrane barriers at the peri-radicular area [4]. In combination with zinc oxide can successfully eliminate biofilms [29]. Also, these NPs can enhance the activity of

NaOCl by helping its penetration deeper into dentinal tubules, as it was found that it normaly penetrates only 130mm [24]. So, owing its antibacterial properties Chitosan NPs can be efficiently incorporated into sealers.

3.2. NON-ORGANIC NANOPARTICLES

3.2.1. Bioactive glass nanoparticles SiO_2, Na_2O, and P_2O_5

Bioactive glass [BG] is formed of variable concentrations of SiO_2, CaO, Na_2O and P_2O_5 [30]. SiO_2-CaO-Na_2O-P_2O_5 are biomaterials, with ability to substitute or regenerate damaged tissues [27,31]. These NPs have some advantages, like an alkaline pH, high osmotic pressure more than 1% which is fatal for many microorganisms, are highly amorphous in nature, can induce the neoformation of bone by inducing apatite formation and also has antibacterial properties [30–33]. When incorporated into bio-glasses, magnesium is beneficial for cell interactions, one example being SiO_2-MgO-CaO system, which is mainly known because of improved mechanical properties and osteoinductivity [30].

3.2.2. Hydroxyapatite nanoparticles

Hydroxyapatite (HA; $Ca_5(PO_4)_3OH$) possess superior qualities such as high biocompatibility, properties similar to human hard tissues, antibacterial properties and bioactivity [31].

Their main function is to integrate into the dentinal tubules and to seal their opening, preventing the exposure of nerves to external stimuli. Also, they have an important role in decreasing dentin hypersensitivity. HAp is very biocompatible and is capable of reducing local or systemic inflammatory reactions and can be used as an agent in periapical healing [34].

In a study made by Al-Bakhsh et al, it was found that the incorporation of nano-structured bioactive glass, hydroxyapatite NPs and fluorohydroxyapatite fillers into an epoxy-based dental sealer could improve its antimicrobial properties [31].

3.2.3. Bioactive Mesoporous Calcium Silicate NPs

These NPs are 100 nm in size, possessing particular properties: antibacterial effect, injectability, osteostimulation, apatite mineralisation [6]. In a study conducted by Ching-Yuang Huang et al, it is mentioned that calcium-silicate-based materials can promote hard tissue regeneration and stimulate odontogenic and osteogenic differentiation in various types of cells [35].

Also, mesoporous calcium-silicate nanoparticles (MCSNs) possess other qualities like great physicochemical characteristics, antibacterial activity and drug delivery capacity, special nanostructure, injectability, mineralization of apatite, promotion of bone generation and defect repair. However, their antibacterial activity is limited, because they cannot completely remove the bacteria and their biofilms in the root canals, such as *Enterococcus faecalis* and *Staphylococcus* [36].

3.3. METAL NPs

Compared to CHX, metal NPs can penetrate deeper into the layers of the biofilm due to their small size and can be mechanically trapped in plaques. Unlike the irrigants that cannot reach all the complex anatomy of the root canals, NPs have small-sized molecules and might have better penetration into these areas. The average diameter of dentinal tubules [1 μm] is ten times greater than the average diameter of nanoparticles (0.1 μm) leading to the easier penetration of NPs into the tubules and longer antibacterial effects [37].

3.3.1. Gold NPs

Gold NPs can be obtained with dimensions between 1-100 nm [38].

It is advantageous due to multifunctional effects, it is non-toxic, easy to be detected and it has photothermal activity [24,39].

Silver [Ag], gold [Au], Zinc [Zn] NPs have been found to be the most effective against bacteria. Only few literature records are available evaluating the effect of Au NPs against *E. faecalis* [14].

Gold is known for its optical properties and the absorption of proteins. The combination between silver and gold NPs creates new possibilities and applications for biomaterials with improved antibacterial activity [40].

3.3.2. Silver NPs

Studies have shown that AgNPs that are between 1-100 nm in size have a great bactericidal efficacy against *Gram-positive* and *Gram- negative* bacteria [41,42].

Silver ions (Ag^+) possess great antimicrobial properties and show no resistance to bacteria, resulting in being a great substituent to antibiotics. These NPs can be efficiently incorporated into biomaterials and they prevent or reduce the formation of biofilms. Also, they do not affect the mechanical properties of the materials [36].

Also, they are considered a 'natural antibiotic', are non-toxic for human cells, but they are highly toxic for some bacterial species, such as E. coli, Staphylococcus aureus and others. Their antimicrobial activity is very similar to that of antibiotics with a large spectrum.

AgNPs are used in Endodontics, in combination with different materials like sealers, gutta-percha or cements. They can efficiently prevent the recolonization of bacteria. They possess some qualities in comparison with NaOCl like maintaining their antibacterial efficacy in the presence of dentin. Studies have shown that bacteria are not capable of developing resistance to AgNPs compared with antibiotics [43].

AgNPs have the same bactericidal effect as 5.25% NaOCl against *E. faecalis* even at low concentration. AgNPs have been proposed as a non-toxic endodontic irrigant [37].

4.4. METAL OXIDES NPs

4.4.1. Zinc Oxide Nanoparticles

Zinc oxide nanoparticles possess high antibacterial properties [44]. Because of its high pH it can destroy microbial cells. Their bactericidal effect is related to their size, meaning that the smaller the size, the higher the effect. Also, their antibacterial effect is in direct connection with their concentration. A maximum effect can be reached at higher levels, ZnO NPs being very effective against *E. faecalis* [34].

Zinc is an important trace element for protein and DNA synthesis, cell mitosis and proliferation and the proliferation and differentiation of osteoblasts. Also, Zn can depolarize the cell membrane of bacteria and Ag^+ and Zn^{2+} may have synergic antibacterial effects on *E. faecalis* and its biofilms [36].

4.4.2. Titanium Dioxide

Titanium dioxide NPs are highly stable particles with suitable photocatalytic properties. It causes oxidative stress due to the generation of reactive oxygen species. The mechanism is related to its superior membrane fluidity and cell membrane disruption due to lipid peroxidation. It is also used as an effective antifungal for fluconazole-resistant strains [4].

As advantages, it can be mentioned: photocatalytic properties, stability, effective fungicidal for fluconazole-resistant strains [34]. TiNPs are another example of NPs used in dentistry as an antibacterial agent. They possess higher antibacterial properties than chlorhexidine, a pleasing color and high biocompatibility [37].

4.4.3. Magnesium Oxide and Calcium Oxide

Magnesium Oxide NPs have proven multiple antimicrobial effects and because of this are used in Endodontics. MgO and CaO nanoparticles were proven to be efficient against both Gram-positive and Gram-negative microorganisms.

Both MgO Nanoparticles at a concentration of 5 mg/L and Chitosan Nanoparticles were demonstrated to a long-lasting efficacy in the eradication of *Enterococcus faecalis* [37].

Metal oxides like Zinc Oxide and Magnesium Oxide have great antibacterial properties in various forms.

There were tested the antibacterial and mechanical properties of the materials in which were incorporated MgO NPs showing a significant reduction in the growth of *Staphylococcus aureus* [45].

4.4.4. Zirconia

Zirconia is a chemical oxide and has optical and metallic properties similar to the natural tooth [46]. One proven advantage is that it eradicates bacterial colonization with low cytotoxic effects because of its insolubility in water. Also, Zirconia NPs are very efficient against specific microorganisms such as *E. faecalis* being successfully used as an antimicrobial agent in Endodontics [4].

MTA is formed of bismuth oxide (Bi_2O_3) as a radiopacifier agent and this association changes the microstructure of the cement, increasing the porosity and solubility and reducing its resistance [47]. There was a study of the association of portland cement (PC) with other radiopacifier agents and one example that has demonstrated satisfactory results was zirconium oxide (ZrO_2) [48].

The association between PC and 30% of ZrO_2 exhibits radiopacity, compressive strength, setting time, water absorption, and solubility similar to MTA ProRoot. Also, it promotes satisfactory pH, solubility, calcium ions release, and setting time.

According to Tanomaru et al. the addition of ZrO_2 does not interfere in the antibiofilm activity and provides radiopacity to Portland cement, but the presence of ZnO significantly decreased the compressive strength of the material [49].

4.4.5. CuO nanoparticles

CuONPs cross the bacterial cell membrane and are very effective against *Gram-positive* and *Gram-negative* bacteria. Also, they have some antifungal properties [4].

Copper surfaces or copper-based alloys can eliminate 99.9% of pathogenic bacteria in hours, including methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella enterica*, *Campylobacter jejuni*, *Legionella pneumophila*, *Clostridium difficile* and *Mycobacterium tuberculosis*. According to Sánchez et al. copper can be used to disinfect the root canal system [50]. Copper NPs are toxic for the cell membrane of *Gram-negative* and *Gram-positive* bacteria, being employed as intracanal medication or as irrigant to decontaminate and disinfect the root canal.

CONCLUSIONS

Nanotechnology is constantly evolving and its applications in Endodontics emerge with great advantages.

Currently available materials for endodontics have some limitations, both the irrigants and sealers. To overcome these limitations, nanotechnology have shown promising results, offering substantial help in treatment of oral diseases and eradication of smear layer and biofilms. The most used irrigant in the current endodontic treatments is NaOCl, but EDTA and CHX are also required. EDTA in order to eliminate the smear-layer and CHX for its most efficient properties against *E. Faecalis*.

These three irrigants are the most used in nowadays Endodontics, but all three of them have limitations.

Also, a comparison was made between the most frequently used endodontic sealers. Calcium-silicate-phosphate-based sealers were found to fulfill the most important criteria: biocompatible, antibacterial, radiopaque, noncarcinogenic, nontoxic, excellent sealing ability when set, sufficient setting time to ensure working time, dimensional stability, insolubility against tissue fluids, good adhesion to canal walls.

However, more *in vitro* and *in vivo* studies are required in order to create an ideal root canal sealer.

As a new trend, nanotechnology and nanomaterials have recently proved superior properties in terms of physical, mechanical, chemical and biological activity, resulting in better performances as compared to the conventional materials used in endodontics.

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