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Analysis of the antimicrobial and antibiotic activity of nanoparticles for endodontic use

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Abstract
Introduction: Great success has been reported with the use of broad-spectrum antibiotics in the treatment of complex bacterial diseases, yet new and recurring bacterial infections continue to pose significant challenges to global health care.
Objective: To review the literature on the antimicrobial and antibiotic activity of zinc oxide, gold, silver, and chitosan nanoparticles.
Methodology: In this article, important literature on the antimicrobial and antibiotic activity of endodontic nanoparticles is reviewed through a comprehensive search in the PubMed, Web of Science and Google Scholar databases.
Results: Zinc oxide nanoparticles do not offer significant improvements when compared to conventional or non-nanometric materials, gold nanoparticles lack clinical applications and their effectiveness is lower than silver nanoparticles. The best options are silver and chitosan nanoparticles due to their low required concentrations and results.
Conclusion: Silver and chitosan nanoparticles are the best options for use in endodontic treatment because they demonstrate better antimicrobial and antibiotic film results. Research with AgNP's provides us with its usefulness in the application of sealing cements and intraconduct medicines, while chitosan nanoparticles offer advantages in healing.

Keywords: Nanoparticles, silver, chitosan, gold, titanium, zinc oxide, antimicrobial, antibiofilm

1. Introduction

Despite the profound success achieved by the use of broad-spectrum antibiotics in the treatment of complex bacterial diseases, new and recurrent bacterial infections continue to pose significant challenges to global health care [1]. Global bacterial drug resistance has become a serious threat, minimizing effective antibiotic options; therefore, novel methodologies that improve antimicrobial action are urgently needed [2]. Antibiotic resistance has different mechanisms such as the use of beta-lactams, acetyltransferases, aminoglycosides, etc. [3]. In February 2017, the World Health Organization published a List of Global Priority Pathogens (PPL). Among others, bacterial infections were considered the greatest public health concern. For this reason, it is very clear that there is an urgent need for new substances with antibacterial properties [4]. Today, nanotechnology offers great potential for both diagnosis and treatment of diseases caused by pathogenic microorganisms [5]. Metal nanoparticles represent a solution to resist to conventional antibiotics having different mechanisms of action, and they also attack multiple biomolecules that compromise the development of resistant strains*)* [6]. The antibacterial nanoparticles have a greater antibacterial activity than the antibacterial powders. This is due to the greater surface area and charge density of the nanoparticles, which allow them to achieve a higher degree of interaction with the negatively charged surface of the bacterial cells [7]. Chitosan is a deacetylated form of chitin, the second most abundant natural biopolymer. Chitosan is used in biomedicine because of its biocompatibility, biodegradability and antimicrobial properties. In addition, chitosan-based biopolymers are also structurally similar to the components of the extracellular matrix [8].

Chitosan has a wide range of antimicrobial activity against fungi, as well as Gram-positive and Gram-negative bacteria [9]. AuNP's are colloidal or clustered particles composed of a gold nucleus, an inert and biocompatible compound [10]. The present research will focus on analyzing the antimicrobial and antibiotic effect of different groups of nanoparticles, because we are currently facing strains that are increasingly resistant to conventional antibiotics and it is necessary to obtain more information about new therapeutic alternatives. The objective of this review is to evaluate the literature on the antimicrobial and antibiotic activity of endodontic, zinc oxide, gold, silver, and chitosan nanoparticles.

2. Materials and Methods

Articles on the subject published through the PubMed, SCOPUS and Google Scholar databases were analyzed, with emphasis on the last 5 years. The quality of the articles was evaluated using PRISMA guidelines, i.e., identification, review, choice and inclusion. The quality of the reviews was assessed using the measurement tool for evaluating systematic reviews (AMSTAR-2) [11].

The search was performed using Boolean logical operators AND, OR and NOT.

It was realized with the words "silver nanoparticles", "chitosan nanoparticles", "gold nanoparticles", "titanium nanoparticles", "antimicrobial", "antibiofilm".

The keywords were used individually, as well as each of them related to each other.

Initially, the titles of all the articles were selected, the abstract of each one was evaluated, and the articles were chosen for a complete reading review.

3. Results and Discussion

3.1 Antimicrobial and antibiotic activity of zinc oxide nanoparticles

Although recent studies have been conducted on the antimicrobial activity of nanoparticles, their effect on bacterial biofilm when it was incorporated with endodontic sealants or drugs, particularly calcium hydroxide, has not been consistently studied. Zinc oxide nanoparticles (ZNO-NP) have antibiotic film activity against strains of Enterococcus faecalis [12]. Besides, when supplementing a calcium hydroxide based sealing cement with ZnONP's, it was obtained a significant increase in the antibiofilm activity and reduction in its thickness, compared to chitosan nanoparticles [13].

Recently, ZnO (ZnO-Np) nanoparticles were added to the original composition of sealants containing zinc oxide. This modification inhibited the formation of biofilms within the sealant-dentine interface, reduced cytotoxicity [14] and improved the sealing capacity [15] and the antibacterial properties [16].

By evaluating the antibacterial activity of pastes with microparticles (micro) or nanoparticles (nano) of calcium hydroxide (CaOH 2) and zinc oxide (ZnO) with 0.4% chlorhexidine against Enterococcus faecalis. The antibacterial activity against E. faecalis was evaluated by means of an agar diffusion test. The direct contact test on planktonic cells of Enterococcus faecalis Calcium hydroxide and zinc nano-oxide and 0.4% chlorhexidine pastes were more effective in the agar diffusion test. In the direct contact test, the pastes with chlorhexidine showed the greatest effect after 30 seconds, concluding that there are no significant differences between the different groups [17, 18].

The antibacterial activity of ZnONP's was examined against Porphyromonas gingivalis and Actinomyces Naeslundii *in vitro*. The results indicated that ZnONPs have excellent antibacterial activity against P. gingivalis and A. naeslundii and also had low cellular cytotoxicity *in vitro*. The maximum inhibitory radii of ZnONP's in P. gingivalis and A. naeslundii were 18.09 mm and 12.05 y. These results showed that ZnONP's in P. gingivalis and A. naeslundii had a higher antibacterial activity statistically significant than commercial AH-Plus [19]. Antimicrobial effect of two ZnONP's gels and zinc/silver oxide nanoparticles and a mixture of calcium hydroxide and chlorhexidine at 0.12% as intraconduct drugs on Enterococcus faecalis at different time intervals. There were no statistically significant differences between the percentage reduction in colony counts. The calcium hydroxide / chlorhexidine mixture was more effective than zinc oxide and zinc oxide / silver nanoparticles gels [20].

We can conclude that within the nanoparticle options, the zinc oxide ones have an antimicrobial and antibiofilm activity very similar to the commonly used materials.

3.2 Antimicrobial and antibiotic activity of gold nanoparticles

Metal nanoparticles have achieved good antimicrobial properties against bacterial and fungal pathogens [21]. Focusing on AuNP's in endodontics, there are not enough studies to prove their effectiveness in treatment. The AuNP's and levofloxacina conjugates were more efficient than levofloxacina alone and improved the antibacterial efficacy against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa by 1.94, 2.89 and 1.46 times, respectively [22].

AuNP are active against Gram-negative and Gram-positive bacteria, namely E. coli, P. aeruginosa, S. typhi, Serratia sp, K. pneumoniae, S. aureus, B. subtilis and E. faecalis, among others. Their main mechanism of bacterial toxicity is based on the direct adherence of AuNP to the bacterial surface driven by electrostatic forces. This mechanism depends largely on the size of the nanoparticles, usually with smaller nanoparticles showing a lower MIC [23]. An evaluation on Candida revealed that AuNP's can act on both the plasma membrane and the cytoplasm. AuNP's inhibit proton pumping, which is fundamental for the growth of Candida, and can alter normal cell conformation, leading to a loss of activity [24].

Vancomycin conjugated with AuNP's can be active against vancomycin-resistant enterococci [25]. It showed enhanced activity with a minimum inhibitory concentration (MIC) of 2-4 µg / ml (based on vancomycin molecules) while the MIC of free vancomycin was much higher (> 64 µg / ml). Similarly, AuNPs coated with vancomycin by electrostatic interaction have improved activity against vancomycin-resistant Staphylococcus aureus [26].

The MICs of vancomycin-coated AuNPs were not only significantly lower than those of vancomycin in free form, but also inhibited the growth of vancomycin-resistant strains, including Enterococcus faecalis, Enterococcus faecium, methicillin-resistant Staphylococcus aureus [27]. Due to their low reactivity, AuNPs show ion release and ROS production as a minor mechanism of action.

Therefore, they must reach higher concentrations to produce the same antibacterial effect as other metal nanoparticles (e.g., AgNP). In this sense, to produce the small inhibition zone of S. aureus, AuNP need a concentration of 197 µg / ml, while AgNP need small concentrations such as 4.86 µg / ml [28].

Taking into account that it exhibits very good results against pathogens frequently related to oral diseases and intraradicular infections, studies are not sufficient and apparently are less effective than silver nanoparticles when talking about antimicrobial effect.

3.3 Antimicrobial and antibiotic activity of silver nanoparticles

Silver is the most widely used metallic nanomaterial for the control of several types of microorganisms due to its well-known antimicrobial properties even in drug-resistant microorganisms [29] including the Enterococcus faecalis strain [30, 31]. Its effectiveness as an irrigant was evaluated, biofilms of Enterococcus faecalis were cultivated in root canals and exposed to 5 ml of Ag NP's suspension at a concentration of 100 ppm obtaining as a result a 94% elimination of the biofilm [32]. Besides, it was evaluated the antimicrobial activity of 20 microliters of silver nanoparticles added to resinous, bioceramic and calcium hydroxide sealing cements on Enterococcus faecalis through collagen membrane assay, obtaining as a result a 9.9% increase in the antimicrobial activity when supplementing the cements with these nanoparticles [33]. On the other hand, in order to evaluate the antibiotic film activity, dentin blocks were used, a 21-day biofilm was developed and sodium hypochlorite (NaOCl) at 2 was used as irrigant. 5%, 2% chlorhexidine and AgNP's with 94 ppm to later analyze the results through a LIVE/DEAD assay, where the results indicate that AgNP's was statistically more effective in dissolving biofilms than chlorhexidine, however, it showed less antimicrobial effectiveness in infected tubules than the results obtained with 2.5% NaOCl [30]. In 2016, Portland cement supplemented with AgNP's was exposed through a direct contact assay (DCT) to planktonic populations and biofilm of Enterococcus faecalis on teeth, showing a statistically significant increase in antimicrobial and antibiotic activity compared to cement alone [34]. On the other hand, the antibacterial efficacy of AgNP's was evaluated as an irrigant and as an intraconduct drug against Enterococcus faecalis biofilms. The biofilms formed were first tested by irrigating with 0.1% AgNP solution, 2% sodium hypochlorite and sterile saline solution for 2 min. In stage two, they were treated with Ag NPs gel (0.02% and 0.01%) and calcium hydroxide for 7 days and evaluated by scanning electron microscopy and confocal laser microscopy with viability staining. Irrigation with 0.1% AgNPs did not damage the structure of the biofilm and was not different from saline. On the other hand, biofilms treated with 0.02% AgNP's gel as a drug for 7 days significantly eradicated the structure with the lowest number of viable residual E. faecalis cells compared to 0.01% Ag NP gel and calcium hydroxide groups [35].

Although its antimicrobial effectiveness is very good, compared to conventional irrigants, it does not offer a significant difference, instead as an intraducted drug or sealing cement, it shows potential activity against residual biofilms.

3.4 Antimicrobial and antibiotic activity of chitosan nanoparticles

Chitosan is a non-toxic, economical and highly biocompatible product. It promotes positive biological effects such as bactericidal, anti-inflammatory, antioxidant, anti-tumor and healing properties [36]. The chitosan nanoparticles incorporated in dental varnishes result in products that demonstrate more powerful antimicrobial activity than varnishes embedded in propolis or chlorhexidine against S. mutans [37]. Using a collagen membrane test, when supplementing with chitosan the sealant cement AH Plus against Enterococcus faecalis the following numbers of inhibition halos are obtained, AH Plus 7.7±1.0 mm and AH Plus with CsNP's 8.5±0.9 mm, registering an increase of 13.5% of effectiveness [33]. A chitosan supplement to calcium hydroxide, exposed to a 21-day biofilm of Enterococcus faecalis and in multispecies biofilms, through microbiological analysis of colony formation and LIVE/DEAD assay, obtained a significant reduction of CFU's and bacterial viability up to 14 days later, indicated that this combination can be potentially beneficial [38]. For a greater approximation to the clinical environment, teeth were filled with sealing cement supplemented with CsNP's. 7 days after filling they were exposed to inoculations of Enterococcus faecalis for growth at the cement-dentine interface and the results were evaluated by laser confocal microscopy; the results were indicating that supplementation significantly reduced the formation of biofilm at the cement-dentine interface compared to the original sealing cement [39]. In a study of DCT and membrane restriction, the addition of CsNP's to ThermaSeal significantly improved the antibacterial capacity of the sealants even after a time of 4 weeks in addition to inhibiting bacterial adhesion [40]. 2- and 6- week biofilm models of Enterococcus faecalis were used and characterized by scanning electron microscope and laser confocal microscope. In addition, the interaction with macrophages was verified, the CsNP's exhibited lower residual bacterial load and an increase in anti-inflammatory markers, concluding in nanoparticles capable of inactivating biofilms and promoting healing by altering the inflammatory response [41]. It was investigated the percentage of CsNP's inhibition in the formation and reduction of C. albicans biofilms, a Candida biofilm was developed on resin surfaces and CsNP's were applied every 8 h for 5 days. Afterwards, fungal cells (CFU / mL) were counted and shown complete inhibition in time elimination assays [42].

Chitosan is a material that has good antimicrobial and antifungal results and clinical studies, also it shows critical biological interaction in healing, stimulation of anti-inflammatory mediators and rapid recovery.

4. Conclusions

The use of antimicrobial nanoparticles has many opportunities for study and improvement to pinpoint which ones offer a better outcome in endodontic treatment, zinc oxide nanoparticles have antimicrobial and antibiotic film activity very similar to commercial non-nanomeric materials, so it does not offer many advantages, gold nanoparticles work against oral and endodontic pathogens but their reported effectiveness is less than that offered by silver nanoparticles, Talking about chitosan nanoparticles, besides having good antimicrobial activity, they have already reported antifungal activity, added to the fact that it promotes a better healing and fast recovery, finally silver nanoparticles seem to be the best option since they have the largest amount of studies and approaches to the clinical environment of endodontics, reporting their use as a supplement of intraconduct medicines and sealing materials in definitive fillings.

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