Copper Nanoparticles as Potential Antimicrobial Agent in Disinfecting Root Canals. A Systematic Review.

Nanopartículas de Cobre como Potencial Agente Antimicrobiano en la Desinfección de Canales Radiculares. Revisión Sistemática

Sánchez-Sanhueza Gabriela*; Fuentes-Rodríguez Daniela** & Bello-Toledo Helia***

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ABSTRACT: Copper was registered as the first solid antimicrobial material. Its availability makes it an important option as an antibacterial agent. At nanoparticle size it does not exceed 100 nm, allowing close interaction with microbial membranes, enhancing its effect even more. Copper generates toxic hydroxyl radicals that damage cell membranes of Gram-negative and Gram-positive bacteria, among the latter, *Enterococcus faecalis*, which are present in infected radicular canals. Synthesis of metal nanoparticles with antimicrobial properties has become a viable alternative and has promising applications in the fight against pathogenic microorganisms. Furthermore, the use of some polymers to stabilize nanoparticles increases their release time and may as well decrease the risk of bacterial recolonization and biofilm formation within the ducts, enhancing the antimicrobial properties of these compounds. The aim of this article is to conduct a systematic review of the literature on antimicrobial copper nanoparticles, their current applications and their potential use in the area of oral health, specifically in the field of endodontics.

KEY WORDS: copper nanoparticles, antibacterial, endodontics.

INTRODUCTION

Copper is an essential element for the metabolism of animal and plant cells. It is a trace element present in most organisms, and there are more than 30 types of proteins that contain it. Copper, along with gold, were the first metals used by man more than 9000 years ago. Today copper consumption worldwide is about 18 million tons per year.

Its antimicrobial activity is recognized worldwide and has recently been registered by the US Environmental Protection Agency as the first solid antimicrobial material. As early as 2200 B.C. it was used to sterilize wounds and drinking water. In the nineteenth century it was observed that copper workers were immune to cholera, and its use became widespread in the twentieth century.

Laboratory and clinical studies have been conducted to demonstrate its effectiveness. In recent years, copper has been used as a building material for hospitals and medical centers as its antimicrobial properties proved useful in the fight against infections. These findings are particularly important for Chile, the main copper producer in the world (Prado *et al.*, 2012). The synthesis or extraction of compounds such as nanoparticles with antimicrobial properties is important and has potentially promising applications in the fight against the increasing number of pathogens resistant to currently available antimicrobials. These pathogens pose a continuous threat to human and animal health (Usman *et al.*, 2013 & Kruk *et al.*, 2015).

This paper presents a systematic review of the literature on antimicrobial copper nanoparticles, their

* Assistant Professor. Discipline of Endodontics. Department of Restorative Dentistry, Faculty of Dentistry, Universidad de Concepción, Concepción, Chile.

** Undergraduate Student, Faculty of Dentistry, Universidad de Concepción, Concepción, Chile.

*** Associate Professor. Research Laboratory of Antibacterial Agents, Department of Microbiology, Faculty of Biological Sciences, Universidad de Concepción, Concepción, Chile.

current applications and their potential use in the area of oral health, specifically in the field of endodontics.

MATERIAL AND METHOD

Eligibility criteria: Original papers from all levels of evidence according to the classification of the Oxford Centre for Evidence-Based Medicine (<http://www.cebm.net/ocebml-levels-of-evidence>) published between 2005 and 2015 (including both years). The most important journals in English and Spanish were included in this review. These papers should contain data about the use of antimicrobial copper nanoparticles and their applications in the area of health care. The period reviewed allowed an assessment of the topic that included research and publications supported by recent findings. The exclusion of non-on line publications was based on the need of conducting a systematic and replicable review.

Sources: Considering the main databases and languages of important journals in biomedical sciences, especially dentistry, the following databases were used: MEDLINE, EMBASE, LILACS, SciELO, Science Direct, IBECs, Cochrane and CENTRAL. Search engines LILACS and EMBASE were used, as they give access to all the above mentioned databases. The search was conducted between April and June 2015.

Search: The preliminary search algorithm used in EMBASE was the following: (nanoparticles*ab,ti OR cooper*:ab,ti OR antimicrobial*:ab,ti OR dentistry*:ab,ti OR endodontics *:ab,ti) AND [2005-2015]/py. In LILACS: tw (nanoparticles* OR cooper* OR antimicrobial* OR dentistry* OR endodontics*) and year_cluster (2005-2015). The search was refined in successive iterations to consider the inclusion and/or modification of key words found in papers during previous iterations. The algorithm was considered completed when new modifications and/or inclusions gave different results from the previous iteration.

Study selection: From the results in search engines EMBASE and LILACS, the first filtering was performed according to the title of the papers; the second, by the abstract and finally by the full-text. The full application of the inclusion and exclusion criteria described above was considered in the review of the full text. The selected papers were finally classified and registered on an electronic spreadsheet (MS Excel, Microsoft Corp., USA) with their respective bibliographic information.

RESULTS

Fifty-one articles were selected by the search methodology. Nine corresponded to literature reviews and forty-one to experimental studies. Only four of them were related to dental literature containing data about research on copper nanoparticles. Evidence shows that there is preliminary emerging research regarding the use of nanoparticles of copper in dentistry, especially in endodontic. Regarding its antimicrobial properties and its use in the field of human health, some studies support its application as an alternative to treat multi-resistant bacteria.

DISCUSSION

Copper as Antibacterial. Copper has bactericidal action, mainly related to its ability to give and accept electrons in a continuous process. It produces a hydroxyl radical that can participate in a number of adverse reactions to cellular macromolecules such as protein and lipid oxidation. The generated hydrogen peroxide can lead to increased production of toxic hydroxyl radicals. It causes displacement of iron from iron-sulfur clusters, it can compete with zinc or other metal ions, important in protein binding sites; it destroys the membrane, denatures DNA and blocks cellular respiration. In turn bacteria developed tolerance mechanisms to copper such as the extracellular sequestering of ion, impermeability of the outer and inner bacterial membranes, removing copper proteins (metallothionein) in the cytoplasm and periplasm, and efflux of copper from cell (Grass *et al.*, 2011).

Copper alloys acting on abiotic surfaces showed that besides the rapid death of antibiotic resistant strains, it caused destruction of plasmid and genomic DNA, which has an implication in preventing the spread of infections and gene transfer (Warnes *et al.*, 2013 & Bagchi *et al.*, 2013). Copper surfaces or their alloys are able to 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*. Exposing bacteria to copper surfaces makes the cells undergo extensive membrane damage within minutes. The sharp contact with metallic copper surfaces did not result in an increase in mutation rates

or bacterial DNA lesions (Santo *et al.*, 2011). The starting point for the clinical use of copper, specifically for disinfecting the root canal system is reported in a *ex vivo* study where the canals treated with copper sulfate pentahydrate showed a reduction of 6 logarithms in the count of colony-forming units on the fourth day (Sánchez-Sanhueza *et al.*, 2015).

Antibacterial Nanoparticles. Nanoparticles are particles with a size not greater than 100 nm, with spherical, cubic and needle-shaped forms (5-100 nanometers to micrometers) (Allaker *et al.*, 2014). They are formed from different materials, either metallic or polymeric and their active surface area, chemical reactivity and biological activity, are often radically different from larger size particles. These features allow the nanoparticles interact closely with microbial membranes and, therefore, their antimicrobial effect is not due exclusively to the release of metal ions (Morones *et al.*, 2005). Nanoparticles are potentially useful in this context, since it is possible to alter their surface charge, hydrophobicity, and other physical and chemical characteristics. This information is important for the safe use of nanomaterials (Nel *et al.*, 2009).

An inverse relationship between the size of the nanoparticles and antimicrobial activity has been clearly demonstrated, where particles in the range of 1-10 nm in size have demonstrated greater killing activity against bacteria compared to larger particles. It is reported that in Gram-negative bacteria nanoparticles acted mainly in the range of 1-10 nm. In addition, by adhering to the surface of the cell membrane, they drastically disrupt its functions, such as permeability and cellular respiration. They are able to penetrate into the bacteria and cause further damage by possible interactions with sulfur and phosphorus-containing compounds such as DNA. They release ions, which will make an additional contribution to the bactericidal effect of nanoparticles. However, in the case of silver nanoparticles, it has been shown that smaller nanoparticles are more toxic than larger particles, even when they are oxidized (Lok *et al.*, 2007).

The shape of the nanostructure can also affect the activity of the nanoparticles, as demonstrated against *Escherichia coli*. Silver triangular nanoplates truncated with a plane (1, 1, 1) showed better biocidal activity than spherical and rod-shaped nanoparticles. The differences appear to be explained by the proportion of active facets in nanoparticles with different forms (Pal *et al.*, 2007).

It is suggested that bacteria are much less likely to develop resistance to metal nanoparticles in comparison to conventional antibiotics. This is because metals can act on a wide range of microbial targets, and many mutations would have to occur for the microorganisms to resist their antimicrobial activity. Metals have been used for centuries as antimicrobial agents. Silver, copper, gold, titanium, zinc have gained particular attention, each having different properties and spectra of action (Allaker, 2010).

With respect to nanoparticles, the antimicrobial properties of silver and copper have received the most attention. Both have been incorporated into various materials including polymethylmethacrylate (PMMA) and hydrogels.

Bacterial susceptibility to nanoparticles depends on several factors such as the bacterial strain, the type and size of nanoparticles, the nature of the initial growth media and cell concentration. In general, Gram-negative organisms are more resistant to the ion effect of copper nanoparticles than Gram-positive bacteria (Yang *et al.*, 2015).

There is growing interest in the application of nanoparticles as oral antimicrobials to control various infections, for their biocidal properties and anti-adhesive capabilities against biofilms. In dentistry there are some studies reporting the use of antimicrobial nanoparticles as components of prosthetic coatings, and topical agents within dental materials, but there are still very few reports about their use as medication in endodontics (Kishen, 2010). Bacteria isolated from root canals can be set in intracanal biofilms in the dentinal tubules of 1-5 microns in diameter, and the uptake and penetration of antimicrobial agents in them are key considerations in therapeutic results.

Copper Nanoparticles. In the form of nanoparticles, the antimicrobial properties of copper are among the most described (Ren *et al.*, 2009). Compared to traditional antibiotics, nanoparticles are effective at concentrations 1000 times lower. Periodontal pathogens like *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans* were found to be susceptible to silver nanoparticles and copper oxide (CuO) in anaerobic conditions, with minimum bactericidal concentrations (MBC) ranging from 0.025 to 2.5 mg/ml (Prado *et al.*, 2012). However, copper is cheaper than silver, readily miscible with polymers, and relatively stable chemically and physically.

CuO is a compound semiconductor with a monoclinic structure and is the simplest member of the family of copper compounds. It has a range of useful properties such as high temperature superconductivity, correlation effects of electron and dynamic rotation. While copper may have a similar mode of action to silver, it is believed that the key is the action of copper in SH-groups of microbial enzymes. It has been shown that copper nanoparticles have higher affinity to amines and carboxyl groups in a high density on the surface of *Bacillus subtilis* than silver nanoparticles, resulting in a higher antimicrobial activity. Another report reinforces the finding that copper acts better on *B. subtilis* when compared to silver nanoparticles (Yoon *et al.*, 2007), but not on *S. aureus* and *E. coli* (Ruparelia *et al.*, 2008). CuO nanoparticles in suspension show activity against a variety of bacterial pathogens including MRSA and *E. coli*, with minimum inhibitory concentrations (MIC) ranging from 0.1 mg/ml to 5.0 mg/ml. Copper oxide (I) or cuprous oxide (Cu₂O) is a red powder and can also be produced as nanoparticles, which has shown activity against a range of species and strains of bacteria.

Regarding biosafety, one study compared the toxicity and accumulation of two different copper compounds, CuO nanoparticles and soluble CuSO₄, in erythrocytes and different tissues in rainbow trout (*Oncorhynchus mykiss*). The results of *in vitro* toxicity indicate that both copper compounds increase the rate of hemolysis in a dose-dependent manner, but the effect is lower when cells are treated with CuO nanoparticles. Furthermore, both copper compounds cause DNA damage, which was more pronounced in cells treated with CuSO₄. Copper concentrations in kidney and gill were significantly higher in fish exposed to CuSO₄ compared with CuO nanoparticles. Significant DNA damage was detected only when Cu was injected as CuSO₄ in comparison to controls. These data suggest that Cu nanoparticles are less toxic than at their macroscale size (Isani *et al.*, 2013). Another report noted that copper toxicity to A6 cells of a frog (*Xenopus laevis*) depends on the form of copper and the state of the cell, so that the effects were produced before stages of differentiation and proliferation. The three forms of copper tested, (Cu²⁺; CuO nanoparticles (6 nm) and CuO nanoparticles polydisperse (poly-CuO) caused an increase of cell death and altered cell cycle progression, being poly-CuO the one that caused the most serious effects. It may be considered that the poly-CuO particles were highly polydisperse, with sizes ranging from 40 to 500 nm in deionized water, with a majority measuring

around 100 nm, and therefore containing particles, both within and outside the defined nano scale (Thit *et al.*, 2013). To test the consequences of the exposure of brain cells to CuO nanoparticles, with a diameter of about 5 nm, cultures of primary brain astrocytes were exposed to CuO nanoparticles, where increased levels of intracellular copper were observed, affecting the cell viability which depends on concentration and temperature. Their application in concentrations above 100 mM copper (6.4 g/ml) severely compromised the viability of cells. Induced toxicity by CuO nanoparticles was accompanied by an increase in the generation of reactive oxygen species (ROS) into cells (Bulcke *et al.*, 2014). Conventional physical and chemical methods of synthesizing metal nanoparticles may be one of the possible reasons for their toxicity. This problem may be overcome by synthesizing nanoparticles from biological sources.

The method of chemical reduction appears to be the most versatile for producing metal nanoparticles because it is simple and inexpensive. It usually involves the reduction of metal salts with a reducing agent in any type of solvent and a capping agent to keep growth of a nanoparticle at specific and required size. One of the most challenging aspects of the synthesis of copper nanoparticles is their high tendency to oxidation (Zain *et al.*, 2014).

Copper nanoparticles have important limitations, including the rapid oxidation in air exposure. Copper is oxidized to CuO and Cu₂O, and becomes Cu²⁺ during preparation and storage, so it is difficult to synthesize copper nanoparticles in a natural environment. Moreover, an excess of copper in the human body leads to the generation of the most detrimental radicals, such as the hydroxyl radical. However, there are enzymes such as copper triphosphatases adenosine (Cu-ATPases), which play an important role in the homeostasis of copper, and the excess is exported through the intestine as feces, through the liver as a product of bile, and through the breast gland in the milk.

It is known that bacteria have strict control over the transport of copper (Input-Output), ensuring the homeostasis required for protein synthesis and prevention of toxic effects, by a highly controlled system of transcriptional regulators, soluble chaperone, membrane transporters, and copper proteins distributed in various bacterial compartments (Rademacher *et al.*, 2012; Elguindi *et al.*, 2011). Most bacteria synthesize P-type ATPase export of copper

as main determinants of defense when copper concentrations are higher than average. In addition, many bacteria use the efflux systems RND and multicopper oxidases to deal with excess copper (Shrestha *et al.*, 2014).

An attempt to establish metal nanoparticles with physiological importance as the best candidates for future nanomedicine can only succeed if these particles are synthesized with improved biocompatibility and low or no toxicity (Sengupta *et al.*, 2014). Today we talk of "Green Nanotechnology" and "nanotoxicology" as two important areas of research on nanomaterials. A report showed that colloids of silver nanoparticles supported on biopolymers, stabilize nanoscale particles, making them minimally toxic and maintaining their antimicrobial activity, which has profound biomedical implications (Barua *et al.*, 2013). Cu₂O nanoparticles were prepared, using agricultural waste *Arachis hypogaea* (peanuts), leaf extracts containing reducing sugars, which act as reducing agent at room temperature (Ramesh *et al.*, 2011). The aldehyde group present in reducing sugar plays an important role in the formation of cuprous oxide nanoparticles in the solution. Another report notes that morphology of 15-30 nm copper oxide nanoparticles can be controlled by regulating the amount of Aloe vera extract. This ecological approach for synthesis is suitable for commercial scale production and related health CuO nanoparticles applications (Gunalan *et al.*, 2012; Kharissova *et al.*, 2013.).

Other alternatives developed to synthesize metal nanoparticles are the use of polymers, for example polyvinylpyrrolidone, polyethylene glycol, and chitosan. Nanoparticles stabilized by biopolymers prolong their release time, which improves, among other things, their antimicrobial properties. Chitosan is the second most abundant biopolymer of natural origin after cellulose and is obtained by removing an acetyl group from chitin. It consists of glucosamine units and N-acetylglucosamine. It is a biocompatible, biodegradable, nontoxic polymer, and has various applications in pharmaceutical and biomedical fields. It presents positive charge and is soluble in acidic to neutral solution, allowing it to bind to mucosal surfaces. These properties make chitosan a good candidate for medical applications and research (Sani-Usman *et al.*, 2012).

Chitosan-copper nanoparticle surfaces are covered by fragments of chitosan that protect against aggregation and oxidation, increasing the release time of the compound. It was shown that the size of

nanoparticles could be controlled by manipulating the concentrations of chitosan and silver, and copper nitrate used in their synthesis. Particle size could be increased either by decreasing the concentration of chitosan or increasing the concentration of metal ions. Nanoparticles produced had a positive surface charge and chitosan used in their synthesis contributed to the stability of suspensions of such particles and had a bactericidal effect (Mallick *et al.*, 2012).

Although antimicrobial irrigants (without incorporation of chitosan) currently used to disinfect root canals in treating endodontic infections are able to kill *Enterococcus faecalis*, they often fail in endodontic restorations. An *in vitro* study by Kishen *et al.* (2008) showed that the root canal surfaces treated with cationic antibacterial nanoparticles combined with chitosan significantly reduced the adhesion of *E. faecalis* to dentin. In theory, such surface treatment may prevent bacterial recolonization and biofilm formation.

One research established a starting point for the potential clinical use of copper in endodontics. In this study the drugs commonly used in endodontics and copper sulfate pentahydrate were compared. It was observed that copper sulfate presented an antimicrobial activity superior to concentrations lower than Chlorhexidine (Sierra *et al.*, 2013). Therefore, the antibacterial properties of copper at nanoscale could also have applications in endodontics, since the environment in the root canal, which serves as ecological niche for microorganisms, is a surface made up of small dentinal tubules composed of collagen and hydroxyapatite; and they are established in the form of biofilm, and as copper is considered a contact antibacterial, its usage time could be reduced to the minimum necessary (Kim *et al.*, 2013; Reyes-Jara *et al.*, 2010).

CONCLUSION

Copper nanoparticles are a group of toxic antimicrobials for the cell membrane of Gram-negative and Gram-positive bacteria, so it could be a therapeutic alternative in the future, either as intracanal medication or as an irrigant to decontaminate and disinfect the root canal. Moreover, this compound may be enhanced with polymers as chitosan, which stabilizes these nanoparticles, increasing their biocompatibility, release time, reducing the risk of bacterial recolonization and

biofilm formation within the ducts, enhancing its antimicrobial properties. Although more research on the effectiveness of copper nanoparticles in disinfecting root canals is required, the antimicrobial potential of this metal, together with its high availability and low cost, make it worthy of consideration as a therapeutic alternative for successful future root treatments.

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RESUMEN: El cobre fue registrado como el primer material antimicrobiano sólido y su disponibilidad permite que constituya una importante opción como agente antibacteriano. Al tamaño de nanopartícula no supera los 100 nm, lo que permite una interacción estrecha con las membranas microbianas, potenciando aún más su efecto. Genera radicales hidroxilos tóxicos que provocan daño en la membrana celular de bacterias Gram negativas y Gram positivas, entre estas últimas, *Enterococcus faecalis*, presente en conductos radiculares infectados. Por lo que la síntesis de nanopartículas metálicas con propiedades antimicrobianas se ha vuelto una alternativa viable con aplicaciones prometedoras en la lucha contra microorganismos patógenos. Además, utilizando algunos polímeros para estabilizar las nanopartículas se aumenta el tiempo de liberación de éstas y se disminuye el riesgo de recolonización bacteriana y la formación de biopelículas al interior de los conductos, mejorando las propiedades antimicrobianas de estos compuestos. El propósito de este artículo es realizar una revisión sistemática de la literatura acerca de nanopartículas de cobre como antimicrobiano, sus aplicaciones actuales y su potencial uso en el área de la salud oral, específicamente en el campo de la Endodoncia.

PALABRAS CLAVES: nanopartículas de cobre, antibacteriano, endodoncia.

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Correspondence to:
Gabriela Sánchez-Sanhueza
Departamento de Odontología Restauradora
Facultad de Odontología
Universidad de Concepción
Roosevelt #1550
Fono: 56- 412204481
Concepción
CHILE

Email: gasanchez@udec.cl

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