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Original Research

Synthesis, characterization and antibacterial activity of copper, nickel and bimetallic Cu–Ni nanoparticles for potential use in dental materials

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Abstract

The antibacterial effect is a desirable property in dental materials. Development of simple methods for the preparation of nanosized metal particles has attracted significant attention because of their future applications due to unusual size-dependent antibacterial properties. Copper (Cu), Nickel (Ni) and bimetallic Cu–Ni nanoparticles were prepared by a simple chemical method and their antibacterial activity was tested against the widely used standard human pathogens *Staphylococcus aureus* (gram-negative) and *Escherichia coli* (gram-positive). Additionally, these nanoparticles were tested against the dental pathogen *Streptococcus mutans*. Our results are promising for potential use in dental materials science.

Keywords: Antibacterial properties; Metal nanoparticles; Copper nanoparticles; Nickel nanoparticles; Nano-alloy

1. Introduction

In dentistry, the persistent microorganisms for various clinical conditions are a common problem. If bacterial contamination is severe, it can lead to treatment failure. For example, several studies have demonstrated that microorganisms can remain viable beneath non-antiseptic fillings for considerable periods of time. This persisting microbial presence beneath restorations is a major factor in the development of recurrent caries [1–3]. It has been suggested that this microbial presence may be eliminated if the cut dentine surface is sterilized, prior to cavity restoration, by antimicrobial solutions which are harmless to the dental pulp. Consequently, it has been realized that there is a need for dental restorative material or cement which could act as an effective inhibitor of bacterial growth. On the other hand, the role of bacteria in the endodontic treatment failure has been well established. Apical periodontitis can develop after treatment, due to bacterial contamination of the filled root canal system resulting from coronal leakage. Due to this, it is important to develop other dental materials with antibacterial activity, without compromising the mechanical properties, which could be fabricated and might be applied in future clinical applications [4,5].

Nanoparticles (NPs) usually refer to spherical particles with diameters in the range 1–100 nm [6]. NPs have higher surface to volume ratio compared to particles constituted for the same material that are not at the nanoscale and therefore, NPs are more reactive [7]. Because of this large fraction of surface atoms, nanoparticles show unusual physical, chemical, and biological properties. This way, a confluence of nanotechnology and biology can address several biomedical problems and can revolutionize the fields of health and dentistry. Currently, some noble metal NPs have been extensively investigated and they are well known for their antibacterial effects [8]. For NPs

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synthesis the noble metals such as gold and silver are being used, despite their cost. In this context, copper (Cu) and nickel (Ni) are good alternative materials because they are more economical than gold and silver. Recent investigations have been extended to the study of other metals, such as Cu and Ni, that could have antibacterial activity. However, little attention has been paid to the study of bimetallic Cu–Ni NPs, although some studies have already shown some features of alloy NPs that distinguish them from the pure ones [9].

It has already been reported that Cu NPs [10–13] and copper oxide NPs [14–18] have antimicrobial activity. Similar findings have been reported for Ni NPs [19,20]; however, there are very few studies available on this topic. Studies have shown that Cu and Ni NPs have bactericidal activity. Nevertheless, they have not been synthesized in aqueous solution without using stabilizers as polymers, ligands, salts, etc. that can hinder their properties. Finally, it is important to note that until now, the antimicrobial properties of Cu–Ni bimetallic NPs have not been studied. Therefore, the purposes of this study were the synthesis and characterization of Cu, Ni and bimetallic Cu–Ni NPs as well as to investigate their antimicrobial activity.

2. Material and methods

2.1. Synthesis of NPs

The salts used were: copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), nickel sulfate hexahydrate ($NiSO_4 \cdot 6H_2O$). A solution consisting of deionized water and the corresponding metal salt with concentration 1×10^{-2} M was prepared. In the case of bimetallic particles an equal mole amount of each salt was used to complete 1×10^{-2} M. The pH of the solution was fixed with NaOH, depending on each metal. The metal salt solution was bubbled with nitrogen (N_2) for 30 min. After that, the reducing agent, sodium borohydride ($NaBH_4$) was used in two concentrations: stoichiometric concentration (4×10^{-2} M) and excess concentration (8×10^{-2} M), as appropriate. To achieve a rapid chemical reduction with $NaBH_4$, it must be added quickly on the metal salt solution[21]. After the addition of the reducing agent the solution was stirred for 120 min to complete the reaction. After that, the precipitate was filtered and washed three times with distilled water, and once more with acetone to displace deionized water. Finally, to store the nanoparticles and avoid oxidation isopropyl alcohol was added in a sufficient amount to submerge them completely. Table 1 lists the preparation conditions of the NPs.

Table 1
Synthesis conditions for Cu, Ni and Cu–Ni bimetallic NPs.

Nanoparticles	pH	Concentration of reducing agent
Copper	Free	4×10^{-2}
Nickel	8 (Controlled)	8×10^{-2}
Cu–Ni bimetallic	8 (Controlled)	4×10^{-2}

2.2. Characterization

The synthesized Cu, Ni and bimetallic Cu–Ni NPs were characterized by Scanning Electron Microscopy (SEM), Energy Dispersive Energy (EDS), X-ray diffraction (XRD) and Transmission Electron Microscopy (TEM). Final product was sonicated for 30 min to break big nanoparticle agglomerates. The particles were then dried in vacuum at room temperature (20 °C) prior to analysis. The SEM and EDS analysis were obtained using a Scanning Electron Microscope JEOL, JSM-6510LV at 20 keV (Tokyo, Japan).

The crystalline phases and composition of the Cu, Ni and Cu–Ni NPs were investigated using a Bruker D8 Advance X-ray diffractometer (Frankfurt, Germany), operated at 35 kV, 30 mA., with CuKα1 radiation (wavelength $\lambda = 1.5406$ Å) and copper filter. The X-ray diffractogram was recorded in the 2θ range from 10° to 80° at scanning steps of 0.049°.

The transmission electron micrographs (TEM) were obtained with a JEOL JEM-2100 microscope (Tokyo, Japan). Samples for the TEM examination were prepared by placing a drop of the sample suspension on a copper grid (300 mesh) coated with carbon film and let dry in ambient air.

2.3. Antibacterial activity

The bacterial strains used in this study were obtained from the stock-culture collection of the Biochemistry Laboratory of the School of Dentistry, National Autonomous University of Mexico (UNAM). The strains used are endemic to the region from central Mexico and each one was characterized by a battery of cultural and biochemical tests. These strains included gram positive and gram negative bacteria commonly used as standards.

The experiments on the antimicrobial activity were carried out as described by the Clinical and Laboratory Standards Institute [22]. Antimicrobial activity of the synthesized NPs was tested against the human pathogenic bacteria *Staphylococcus aureus, Escherichia coli* and *Streptococcus mutans* by determining the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) following the broth dilution method. Selective media were used to culture each strain. For culturing *S. aureus*, *E coli* and *S mutans*, the agars used were: Eosin methylene blue agar [23], Mannitol salt agar [24] and Mitis saliviarius agar enriched with 150 g/L sucrose, potassium tellurite 1 mL (1%) and 1 mL of bacitracin (200 U/mL) [25], respectively. The samples were initially incubated at 37 °C for 24 h for the bacterial cultures, which were used to prepare McFarland standards. The 10 mL nutrient broth medium was prepared. Each set was inoculated aseptically with 10 mL of the respective bacterial suspension (approximately 10^8 CFU/mL). Six dilutions of NPs were prepared for testing: 0.01, 0.10, 1.0 10.0, 100.0 and 1000.0 μg/ml. We used a positive control (only bacteria) and a negative control (only NPs). Tests were performed three times for each strain. The inoculated sets were incubated at 37 °C for 24 h. The presence or absence of turbidity in each tube was recorded. Tubes showed no turbidity when cultured on agar plates. Viable