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Comparative effectiveness of $NiCl_2$, Ni- and NiO-NPs in controlling oral bacterial growth and biofilm formation on oral surfaces

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ABSTRACT

Objective: Oral ailments are often treated with antibiotics, which are rendered ineffective as bacteria continue to develop resistance against them. It has been suggested that the nanoparticles (NPs) approach may provide a safer and viable alternative to traditional antibacterial agents. Therefore, nickel (Ni)- and nickel oxide (NiO)-NPs were synthesized, characterized and assessed for their efficacy in reducing oral bacterial load in vitro. Also, the effects of bulk compound $NiCl_2$ (Ni ions), along with the Ni- and NiO-NPs on bacterial exopolysaccharide (EPS) production and biofilm formation on the surface of artificial teeth, and acrylic dentures, were investigated.

Methods: Total bacteria from a healthy male were collected and adjusted to 4×109 cells/ml for all the tests. Effect of the NPs on growth, biofilm formation, EPS production and acid production from glucose was tested using standard protocols.

Results: Data revealed that the Ni-NPs (average size 41.23 nm) exhibited an IC50 value of 73.37 μg/ml against total oral bacteria. While, NiO-NPs (average size 35.67 nm) were found less effective with much higher IC50 value of 197.18 μg/ml. Indeed, the Ni ions exhibited greater biocidal activity with an IC50 value of 70 μg/ml. Similar results were obtained with biofilm inhibition on the surfaces of dental prostheses. The results explicitly suggested the effectiveness of tested Ni compounds on the growth of oral bacteria and biofilm formation in the order as $NiCl_2 >$ Ni-NPs $>$ NiO-NPs.

Conclusion: The results elucidated that Ni-NPs could serve as effective nanoantibiotics against oral bacteria.

1. Introduction

Microbial community in the oral cavity is represented by hundreds of microorganisms forming dental plaques and colonizing a variety of surfaces.[1,2] Dental plaque is a dynamic and complex oral biofilm ecosystem consisting of at least 800 bacterial species.[1] The toxic substances like exotoxins, endotoxins and metabolites like hydrogen sulfide, methyl-mercaptan and polyamines released by oral bacteria evoke a number of oral diseases.[3–5] Bacteria such as *Streptococcus mutans* and *Streptococcus sobrinus* are well-known causative

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agents of dental caries. Similarly, *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* are known to cause halitosis and periodontitis.[5] Presence of *Exiguobacterium oxidotolerans*, *Prevotella melaninogenica*, *Staphylococcus aureus* and *Veillonella parvula* in the oral cavity is being related to oral cancer.[6] Prevention and treatment of oral diseases require the reduction of bacterial accumulations at retentive sites in the oral cavity, which ultimately leads to biofilm formation on these surfaces. To maintain good oral hygiene a number of antimicrobial formulations in the form of tooth pastes and mouth washes are being used. These formulations contain bisbiguanide, enzymes, essential oils, metal ions, phenols, plant extracts, quaternary ammonium compounds and surfactants[7] to inhibit bacterial growth. Also, for treatment of oral infections, the antibiotics such as penicillin G, penicillin V, erythromycin and tetracyclines are often used.[7,8] However, the growing resistance in bacteria against these drugs[9] has become a serious public health problem, and has evoked interest in finding ways to formulate new types of safer and cost-effective biocidal materials.

Metal nanoparticles (NPs) are regarded as new generation antimicrobial agents, as bacteria are less likely to develop resistance against metal NPs than conventional antibiotics. Besides, some antimicrobial agents are awfully irritant and toxic, which necessitates the search for new alternatives. Previous studies have shown that nanoparticles can serve as effective bactericidal materials.[10,11] Antimicrobial activity of Al_2O_3, Fe_2O_3, CeO_2, ZrO_2 and MgO against urinary tract infecting microorganism (*Pseudomonas* sp., *Enterobacter* sp., *Klebsiella* sp., *Escherichia coli*, *Proteus morganii* and *S. aureus*) was tested by Ravikumar et al.[12] Al_2O_3 nanoparticles were found to be most effective against these microorganisms. In another study the effect of silver NPs against water borne pathogens namely *E. coli* and *Vibrio cholerae* was tested.[13] Stoimenov et al. demonstrated that highly reactive metal oxide NPs exhibit excellent biocidal action against Gram-positive and Gram-negative bacteria.[14] When biocidal properties of silver NPs were compared with chlorhexidine, a traditionally used antimicrobial agent, silver NPs were found to be more effective.[15] Also, the effect of ZnO, CuO, Ag, Au and Bi on dental caries causing bacteria *S. mutans* has been widely studied.[16–18] Our earlier studies have also demonstrated the biocidal effect of ZnO- and CuO-NPs on total oral bacteria.[19] It is strongly realized that the preparation, characterization, surface modification, and functionalization of metal NPs may unwind the possibility of formulation of a new generation bactericidal materials. Therefore, we have chosen Ni-NPs for assessment of their anti bacterial effect on oral microflora. To the best of our understanding the effects of NPs on oral microbiome has not been thoroughly investigated. Furthermore, the nickel-containing metal alloys are being extensively used for dental prostheses and orthodontic appliances. It has been suggested that reducing total bacterial load in the oral cavity is of primary importance and crucial for the prevention of oral ailments. Therefore, in this study, the biocidal activities of Ni- and NiO-NPs have been investigated on total oral bacterial growth, metabolism and biofilm formation on artificial acrylic teeth and dentures with aim of elucidating the efficacy of Ni ions, Ni- and NiO-NPs, as future nanobiotics in biomedical and dental applications.

2. Materials and methods

2.1. Synthesis of Ni and NiO-NPs

Ni-NPs were synthesized through a solution reduction process using hydrate hydrazine as a reducing agent.[20] Hydroxyethyl carboxymethyl cellulose (HECMC) was added to aqueous solution of $NiCl_2{\cdot}6H_2O$ to a final concentration of 0.2% (w/v) NaOH solution. Subsequently, hydrate hydrazine was added to above mixture to achieve a pH value of 11.0. The resulting solution was kept in a thermostatic bath till the black colour precipitate is developed completely. The product was washed with distilled water and 75% ethanol for three times, and dried in a vacuum drying oven at room temperature.

NiO-NPs were synthesized using nickel acetate and polyvinyl acetate (PVAc) precursor, as described by Dharmaraj et al.[21] In brief, nickel(II) acetate tetrahydrate (0.1 M) was dissolved in 2-methoxy ethanol (1 M) by heating at 72 °C for 2 h under stirring. PVAc (14% w/v) solution was prepared in N,N-dimethyl formamide by stirring at room temperature. Then nickel acetate and PVAc solutions were mixed in a ratio of 1:4 (v/v) and stirred for 3 h at room temperature. The resulting mixture was heated at 152 °C to remove the solvent and water. Solid mass obtained was ground well and heated in a crucible at 450 °C for 3 h in air to get the NiO-NPs.

2.2. Characterization of Ni- and NiO-NPs

The crystalline nature of Ni- and NiO-NPs was determined by X-ray diffraction (XRD) pattern. The XRD pattern of Ni and NiO nanopowder was acquired at room temperature with the help of a PANalytical X'Pert X-ray diffractometer (Spectris plc, England) equipped with an Ni filter using Cu Kα (λ = 1.54056 Å) radiations as an X-ray source. Structural studies of Ni- and NiO-NPs were performed by transmission electron microscopy (JEM-2100F, JEOL Inc., Japan) at an accelerating voltage of 200 kV. Briefly, the dried powder of Ni and NiO-NPs were suspended in deionized water at a concentration of 1 mg/ml, and then sonicated by use of a sonicator bath at room temperature for 15 min at 40 W, to prepare a homogeneous suspension. For size measurement, the stock solution was further diluted to a final concentration of 100 μg/ml. Transmission electron microscopy (TEM) was used to determine the size and shape of NPs. A drop of aqueous NPs suspension was placed onto a carbon-coated copper grid, air-dried and observed under TEM.

2.3. Effect of $NiCl_2$, Ni- and NiO-NPs on total oral bacterial population

Slurry was collected from the surface of the teeth crown of a healthy male using sterile toothpick and suspended in 1 ml of autoclaved PBS. The number of bacterial cells in suspension was adjusted to 4×10^9 cells/ml in all the experiments. The aliquots of 500 μl were separately inoculated in 5 ml of sterile nutrient broth and brain heart infusion (BHI) broth. The cells were treated with $NiCl_2$, Ni- and NiO-NPs at concentrations of 50, 100 and 200 μg/ml at 37 °C for 16 h. Treated and untreated cultures (0.1 ml) were spread on nutrient agar prepared by