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Antimicrobial Effect of Copper Oxide Nanoparticles on Some Oral Bacteria and Candida Species

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Abstract

Statement of Problem: Acid producing bacteria including Streptococcus mutans and lactobacilli cause tooth demineralization and lead to tooth decay. Also, oral colonization of the species of Candida has been reported in many studies that are resistant to antifungal agents.

Objectives: In this study, antibacterial and antifungal effects of nano-CuO were studied against some oral bacteria and yeast fungi.

Materials and Methods: The minimum inhibitory concentrations (MICs) of copper oxide nanoparticles (CuO NPs) for oral bacterial and fungal test strains were determined in 96-well microtiter plate technique. The agar diffusion test (ADT) was employed to assess the antifungal properties of nystatin.

Results: The MIC_{50} value of CuO NPs was determined at the range of 1–10 µg/ml for *S.* mutans, < 1 µg/ml for L. acidophilus, and 10 µg/ml for L. casei. Higher concentrations of CuO NPs (100-1000 µg/ml) were effective on the bacterial cell growth, resulting in 100% reduction in the optical density in TSB medium. The cells of Candida albicans, C. krusei and C. glabrata were treated with CuO NPs and the results showed a decrease in fungal growth at a concentration of 1-1000 µg/ml in TSB medium. The MIC_{50} value of CuO NPs was determined 1000 µg/ml for three species of Candida. The diameter of growth inhibition zones of 1100 µg/ml nystatin was obtained 15-21 mm for clinical isolates of three species of Candida.

Conclusions: With respect to the potential bactericidal activity of CuO NPs on various cariogenic bacteria examined in this study, these NPs could be introduce as a candidate control agent for preventing dental caries or dental infections. In our study, on the other hand, Nano copper oxide had a weak effect on the candida species.

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Introduction

Antibiotic treatment of oral biofilms is inadequate, often leading to chronic oral infections and constrained tooth extraction or implant removal due to the development of antibiotic resistance [1,2]. Oral biofilms comprise a complex polymicrobial community in which oral streptococci are initial colonizers [3].

Tooth demineralization caused by acid producing bacteria including Streptococcus mutans, S. sobrinus, and Lactobacillus species, which can ferment dietary carbohydrates, leads to dental decay [4-6]. The bacterial gelatinous material adheres to tooth surfaces and becomes colonized by bacteria to form dental plaque. Several factors including dietary carbohydrates, cariogenic bacteria, and many host factors such as teeth and saliva result in tooth decay over time [4,5].

The most common etiological agent of candidiasis in compromised patients is known as Candida albicans. This organism can produce biofilm on the oral mucosa. The exopolymeric substance matrix surrounding the cells of Candida protects the yeast cells against harsh conditions and the antifungal antibiotics [7].

The use of nanoparticles, as new agents for inhibition of microbial growth, has developed due to the development of antibiotic resistance [8-10]. The particle with 1-100 nm size that behaves as a whole unit with respect to transport and properties is called nanoparticle [11-13]. These particles which have much higher surface area than conventional materials are currently considered as antimicrobial agents [8]. Among these nano-materials, nano-metals have been used more because of less toxicity [7,14].

The silver NPs were studied in most related research, exhibiting antibacterial effect at low concentrations [8,10,15].

Ionic nanopariculate metal oxides are among the potentially interesting antimicrobial agents, because of their extremely high surface areas and having unusual crystalline structures with high number of edges and corners and other reactive sites [16]. Copper oxide nanoparticle (CuO NP) is the simplest member of the Cu compounds that reveal a range of potential physical properties and is much cheaper than silver oxide. It can be mixed easily with polymers to provide the composites with unique physio-chemical properties. Also, these nanoparticles have high surface areas and unusual crystalline structures to give CuO NPs with antimicrobial activity that is dose dependent [17].

Metals NPs and other NPs are being combined with polymers (such as: dental composite) or coated onto surfaces which acquired the potential applications within the oral cavity [10,18].

In this study, the antimicrobial activity of CuO NPs against Streptococcus mutans, Lactobacillus acidophilus, L. casei and three species of oral Candida including C. albicans, C. krusei, and C. glabrata were investigated.

Materials and Methods

Preparation of microbial inoculums

Bacterial inoculums were prepared from cultured Streptococcus mutans (PTCC 1683), Lactobacillus casei (PTCC 1608), and L. acidophilus (PTCC 1643) on Tryptic Soy Agar (TSA) (236950 - BD Difco™) medium incubated at 37°C for 48 hours. The bacteria were transferred to Tryptic Soy Broth (TSB) (211825 - BD Difco™) media and incubated at 37 °C overnight. One ml from each overnight broth culture was inoculated to 10 ml broth medium and incubated at 37 °C on a rotary shaker with 180 rpm shaking until optical density adjusted to 0.5 McFarland standard.

Yeasts inoculums equivalent to 0.5 McFarland standard were prepared for antifungal assay of nystatin and CuO-NPs as explained for bacteria.

Preparation of nano-copper oxide solution

For preparation of stock nano-solution, 1 mg CuO NPs was dissolved in 1 ml sterilized double distilled water and kept in Ultrasonic Sonicator bath for 30 min.

MIC of nano-copper oxide

The minimum inhibitory concentrations (MICs) of CuO NPs for oral bacterial and fungal test strains were determined in 96-well microtiter plate technique as described by Padil and Cernik [19]. TSB medium was used for resistance experiments.

MIC values were detected by various concentrations of CuO NPs in the range of 1 µg/ml to 1000 µg/ml. As the first step, 50 µl aliquot of 0.5 McFarland microbial suspension was inoculated to the wells of microtiter plate; then, 50 µl of TSB supplemented with the considered concentrations of CuO NPs was added. The TSB medium without CuO NPs inoculated with the bacterium and TSB medium supplemented with CuO NPs without any bacterium were used as proper controls for these experiments. After incubation at 37

°C for 48 hours, the microtiter plates were scanned with an ELISA reader (Stat Fax -2100, Portland, ME, USA) at 600nm. All experiments were carried out in triplicates with proper blank.

Inhibition effect of nystatin

In this study, the inhibition effect of nystatin was experimented by agar diffusion test (ADT) method using the nystatin concentrations with a range of 0-1100 µg/ml dissolved in di-methyl sulfoxide (DMSO) as antifungal antibiotic for comparison by the CuO NPs. The inhibition zone diameter was measured after 48 hours of incubation at 37 °C.

CuO NPs with a size of 40 nm and purity of 98% were purchased from Nano Avijeh (Nanosav) Co.

Statistical Analysis

Data were analyzed by Excel 2013. All experiments were performed as triplicates. The means of data and standard deviations were analyzed by Excel.

Results

Growth inhibition of Bacteria by nano copper oxide in microtiter plate

Treatment of three oral bacteria including Streptococcus mutans, L. casei and L. acidophilus with CuO NPs resulted in a decrease in bacterial growth in the TSB medium (Figure 1). The MIC_{50} value of CuO NPs was determined at the range of 1–10 µg/ml for S. mutans, < 1 µg/ml for L. acidophilus, and 10µg/ml for L. casei (Figure 1). Higher concentrations of CuO NPs (100-1000 µg/ml) were effective on the bacterial cell growth, resulting in 100% reduction of the optical density in TSB medium (Figure 1).

Inhibition of the growth of oral pathogen candida species by nano-copper oxide in microtiter plate

The cells of Candida albicans, C. krusei and C. glabrata were treated with CuO NPs, and the results showed a decrease in fungal growth at a concentration of 1-1000 µg/ml in TSB medium (Figure 2). The MIC_{50} value of CuO NPs was determined 1000 µg/ml for three species of Candida (Figure 2). In lower concentrations of CuO NPs (1-100 µg/ml), the yeast cells growth was slightly inhibited (almost 30-40% decrease) (Figure 2).

Diameter of growth inhibition zones of 1100 µg/ml nystatin was obtained from 15 mm till 21 mm for clinical isolates of the three species of Candida (Table 1). All the Candida species showed resistance to the concentrations of 0-55 µg/ml nystatin.

Discussion

The inhibition of oral bacteria, S. mutans and Actinomyces viscosus, with ZnO by concentrations of 78 - 312.5 µg/ml was reported by Hall-Stoodley et al. [20]. Also, EC50 value of CuO NPs was obtained as 78 µg/ml for Vibrio fischeri in their study. Khan et al. showed that CuO NPs in the concentration of 50 µg/ml had a higher biocide activity against growth and biofilm formation of oral microbiota than ZnO NPs [3]. Low concentrations (0.0001-1 µg/ml) of CuO

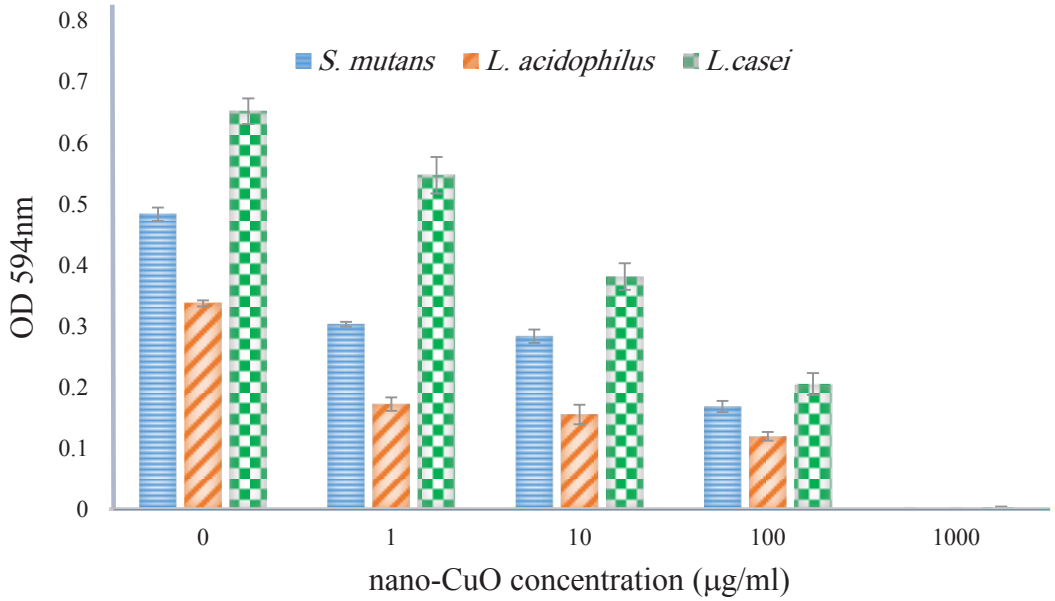

Figure 1: Effect of CuO NPs on the growth of oral bacteria: Streptococcus mutans, Lactobacillus casei, and L. acidophilus by microtiter plate technique in TSB medium incubated at 37 °C for 48 hours.

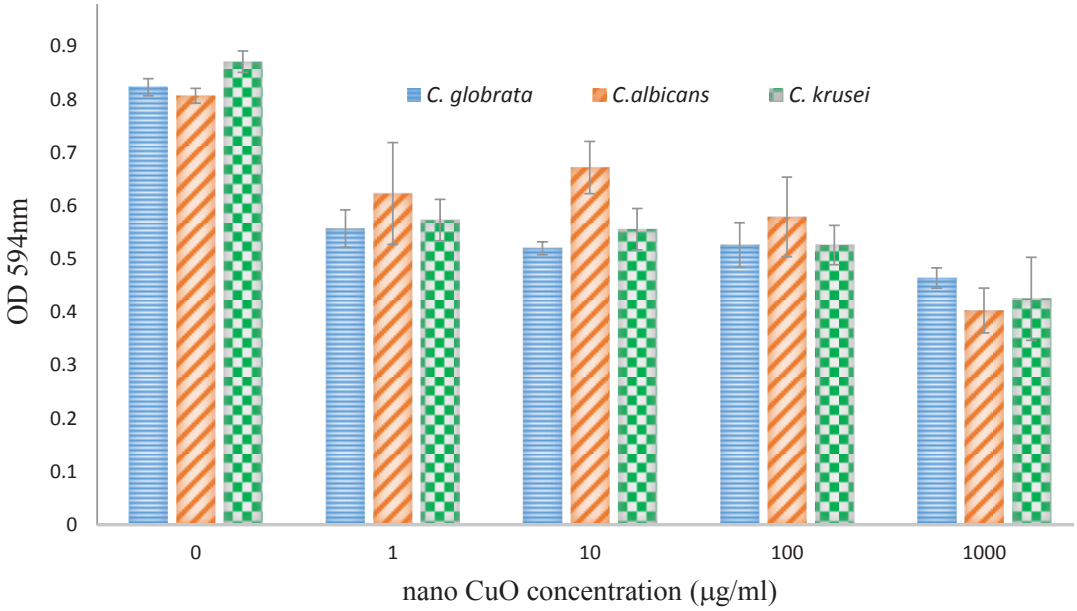

Figure 2: Antifungal activity of CuO NPs against Candida albicans, C. krusei, and C. glabrata by microtiter plate technique in TSB medium incubated at 37 °C for 48 hours.

NPs with 18-20 nm sizes were used by Eshed et al. for inhibition of Streptococcus mutans. The results showed that the growth was not inhibited, but biofilm formation was prevented in these low concentrations of CuO NPs [9].

The mechanism of antibacterial activity of nano-metal oxide is the production of different active oxygen species, like H_2O_2, which inhibit the growth of bacterial cells [21].

Alaker *et al.* showed that CuO NPs in a concentration of 100-5000 µg/ml had no toxic effect on human cells [8]. The micro- and nano-copper oxide up to 50 µg/ml showed no genotoxic and cytotoxic effects on Hella cells [22]. Thus, with respect to the potential bactericidal activity of CuO NPs on various oral bacteria examined in this study and also previous studies and no toxic effect on human cells, this NP could be introduce as a control agent for preventing dental caries or dental infections.

Most frequently isolated organisms from the oral cavity are from Candida species which are detected in 31-55% of healthy population [23]. The major yeast implicated in the esophagitis is C. albicans. C. glabrata or any other Candida species were rarely detected as infectious agents in these patients. Also, oral colonization of C. krusei and C. glabrata with antifungal resistance in vitro is described in compromised patients [23].

MIC_{50} value of Nano-CuO for Candida albicans was obtained 400 µg/ml by Karimiyan *et al.* [24]. The shape and size of nanoparticles can influence their antimicrobial activity [25], which led to different results in these experiments.

The antifungal activity of nystatin was studied on Candida albicans in previous studies. In one of them, the bioactivity of 30 µg/ml nystatin was obtained 18-24 mm in agar well diffusion test [26]. In another study, nystatin showed antifungal effectiveness against C. albicans, and MIC_{50} equal to 25 µg/ml was obtained. MIC50 standard of nystatin for sensitive Candida species is 4-7 µg/ml; thus, the experimented strains of candida in this study were resistant to

Table 1: Mean values and standard deviation (SD) of inhibition effect of nystatin on three species of *Candida* by well diffusion technique on Potato Dextrose Agar (PDA) medium after 48 hours of incubation at 37 °C.

Nystatin (µg/ml)	Inhibition zone (mm) ± SD		
	C. albicans	*C. glabrata*	*C. krusei*
0	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
11	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
55	10.5 ± 0.58	11.5 ± 0.58	0.0 ± 0.00
110	13 ± 1.15	14.5 ± 0.58	11 ± 1.15
550	18 ± 0.00	19 ± 1.15	13.5 ± 0.58
1100	21 ± 1.15	20.5 ± 0.58	15.5 ± 0.58

nystatin. In comparison with the inhibition effect of CuO NPs, the yeast strains showed resistance to nystatin.

The wide and indiscriminate use of antibiotics and different sensitivity of clinical strains of Candida toward the antifungal agents cause different values of drug inhibitory doses [27]. The results of this study showed that the examined yeast strains had considerable resistance to nystatin compared to those strains used by Khoshkholgh-Pahlaviani *et al.* [26]. Also, Amirrajab *et al*. showed high resistance of C. glabrata to some of antifungal antibiotics, such as amphotericin B (the MIC_{50} equal 1100 µg/ml) [28]. In our study, on the other hand, the growth of these strains decreased when treated by 1-1000 µg/ml of nano- copper oxide, and MIC_{50} was seen at a concentration of 1000 µg/ml of nanoparticles.

Compared to organic antibacterial agents such as antibiotics, inorganic substances with antimicrobial activity may have several advantages such as low possibility of resistance, and susceptibility of a broad range of microorganisms to these nano-materials.

Conclusions

Nano-copper oxide used in this study showed a high antimicrobial effect against the examined dental caries bacterial agents, and lower effect on three species of candida. Thus, this NP could be introduced as a candidate control agent for preventing dental caries or dental infections.

Conflict of Interest: None declared.

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